CARNEGIE
INSTITUTION
OF WASHINGTON

Year Book 61

1961-1962

Sixtieth Anniversary

A. D. SINGER

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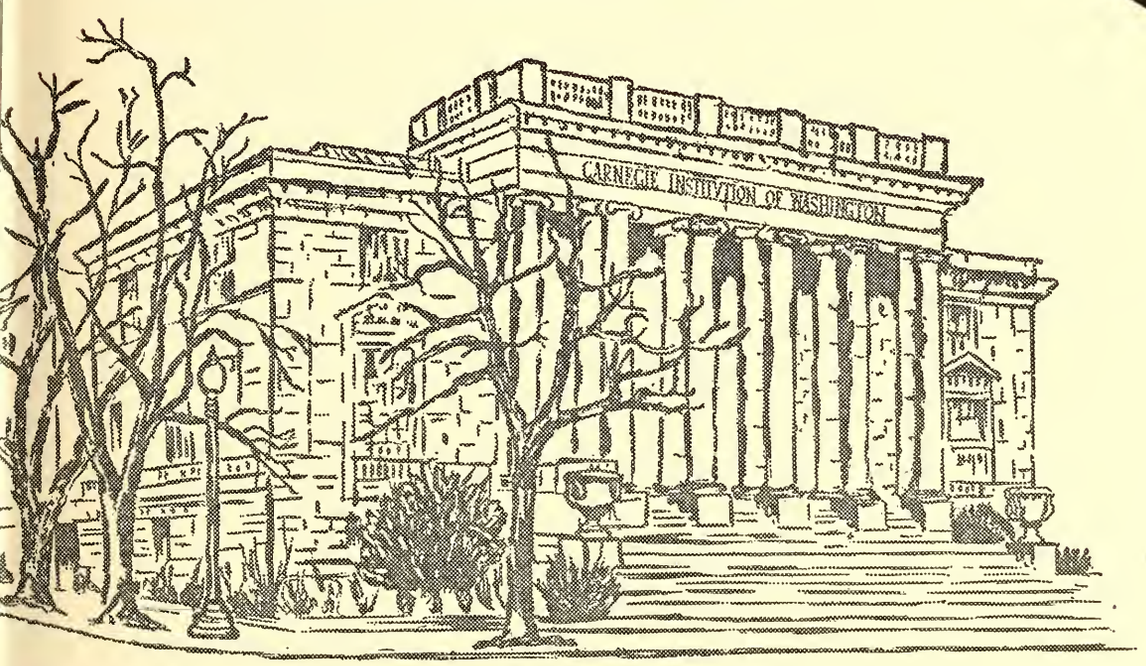
July 1, 1961 - June 30, 1962



Sixtieth Anniversary

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[1] Resigned June 30, 1962.
[2] Appointed September 1, 1961.
[3] Through September 15, 1961.
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The Report of the President

I look upon the Carnegie Institution as the most interesting effort the world has known for the development of a national interest in research.

Henry S. Pritchett
in a letter to Major Henry L. Higginson, May 1904

Without the degree of liberty which culture demands even a perfect society will be no better than a jungle. For this reason all authentic creation is a gift to the future.

Albert Camus
"l'Artiste et son temps"
Actuelles II, chroniques 1948–1953

The difference is infinitely small between a system of labour which leads men to discover the beauty of the world and one which hides it from them. But this infinitely small difference is real, and no effort of the imagination can bridge it.

Simone Weil
"Cette guerre est une guerre de religions"
Écrits de Londres et dernières lettres

THIS YEAR MARKS THE SIXTIETH ANNIVERSARY OF THE CARNEGIE Institution of Washington. Sixty years ago, in 1902, Andrew Carnegie transmitted to a newly elected Board of Trustees a deed of trust conveying the sum of ten million dollars "to found, in the city of Washington, an Institution which with the cooperation of institutions now or hereafter established, there or elsewhere, shall in the broadest and most liberal manner encourage investigation, research, and discovery. . . ." At the end of January in that year, the Trustees elected Daniel Coit Gilman, fresh from the career for which he was already noted as president of the Johns Hopkins University, as first president of the Carnegie Institution, and resolved "to promote original research by systematically sustaining projects of broad scope that may lead to the discovery and utilization of new forces for the benefit of man . . . projects of minor scope that may fill in gaps of knowledge of particular things or restricted fields of research . . . administration of a definite or stated research under a single direction by competent individuals."

It was not the first of Andrew Carnegie's great philanthropic gifts. Far from it indeed. In the last decade of the closing century in Pittsburgh he had established the Carnegie Institute with its natural history museum, its music hall, and its department of fine arts, and had made possible the Carnegie Institute of Technology, grown now to front rank among the

scientific and technical universities of the nation. In the opening years of the new century he had established the Carnegie Trust for the Universities of Scotland, and the Carnegie Dunfermline Trust in benefit of his native town. Nor was it, by many removes, to be the last. There were to follow the Carnegie Foundation for the Advancement of Teaching, the Carnegie Endowment for International Peace, Carnegie Hero Funds in no less than eleven countries, and finally, in culmination, the Carnegie Corporation of New York. And long before all of them—indeed well before the publication of his pioneering "Gospel of Wealth" in the *North American Review* in 1889—he had initiated that career of benefactions which was to be so profoundly influential in all the subsequent shaping of American philanthropic tradition with the gift of a library to his native Dunfermline.

But the establishment of the Carnegie Institution of Washington marked a new direction in the kinds of institutions made possible by Mr. Carnegie's gift. In fact, it established a new kind of institution for America—the first to be devoted wholly and completely, in intent and in philosophy, to the ideal of research scholarship over wide fronts of science in its broadest, most unfettered, most completely uncommitted aspect. This was a novel concept, and quite obviously, from some of the records of the time, one neither everywhere comprehensible nor even everywhere palatable in a youthful nation with a strongly established pragmatic tradition. It represented, indeed, a notably original idea, which six following decades have shown to be both great and enduring.

Four years after the establishment of the Institution, it had been granted a new Charter by special Act of Congress and had been organized into no less than fourteen departments, representing as many subjects. Over the next five years, definitive judgments were made as to where and how the Institution could work most effectively. One of them made during these years of experiment and trial was to prove crucial. It involved the decision to concentrate the resources of the Institution primarily on the research of its various departments; to make of it, in essence, an operating rather than a granting scientific organization. By 1911, its endowment more than doubled by subsequent additions by Mr. Carnegie, its departments firmly established but now reduced to ten, the Institution was molded to the purpose, and had taken on essentially the form of organization, that characterize it to this day. Through the following years new departments have arisen, departments have been consolidated, and some departments have been closed, as the needs and the research frontiers of each decade have dictated. Whole fields that were represented in the Institution in 1911, like economics and sociology, historical research, meridian astrometry, nutrition in the medical sense, no longer are included in its program as the resources of the nation in those areas have strengthened and enlarged.

Other fields not represented then but now on the frontiers of research, like modern embryology, molecular and cellular biology, the study of the mechanisms of photosynthesis, have been included in its purview in more recent years. Today there are five instead of ten departments in the Institution. Most originated in planning going back to the very beginning, though the work they conduct today, under the same general titles with which they began, has expanded far beyond the original concepts embodied in those rubrics, and may have wandered far afield from them as well. The Department of Terrestrial Magnetism was founded in 1904, the Geophysical Laboratory in 1906, and a Desert Laboratory, later to become the Division and then the Department of Plant Biology, appeared in 1903. A Solar Observatory for Mount Wilson was planned as early as 1902. Studies of the sun remain at the pioneering fringes of investigation in that part of the Institution to this day. But now the Solar Observatories have metamorphosed to the complex of giant telescopes included in the Mount Wilson and Palomar Observatories, operated jointly with the California Institute of Technology. To the intensive program of solar investigations of which George Ellery Hale dreamed and which he initiated with his striking discoveries of magnetic fields in the sun have been added a goodly share of the world's most important findings about the farthest reaches of the celestial universe.

But through all the years the major philosophies of the Institution and one major feature of its organizational pattern have stood constant, tested and retested in situation after situation and proved as fresh and relevant today as when they were conceived. The decision made at the outset that flexibility and effectiveness in the kind of research to which the Institution is dedicated can best be achieved through a series of rather small unit laboratories, each mobile and relatively independent, each able to seize the initiative in new and appropriate fields as they appear, yet all sufficiently connected so that they may be of mutual assistance as the needs arise, was a remarkable one, both for its uniqueness at the time and for the subtlety of the vision that dictated it. Over the decades, as research has burgeoned in the nation and groups devoted to research have multiplied, many other experiments in organizational form have been tried. But it is especially interesting that some of the most modern thinking and experimenting in organization for research, in this country as well as abroad, has returned to precisely this pattern as one of the most effective in exploring the dynamic frontiers of scientific knowledge.

Organization, however, is only a framework, vital but at last only supporting. Most significant—and most truly enduring—have been the elements of philosophy and purpose which inaugurated the Institution and which have remained unchanged through all the years: the philosophy that

all its resources, all its deepest purposes, are centered in the creative individual, whatever be his field, that in the truest sense he is the uncommitted investigator, suitably endowed and suitably protected, whose time, quite literally, is bought by the Institution and then returned as unconstrained endowment. And with this goes the philosophy, equally deep-seated and equally important, that this freedom from fixed commitment applies to fields of endeavor as well as to men: that high mobility within specific fields, that the unfettered crossing of fields, that the fashioning of unconventionally wide-ranging programs, are subject only to the limitations imposed by Nature and by the judgment of gifted and discriminating investigators, and that making this mobility and this flexibility possible is a principal objective of the Institution.

Over the years that philosophy, and the programs that have followed from it, have led to many pioneering practical discoveries within the Institution. The elucidation of the genetic principles underlying the development of hybrid corn, first accomplished by Shull in the Department of Genetics at Cold Spring Harbor working with East at Harvard, provided the fountainhead for an agricultural innovation which by 1952 was estimated to have brought an economic gain for the United States of almost forty billion dollars. For many of the predominantly agricultural countries of the world, moreover, the technique of hybrid corn has provided one of those basic resources which, as Galbraith has recently pointed out, is in the truest sense a fundamental contribution to their economic strength—an advance of really general application. At the same Department, during the second world war, studies of mutations occurring under X-ray bombardment in the famous mold *Penicillium* resulted in the development of a strain of that fungus which produced three to five times as much of the vitally needed penicillin as the highest-yielding strains then known.

In 1925, fully fifteen years before the intensive research on radar for combat in the second world war, Breit and Tuve at the Department of Terrestrial Magnetism, experimenting with a modified Navy transmitter, produced radio pulses and for the first time observed their echoes from the ionosphere. In the course of those experiments, moreover, they detected a curious interference of normal echoes by passing planes—prophecy of the field of radar. At the Geophysical Laboratory, Day and Shepherd early undertook studies in the field of low-expansion quartz glasses that proved basic to the evolution of Pyrex—a program which during the first world war supplied the United States with ninety-seven per cent of its requirements for optical glass. In 1935 a modified formula for annealing that same Pyrex glass proved fundamental to the manufacture of the mirror for the two-hundred-inch telescope on Palomar Mountain. Later, in the same laboratory, studies by Morey on lanthanum and borate glasses of high

refractive index led to a whole new family of glasses of great importance in the manufacture of photographic lenses—a development having important implications for the second world war. In the Geophysical Laboratory, again, Rankin and Wright as early as 1915 were able to solve the age-old riddle of cement, and their classic work has served ever since as a guide in the chemical aspects of the cement industry. From the same laboratory in later years have come new refractories for the steel industry, studies of natural geothermometers and geochronometers of fundamental concern to practical mining and oil prospecting as much as to fundamental geology, and, as recently as 1959, synthetic diamonds produced with new substrates and under new conditions of pressure.

Such practical innovation within the Institution has not been confined to the substantive aspects of its concerns. In both world wars the Institution played a major role in initiating forms of research organization for armed conflict. In the first war, the scientific and technical role of the Institution overshadowed its organizational one. But in World War II, through its President, the Institution served as a core of thinking and effort from which, in the following war years, the Office of Scientific Research and Development was to grow and to assume the lead in civilian scientific and technical military development in the nation. Through its activity and its influence, a preponderant share of all the major scientific and technical advances in the military art were achieved, from radar to modern submarine detection to proximity fuzes to nuclear weapons to new and improved prosthetic aids for the war wounded and the war blinded.

But as critical as the technical findings developed from its activities, and in the final analysis perhaps more enduring, was the dramatic and conclusive demonstration of the crucial role that science as a whole must play in our national life in the years to come, in formal peace as in formal war. Experiments in the organization of science were initiated in the O.S.R.D. which were ultimately to find fruition in such government instruments for the furtherance of scientific development throughout the nation as the Office of Naval Research and later the National Science Foundation, and in such bodies as the Atomic Energy Commission, whose present organizational patterns, first tested in the Manhattan Project of the Army Corps of Engineers, were likewise pioneered in the O.S.R.D. They were reflected, too, in such special resources of military thinking and planning as the Rand Corporation, founded shortly after the close of the war. Before those wartime demonstrations of the crucial role of science and technology in the very web of our national life had been made, the greater part of the scientific activity of the nation was prosecuted outside the sphere of government and of public funds. Today, probably sixty-five per cent of the total research of the nation is supported by federal funds, and the proportion is continuing

to grow. It is a dramatic demonstration of how deeply, in the public view, the scientific and technical development of the nation has, in fact, become the whole nation's concern. This situation has brought its own problems, of a wholly new order of scope and depth. They, too, must be important concerns in the future for the Carnegie Institution.

To have initiated such practical contributions to the public welfare on the scientific and technical fronts, to have participated actively and significantly in the initiation of major currents of scientific history whose sweep has now carried us to realms far beyond what was remotely imagined even twenty years ago, to have pioneered forms of organization that are today in the furnace of national trial and test, sum to considerable useful achievement, and might be thought, in and of themselves, to justify the vision upon which the Institution was founded and through which it lives today. Yet, in one sense, they represent mere by-products, mere projecting iceberg tips, as it were, of that vision, indicators only of the submerged seven-eighths. That seven-eighths lies in the kingdom of the mind. It lies in that devotion to deeper patterns, the symmetries, the lights and shades of Nature, wherever the search may lead, to which the Institution was originally dedicated, and which, undeviatingly, it pursues today.

That seven-eighths too has been productive of striking innovations in its own realm, and these, possibly in a truer sense than the practical "firsts," stand as proper signatures of the Institution. They range over many fields. While the thinking which underlay the famous Michelson-Morley experiment on "ether drift" was yet fresh, Professor Michelson, holder of the first Nobel prize in the natural sciences to be awarded in America, within the Institution repeated the experiment with an accuracy hitherto unattained, giving strong support to the theory of relativity, itself still at the stage of question and of doubt. Within the Institution, too, Michelson repeated with greater refinement that classic work that he had first undertaken as Ensign A. A. Michelson of the United States Navy, determining the velocity of light with a new precision, first across a path between the peaks of Mount Wilson and Mount San Antonio, then in one defined by a mile-long line of evacuated pipe at the Irvine Ranch in southern California. At the Mount Wilson and Palomar Observatories Hale's pioneering discovery that sunspots mark strong magnetic fields has been followed in more recent years by studies of solar magnetism of unprecedented refinement, and by the discovery, among the stars, of the most intense magnetic fields ever observed in any astronomical body. Hubble's studies of the phenomenon of the redshift in stellar spectra led to the theory of the expanding universe, culminating dramatically a year ago in the measurement of the redshift of

by far the most distant celestial object yet recorded. At the Observatories, too, Baade's studies of the structure and stellar composition of galaxies, with those of others, have suggested concepts of stellar evolution, of growth and decay, undreamed of as little as a quarter century ago.

At the Department of Terrestrial Magnetism a series of conferences on theoretical physics, held shortly before the second world war in cooperation with the George Washington University, among other things stimulated the suggestion that the source of energy in the sun and the stars is a nuclear reaction involving carbon—a notion leading within the next year to a classical model of the hydrogen-helium reaction now familiar as one of the accepted sources of stellar energy, ancillary to the hydrogen-deuterium-helium reaction recognized in recent years as more important. In the Geophysical Laboratory studies of the biochemistry of ancient sediments have given new dimensions to our concept of the age of terrestrial life, while studies of the artificial synthesis of amino acids from inorganic components under a variety of physical and chemical conditions, besides shedding new light on the probable modes of the origin of life on earth and the nature of its chemical environments, have also carried important theoretical implications for our notions about the existence of life on other planets.

At the Department of Plant Biology, work on photosynthesis has produced suggestive insights about that critical step which, with all the research that has been brought to bear for the last half-century, still eludes our understanding—the initial process by which the energy of light is used in the fixation of carbon dioxide. It has brought suggestions, too, about that further mystery, still elusive, of what it is about the chloroplast that enables it alone, when intact, to bring this about, whereas extracted chlorophyll itself will not. And in that Department, too, investigations of many years' duration have illuminated the detailed bases of plant evolution—of the roles of mutation and selection, of the development of ecological races and of speciation—and have revealed the often enormously complex and exquisitely coordinated detail of the evolutionary patterns they compose, at the levels both of form and of physiological function.

In three laboratories of the Institution—the Department of Terrestrial Magnetism, the Department of Embryology, and the Department of Genetics at Cold Spring Harbor—investigations of cellular metabolism and development, of cellular differentiation, and of the mechanisms of heredity at the molecular level have brought striking new knowledge of the detailed ways in which the materials of heredity and of development interact at the level of the cell nucleus and of its cytoplasm, at the level of the germinal cell and of the body cell of the plant or animal, at the level of differentiation and development of the individual organism, and at the level of its heredity.

Such discoveries and results are but scattered samples taken from a rich

matrix of sixty years of Institution work. But they are fair examples of its most typical fruit—the truest product of the philosophy in which it was founded and through which it lives. It may well be said that all else is in one sense by-product.

In the seventh decade of the twentieth century, it is hard to recast the scientific and technical America in which the Carnegie Institution was founded in 1902. In the America of 1902, few if any corporations in the United States could boast over sixty thousand stockholders. The American Telephone and Telegraph Company, as example, admitted to less than eight thousand. A third of all the manufactured products of the country were produced by partnerships or by individual proprietors. Speech had been transmitted by wireless, but the Fleming valve was still to be produced, and the first audion was not to be developed for eight more years. The first aerial flight, the twelve-second achievement of Orville Wright at Kitty Hawk, was not to occur until the following year. The earliest motion picture to tell a connected story, *The Great Train Robbery*, was yet to be produced. A large proportion of such great technical industries of today as the movie and the aircraft industries had not been born, and even the technical principles underlying the television industry were not yet remotely conceived.

The independent industrial laboratory had been pioneered some years earlier by the Arthur D. Little Company, but the concept of such a laboratory within an industry had just been formulated and put into practice with the establishing of the General Electric research laboratory in 1901 and of that of the du Pont Company in the same year the Institution was founded. Of all the great complex of industrial laboratories that were to transform the nature of American industrial science and technology in the twentieth century, not one other had yet appeared.

For the scope of science in that day, it is worth noting that in genetics it was only two years before that the work of Gregor Mendel had been rediscovered and its significance truly appreciated by Hugo de Vries and Correns and von Tschermak-Seysenegg. The very notion that some genetic characteristics can be dealt with in crosses in numerical ratios was still unfamiliar, while ideas of genetic linkage and dominance, or the notion of the linear array of genes, was still almost a decade away. Indeed, there was no proper science of genetics at all, and the word *gene* itself had yet to be coined. In astronomy, it is probably fair to say that the entire known universe was thought to lie within our own Galaxy. By contrast, within the range of the two-hundred-inch Hale telescope today lie perhaps a thousand million such galaxies.

Only seven years before the Institution was established, Wilhelm Roentgen had given the first demonstration of the X rays that bear his name, and the first Nobel award in science had gone to him for that discovery only a year before the founding of the Institution. The electron had been discovered by J. J. Thomson but five years earlier, and radium and thorium had been isolated by the Curies only four years before. Max Planck had advanced the quantum theory in the year preceding the founding of the Institution. And the special theory of relativity was not to appear for three more years. The Institution was five years old when the first Nobel award in science to be made in the United States came to Albert Michelson.

In the world of technology, plastics, synthetic fibers, vitamins, antibiotics, all were unknown. And in practical medicine, it is striking that the national death rate from influenza and pneumonia was reckoned at one hundred and eighty-two per one hundred thousand of the population—a figure to be reduced to thirty-nine forty-eight years later. In the same period deaths from scarlet fever fell from more than eleven per thousand to a total of sixty-eight for the entire country. It is worth recalling that, when Lord Lister, scientific disciple of Pasteur to whom the whole concept of antisepsis and sterilization in medical practice may be said to have been due, died in 1912, the Institution was already completing its first decade. Such was the world scene of science and technology within which the Institution took its place.

In 1902 science and technology were already familiar concerns within the federal government. They were indeed concerns as old as the nation itself. It was Thomas Jefferson who as Secretary of State in 1790 submitted a "Report . . . on the Subject of Establishing the Uniformity of the Weights, Measures, and Coins of the United States," and who, upon recommendation of the American Philosophical Society, transmitted to the Congress a proposal for the establishment of a United States Coast Survey, which was set up within the Treasury Department seventeen years later. And it was John Quincy Adams, when he was Secretary of State, who personally prepared for the Congress a similar report upon weights and measures. It was Adams, too, who led the fight to accept the bequest from James Smithson, who had died in 1829, to found the organization that was to grow to the Smithsonian Institution of today. The establishment of the Department of Agriculture dated from Civil War days, contemporary with the passage of the Morrill Act. So also did the National Academy of Sciences, from whose recommendations, somewhat later, were to follow the Geological Survey and the Weather Bureau.

These early involvements of the federal government in science and technology, however, gave little hint of the massive and commanding role it would play on the national scene in little more than half a century. Even at the end of the fourth decade of the twentieth century the total federal research program is estimated to have cost annually only about one hundred million dollars—less than the annual budget for the National Science Foundation alone in 1962. Twenty years later, however, yearly federal expenditures for research and development had grown to over a billion dollars out of a total estimated national commitment of about three billion. By 1960 the national total had climbed to fourteen billion dollars or more, of which the federal government supplied some nine billion. Today it may have reached sixteen to eighteen billion. The budget of the National Science Foundation for scientific research and related activities as submitted to the Congress for 1963 will total one hundred and sixty-five million dollars, while the Department of Defense is expected to spend about seven billion dollars on research and development, the National Aeronautics and Space Administration about two and one-half billion, the Atomic Energy Commission approximately another one and one-half billion. The total government funds spent in research and development in 1963 are expected to reach almost twelve and one-half billion dollars, of which expenditures for research alone may attain to one and one-half billion dollars, as compared with approximately one billion for the present year.

It has been calculated that the total funds expended for research and development in the United States over the past decade have increased at approximately fifteen per cent per year, leading to a doubling of volume every five years. If the present rate of increase of our expenditures in the field were to continue, indeed, our projected monetary support of research and development in their current definition could formally exceed our total governmental budget before 1975, and could exceed our gross national product before the end of the century—a reflection, however hypothetical, that vividly illuminates the scientific and technical dynamism and the scientific and technical problems with which we live. How different is this scene from that upon which the Institution entered!

The implications of this astonishing vista are many. One is the degree to which, with almost explosive suddenness since World War II, science and technology have been universally recognized as of major national concern. Another, of course, reflects the depth and intensity of technological competition in the world and our own needs in national defense. A third mirrors both the rate of population growth and, most pointedly, the growth of wealth in the United States. And the climates in which these expenditures on both the private and the public fronts have taken place and the governmental patterns through which they are effected in the public sector—

patterns at present in perhaps their most active phases of evolution and of adjustment—make a compelling chapter in the history of development both of American scientific enterprise and awareness and of American political institutions, and reveal much about their nature.

All these factors—the vast increase in the volume of our scientific and technical resources, in human and in monetary terms and in terms of scientific and technical facilities, the pressing demands of overriding national objectives, economic and military, the consequent larger and larger participation of federal resources in the total funding of the national research and more especially of the national technical effort—have, not unnaturally, had profound impacts on our thinking about science generally. Bit by bit they may have led to some subtle changes, perhaps well-nigh unconscious ones, in our conception of the ways in which, typically, the frontiers of truly new scientific knowledge are pushed back. This evolution could carry implications grave enough to warrant serious thought.

In all the years of American scientific research, from the times of Josiah Willard Gibbs to those of the second world war, we were accustomed to think of the great advances in scientific thought, of the initiation of its great new directions, as being predominantly the product of individual genius, working in environments which, however modest, and in part perhaps because of that very modesty, were especially adapted for flexibility, for absence of constraint, for a maximum of freedom in concept and in execution. We thought of the outstanding scientific conquest as typically an achievement of extraordinary brilliance, originality, and insight in individual innovation, giving significant new dimensions to its time, and ideally climaxing a career of unfettered scholarship. We did not particularly conceive research in this sense as the composite product of large numbers of men working in numerous and highly organized groups.

Since the second world war, however, following the spectacular demonstrations of technical conquest wrought by great organizations, of which the Manhattan Project was but the forerunner, we have sometimes been inclined by analogy to conceive of pioneering research for basically new ideas in rather similar terms—inclined, perhaps, to more than half believe that in the contemporary world it too may require such teams. It is then only logical to reason that if, at this stage of the world's scientific development, pioneering scientific research critically depends upon the large-scale efforts of highly organized and massively implemented teams, its effectiveness may be roughly proportionate to the material resources bestowed upon it—and that cost and magnitude themselves may provide an important index of scientific significance. We have even been tempted at times to imagine that the speed and effectiveness with which new scientific frontiers are breached may be a simple function of numbers of men and rates of

expenditure, and to expect that the attainment of new scientific vision in an area of basic research may be accelerated in direct proportion to the size of teams and the amounts of money committed to the search.

This philosophy, so directly derived from the demonstrated course of practical achievement, appeals especially to that keen pragmatic instinct that has run like a golden thread through all the fabric of our development as a nation, and to the genius for organization which has so long been one of our most pronounced national characteristics. Nor is there lack of evidence that at first sight seems to confirm the idea. It is patent today that the physical equipment required on the frontiers of research in many of the sciences, especially those of the greatest conceptual maturity, is massive, complex, and expensive, and requires the collaboration of sizable teams in designing it, in manipulating it, and in gathering data with it if truly new information is to be obtained. The productiveness of research in many such fields since men and money have made possible the design of powerful new tools and massive teams have been assembled to operate them gives vivid testimony to how powerful, and indeed how indispensable, resources of this kind may be in some of the most highly developed fields of science.

Yet in a deeper sense this judgment may harbor a considerable, and sometimes a positively dangerous, misconception, especially when it is assumed that great teams and high costs are prerequisites for the setting of new *directions* in scientific thought. A part of that misconception doubtless stems from a failure to demark sufficiently two general approaches in research, which, though they are complementary and often intergrade, yet have certain characteristics and pose certain requirements that are quite distinct. In one the basic ends of the investigation are generally evident, if not wholly clear in detail, at or near its beginning. The preeminent challenge to the investigator is to chart the road toward his goal—mapping it, projecting it, building it, all that it may approach a citadel already at least dimly visible on the horizon. The other general kind of research may begin without specific ends or, indeed, without consciously conceived objectives of any kind. Its driving motive is likely to be pure curiosity, the winning from Nature of deeply new knowledge, of knowledge won wholly for its own sake. The talents and the training demanded by these two kinds of research, and the difficulty of the scientific challenges posed by each, are often much the same. At one end of a spectrum of research they intergrade, and any distinction attempted between them becomes formal and unreal. At their extremes, however, the challenges they present are undoubtedly quite different, often to be met in widely divergent ways. Above all, whereas research programs of the first kind can frequently be visualized in a general way ahead of time, and so planned intelligently, the same is rarely true in the second type of research. A very large share of the concerns of such a

great team effort as was involved in the program of the Manhattan Project, for instance, fell into the former category. The deeply underlying theoretical knowledge, the unexpected and radically new ideas about Nature, on which the whole program of the Project was based and on which it turned, had been achieved by investigators like Meitner and Hahn and Strassmann in Europe in 1938, by such individuals as Rutherford and his colleagues at Cambridge in 1914. They had been won through research of the second kind, conducted by a very few gifted scientists working in the settings we have traditionally visualized as consonant with the finest of individual creative effort.

It is no accident that today we sometimes make these distinctions less clearly than we might. At a very deep level it may be a consequence of our peculiar history and circumstances. Throughout our earlier years as a technically developing nation we were able to rely on the older countries of Europe for basic ideas on which to build our applications as implicitly, and often as unconsciously, as we relied upon the British navy for the protection of our seas. It was both natural and adaptive that the kind of scientific and technical contributions at which we early became most adept and developed most highly, and to which perhaps we initially attached greatest attention and attributed greatest value, should have involved the brilliantly organized, the meticulously careful development, often undertaken on the boldest and most breathtaking scale, of basic ideas that had been conceived abroad. Today such ideas are much more often drawn from our own resources. But historically our first attachment was to their execution rather than to their generation. And so it is not surprising that we sometimes fail to distinguish innovation from execution, and have not always recognized the limitations within which we can extrapolate experience from one kind of activity to the other.

But there is more to the matter than this. For it is demonstrably true that gains in our knowledge of Nature as new and fundamental and unexpected as any in the world can come, unbidden, from the investigations of great teams for research and development in many areas. As our resources for team research grow in the coming years, we can properly expect the rate at which such new knowledge is revealed to increase also—if not proportionately, at least very substantially. And so we should not fail to ask an implied question of great importance. The philosophy that envisaged the environment of brilliant, original, unfettered individual research as the *milieu* in which the great new directions of scientific thought were born and nourished, the philosophy which has had such confirmation in recent scientific history, was itself developed in the days of scarcity in science—

scarcity not only of material wealth, but especially scarcity of scientific workers. Now we live and work in a nation committed to an unparalleled rate of growth in the material resources for research, and in a world in which perhaps eighty per cent of all the scientists who have ever lived are our contemporaries. Is it possible that the philosophy itself was adjusted to the needs of other times; that it is not relevant to an era of plenty? May it actually be true today not only that major advances in new knowledge, the setting of radically new scientific directions, *can* be achieved in the environment of great and highly organized research teams, but also that, in practice, such environments are indeed *essential*, or, at any rate, the most favorable, to the process? Is it possible that we are witness to a profound revolution in the very character of research itself? Is it possible that the small and mobile groups to which we earlier looked for some of the most significant scientific innovations, the groups which in the past characteristically had an influence on scientific progress out of all proportion to their numbers or their social cost, can no longer in our day provide such significant approaches to the unknown?

Such a radical query, of course, bears profoundly on the whole philosophy of research. It is far more than a practical question. It touches some of the deepest wellsprings of scientific faith. It touches belief in the very nature and effectiveness of the individual search for truth in our time. In subtle ways it touches on the nature of scientific truth itself. It is an important question for the Carnegie Institution, deeply committed to the faith that the distinguished, unfettered individual can bring unique gifts to his society, and deeply committed, too, to belief in the uniqueness and the importance of the influence which a community of independent scholars can exercise on scientific progress.

For a question of such magnitude and gravity, abstract analysis will not suffice. Contemporary evidence alone can give convincing answers. Have the recent great advances in our knowledge of the universe and of our own more immediate environment, the original ideas of scientific stature achieved in the last few years which promise to open truly novel avenues of thought for the future—have these been necessarily, or even primarily, associated with the massive programs of great teams? Or do the basic contributions of small and mobile research groups continue in our day to have their old significance?

Such an abundance of evidence springs to mind, provided by striking advances no more than a half-dozen years old, in so many regions of scientific inquiry, that its very selection poses a problem and must necessarily be arbitrary. But three outstanding areas of recent investigation are particu-

larly interesting to consider from this standpoint, because their environments and circumstances span such an extraordinary range of magnitude and character and form.

The first example may comprehend that immense complex of research and development dedicated to the placing of man in outer space and ultimately on the moon or on neighboring planets, its present great achievements in our country vividly symbolized by the voyages of Shepard and of Grissom, of Carpenter, Glenn, and Schirra. The second is of quite a different kind. It involves an achievement in astronomy of the year just past which in the staggering distances with which it deals emphasizes anew what a thin terrestrial shell is the outer space so far entered by man. It is the identification of what has proved to be by far the most remote celestial object ever discovered in the heavens—an object certainly billions of light years distant from us—and the measurement of the redshift of its spectrum. The third selected area of advance may in some ways be the most profound of all, though it is far from the best known. It includes the experimental evidence so brilliantly obtained in the last few years, and the reasoning directing the search for it, indicating beyond reasonable doubt that the information governing the inheritance of all the qualities of living things is structurally graven on the chromosomes within their germ cells in the form of a genuine code. It includes, as a climax, the demonstration of the general nature of that code, which the year just past has witnessed. These findings may well mark the greatest single advance in genetics since the demonstration five decades ago that the genes of heredity lie in the chromosomes in a linear array.

These three advances in natural knowledge bear much resemblance in certain fundamental qualities. All have won important and striking new knowledge. In all of them, the research for that knowledge has included a variety of scientific disciplines apparently far removed from the main concern—in the case of the third as far removed as crystallography seems to be from conventional genetics. Profoundly new directions of thought have resulted from all three. Possibly the third has produced the most thoroughly revolutionary new insights. The first has brought a sense of liberating conquest and a wealth of first-hand information about regions known hitherto only palely and at second hand.

But in many features of the modes and environments of research characterizing them, the three examples diverge about as much as scientific activities can differ. The contrast is particularly vivid when cast in terms of the parameters under special consideration: the relative size of the efforts, the sheer volume of human and material sources brought to bear, the kinds and degrees of organization. The enormous magnitude of the space program and the tremendous cooperative efforts currently involved in its prosecution and planned for the future need little emphasis. In this respect, indeed,

Project Apollo is much in the tradition of a Manhattan Project, though yet bolder in both variety and scale. It is estimated that by the close of the budget for 1963 the National Aeronautics and Space Administration will have spent more than four thousand millions of dollars for the conduct of research and development. For research facilities alone it will have expended more than eight hundred and twenty millions. Behind the great individuals who have manned the space vehicles, and have recorded and analyzed the data of research, and who will do so in the future, lie the years of development on a scale of unprecedented magnitude and the immense organizations required for its successful prosecution. Behind the fashioning of the tools the final explorers command lie combinations of highly specialized disciplines and intricate techniques of the most varied kind—chemical, electronic, mechanical—ranging from the arts of propulsion engineering to those of miniaturization. It is interesting to notice in this connection that the cast of the effort at present is, as it perforce must be, importantly oriented about the design and use of *tools*. In considerable measure it is basically an engineering effort—perhaps the most exciting and compelling engineering effort of this century.

Shortly after the second world war, when instruments of radio detection were being put to a new use in the service of astronomy, several surveys of the skies were undertaken to detect and locate the positions of celestial bodies that were emitters of radio waves. The equipment then available, however, was relatively poor in both resolution and accuracy. It could not effectively complement the far more precise tools of optical astronomy. Resolution and precision were often too low to permit a reliable identification of radio sources with corresponding objects observed optically, though sometimes they were suspected to be the same. As the techniques of radio astronomy sharpened, however, as larger dishes were built and manned and put into use, both penetration and resolving power improved greatly. At the radio observatory of the Cavendish Laboratory in England and at the observatory of the California Institute of Technology at Bishop in the Owens Valley, instruments of outstanding capacity were built. During 1959 and 1960 two fresh surveys of the skies were undertaken with them: in Cambridge at 169 and 189 centimeters, in California at about a sixth that wavelength (31.2 cm). In the course of these surveys the celestial positions of certain emitters of radio waves were determined with a new precision. So precise was the location of one of these objects, indeed, that the two-hundred-inch Hale telescope could be brought to bear upon it. The peculiar color characteristics of the object suggested that it might include a pair of galaxies in collision, and so might be expected to have one or more emission

lines in its spectrum. And so it happened that a prescient astronomer of the Mount Wilson and Palomar Observatories was able to obtain two spectra of the visible light from this source and to measure the degree of redshift in them. At the same time another observer, obtaining multicolor photometric observations of two of the fainter galaxies of the same cluster and constructing their curves of continuous emission, confirmed this measurement of redshift. It corresponded to a recession velocity of nearly half the speed of light. This heavenly body defines a new boundary for the universe comprehended within human ken. It marks by far the most searching probe into unplumbed reaches of space that the mind and hand of man have yet accomplished, ranging certainly to the order of several billion light years. When it is recalled that a single light year amounts to almost six million million miles—about sixty-three thousand times the distance of our own world from the sun—it makes the orbits of earth satellites, spectacular as they are, yet appear as comparatively near-neighborhood adventures.

Perhaps the greatest ultimate significance of this achievement will lie in the contribution it can make to our ideas about the basic nature of the universe. Indeed, this newly determined point of distance, so far beyond any other yet obtained, has already offered suggestive evidence on the great question of whether our universe is a continuously expanding one, or a universe in which the continuous creation and destruction of matter stand in equilibrium, or whether the universe in fact may experience alternate expansion and contraction extending over astronomic periods of time.

In sharp contrast to the first example, the planning of these observations, their confirmation, and the deductions from them were not the work of great teams of highly coordinated technical workers. These were the fruits of observations and calculations made by a few individuals laboring in relative solitude, the fruits of work of a relative handful of gifted astronomers. Perhaps never in science has the work of individuals been more clearly identifiable. The contrast with the first example is sharp.

Yet behind this classical achievement of gifted individuals lay many decades of research and engineering focused on the design of the powerful modern tools of optical and radio astronomy. Without them the achievement itself would have been quite impossible. These tools, like those involved in the space effort, were the products of hands and minds and toil in literally hundreds of specialized skills. And it was not skill and art that alone were brought to bear, but with them the magnificent resources of intellect and materials and time and research that gave them scope and effectiveness. The achievement itself dramatically underlines how significant and how essential the gifted and untrammeled individual investigator is today on some of the most advanced frontiers of the physical sciences. It was primarily focused on the gathering and the interpretation of information about

nature, not on the design of tools. Yet its success depended in turn on a panoply of instruments brought to perfection in other times and other places, the development of which had required a structure of science and technology of whose cumulative magnitude and scope no scientist of an earlier generation could have had the faintest dream.

The third example embodies yet a different pattern. It would be hard to imagine a more fundamental or more sweeping discovery than one elucidating, at a deeper level than had hitherto been imagined, the manner in which the information governing all the qualities of inheritance may be recorded and stored in the chromosomes of plants and animals and men—stored with such extraordinary effectiveness and such enduring stability that there are organisms living today whose hereditary characteristics have been maintained more durably than the very rocks within whose strata the fossils of their remote ancestors are preserved. Yet in terms of magnitude the human and the material resources committed to that search, by comparison with the preceding illustrations, have been positively minuscule.

In 1953 Linus Pauling and Robert Brainard Corey at the California Institute of Technology suggested that the molecular structure of the unit of heredity, the "molecule" of deoxyribonucleic acid, might consist of chains of polynucleotides intertwined in the form of a helix, with four characteristic bases, the purines adenine and guanine and the pyrimidines thymine and cytosine, attached to them and projecting outward, while phosphate groups were oriented to the center. There were features of this model which conflicted with experimental evidence, notably that it was hard to reconcile the fact that DNA is an acid with the existence of bases lying, as it were, on the outside of the molecule. But the model involved one very great idea which, though it was not widely credible in terms of that particular construction, yet was to prove fundamental to all further thinking on the matter. It was the idea that the biological specificity of the unit of DNA, on which its power of determining inheritance must rest, must inhere in the sequence of occurrence of these bases along the molecular chain and the suggestion that the periodic distances at which these bases occur might be of the right order to permit them to order the sequence of amino acids in the construction of a protein. This was a most important foundation upon which to rear what would prove a truly extraordinary arch of reasoning. But for long even the idea that the nucleic acid structure could be locally specific was resisted. Until that idea had been widely accepted, its more detailed consequence could hardly gain effective credence. Both these developments were made possible by a second great idea, which might be likened to a keystone of the arch.

This critical idea was provided by J. D. Watson when, in a flash of insight

reminiscent of Kekule's vision of the structure of the benzene molecule that came to him in a London bus almost a hundred years ago, he imagined the consequences of, in effect, turning the model inside out, pointing the bases inward, and pairing the purine molecules with the smaller pyrimidines. Highly significant correspondences with nature were achieved by this remarkable insight. The first and fundamental rule of the composition of deoxyribonucleic acid, namely that it incorporates purines and pyrimidines in equal ratio, was given a rational basis. And the contradiction between the acidic nature of DNA and its presumed outwardly pointing bases, which had plagued the model of Pauling and Corey, was resolved. But there were impressive difficulties to be met also. The idea that the bases were outward-pointing had not resulted simply from neglecting the alternative that they might point inward. That possibility, indeed, had been carefully examined in formulating the earlier model. But it had been concluded that such a structure was not possible. For the new model to be convincing, the physical possibility of such an arrangement had to be demonstrated, and the details of the linkages between the purines and pyrimidines had to be worked out—formidable tasks requiring concepts and techniques familiar to those dealing with the structure of crystals.

And so it was that, also in 1953, Watson and F. H. C. Crick, working in the Molecular Biology Unit of the British Medical Research Council adjacent to the Cavendish Laboratory at Cambridge, announced their brilliant hypothesis of the structure of the unit of heredity, of the "molecule" of deoxyribonucleic acid, as a pair of "ribbons" wound in the form of a double helix around a common axis and linked by the four bases, the purines adenine and guanine and the pyrimidines thymine and cytosine, paired in a highly specific fashion. The model of Pauling and Corey had suggested that the bases could not be packed in the center of the molecule. The new model proved that indeed they could, and from that demonstration came perhaps the most significant idea in the whole chain—the concept of base pairing itself, and with it the associated and important notion that a maximum of four kinds of base pairs could be involved. The beauty and credibility of the model gave firmness and emphasis to the earlier idea that the biological specificity of the unit of heredity must derive in large measure from the ordering of the pairs of bases along the chain of the deoxyribonucleic acid.

All together, three biological consequences stemmed directly from the model, which must rank among the most important advances of our age in the understanding of the fundamental nature of earthly life. First, the model allowed the extraordinary phenomenon of the replication of the genetic pattern which occurs at every division of every living cell—the mechanism fundamental to the very process of the growth and multiplication of life on earth—to be understood consistently for the first time. Second, the nature

of the phenomenon of the sudden changes in inheritance which we call mutation, intensively studied since the days of de Vries but never understood in their fundamental molecular mechanisms, now for the first time became comprehensible at that level, in terms of known changes in bases which could result in alterations of their sequence to produce such changes. Third, and greatest of all, perhaps, was the full rationalization of the key concept that biological specificity in inheritance must in large part derive from the sequential ordering of the bases in the nucleic acids.

This third great consequence was to lead to a scientific vision of new and unexpected dimensions. That vista was provided by the idea that genetic information might in fact be *coded* in the DNA molecule in the form of a linear message for which the four permissible combinations of bases might serve as alphabet, in a manner, indeed, reminiscent of the coding of a message on the punched tape of a computer. This radical concept was first examined in detail by the astrophysicist Gamow in 1954. Although the precise form of the code suggested at that time has since proved incorrect, the basic idea has become established as one of the great theoretical advances in our view of the nature of the living world. And so was posed the pointed question: if such a code exists, what is its specific nature?

It is that question which theoretical and experimental work of the past two years has done much to answer. An important share of the answer, like the original question, has come once again from the laboratory of the Unit for Molecular Biology at Cambridge; other critical parts have followed from several American university laboratories, from the National Institutes of Health, from the Carnegie Institution of Washington. Suffice it to say that preponderant evidence suggests that the code employs words containing very few "letters," probably not more than three.

A virus may include within its single chromosome something of the order of a hundred thousand base pairs. A billion pairs of bases may be included within the total store of information of our own chromosomes. It is a startling concept that if the DNA strands from all the cells in a single human body were uncoiled their total length might well span the solar system. There is ample opportunity for diversity in the ways that the elements of the code can be combined.

With this conceptual advance, carrying the implication that one of the basic challenges offered by the problem of heredity might lie, in effect, in the decoding of a script, progress in meeting that challenge has come with remarkable speed. What may well prove to be a Rosetta stone has been provided by the development of methods of accomplishing protein synthesis in cell-free systems under the influence of artificial ribonucleic acids composed of only two bases in known ratios and therefore containing specified code words in known frequencies. The composition of the resulting protein

should yield the key to code "letters" in terms of the ratios of specific amino acids corresponding to them. Another highly promising approach involves techniques for investigating the coupling between the base-pair patterns of the deoxyribonucleic acid of an organism and the "messenger RNA" of related forms, which may differ in their coding only in relatively minor, but specific and determinable, particulars. The current year sees work of this kind at a peak of activity. With wing-swift speed, a whole new area in our understanding of the basic mechanisms of heredity at the molecular level is being exploited.

Here, then, are three genuinely great advances marking the technical and scientific progress of the last three years. In a profound sense all three are typical of their age, and, for a variety of reasons, could not have occurred at any earlier time. Obviously neither space exploration nor the astronomical investigations of the new "edge of the universe" now within our ken could have been achieved with the tools of any other era. The peculiar modernity of the third example involves especially a yet different circumstance. For the very idea that the information of inheritance may be recorded as a code is peculiarly consonant with our age—perhaps so characteristic that it should be treated with a caution doubled by this very fact. In the nascence of primitive biological thought fire was a living thing, dangerous and bright, and the expression "vital fires within us" remains to remind us how much we once thought of life as the "inhabiting property" of something that was obviously dynamically alive. In an age when the frontiers of engineering exploration concerned pumps and hydraulics the mechanism of the circulation of the blood was a fascinating and fertile subject of physiological speculation and of physiological research. For the age of Descartes, strings and pulleys provided compelling images for the mechanisms of life, and images of clockwork for the mind. In the early nineteenth century, dominated by the vision of steam power engineering, energy transformations seemed among the most important aspects of life, and the rise of large-scale electrical power engineering in the latter part of the nineteenth and the early twentieth century reinforced the vision. Then, in our own era, with its emphasis on small-current engineering and the modulated control of gigantic mechanical and electrical processes, the aspects of living processes included under the rubric of Cybernetics have occupied a center of the stage. Studies of those fascinating properties of living systems involving, in all their varied and exquisitely elaborate mechanisms, the maintenance of homeostasis, the preservation of balance in dynamic systems, have held a special attraction for our time. And in our immediate day, when communication of new orders of content and of speed, and with it the massive processing of information,

so dominates our lives, when we are inevitably so much concerned with the coding of information and the unraveling of such codes, it is scarcely surprising that a natural process operating upon those principles, which has evidently been central to the evolution of all life, as no doubt it was also in its origin, should only now have so powerfully focused our attention as to be on the threshold of solution. It follows, too, that, just as each of the earlier interpretations of living processes subsequently gave central place to its successor but left the residue of its own unalterable truth to contribute permanently to our basic understanding, we must be prepared to accept—and indeed to welcome—the same fate for the concept of genetic coding.

The likenesses uniting these three examples, then, lie deep. It would be hard to select the most significant among them, though in the achievement of particular new insights the second and especially the third may predominate. What now of the parameters of scale, of magnitude of the resources committed, of the extent of organization of the work, as criteria of its significance? Here it would be difficult to imagine wider contrasts.

At every point in the extraordinary conceptual development that marks the third example, the commitment to it in terms of numbers of workers, in terms of material resources, was extraordinarily modest. The Unit for Molecular Biology of the Medical Research Council at Cambridge began with two crystallographers. Ten years later, when its revolutionary discoveries were well launched, it numbered perhaps a dozen workers and was housed in a temporary building behind the Cavendish Laboratory and in various University rooms—a very minimum of space. It was, indeed, superbly instrumented for its task. But such instrumentation was incredibly modest in both mass and cost compared with that required in either of the other fields. In that free and flexible atmosphere, built about the largely unfettered efforts of a few gifted individuals working within a minimum of formal organization, have been made some of the most important advances in man's concept of his world and of himself possible to the twentieth century. It is striking to compare this situation with that in which the exploration of space must go forward.

This, then, is the character of the contemporary evidence. Such contrasts of size and structure and organization in the modes of some of the most significant assaults on the frontiers of natural knowledge in this decade strongly suggest that these parameters, broadly considered, bear little direct relation to their scientific significance. They inspire compelling reflections about the continuing effectiveness, in our own day, of the scale and

the pattern and the philosophy of research to which the Carnegie Institution is so deeply committed. It seems abundantly clear that the essential qualities and requirements of inquiry at the very frontiers of man's knowledge of his universe do not now, and in all probability will not in the foreseeable future, differ significantly from those of our classical scientific past. Such inquiry will surely continue to bear the unmistakable stamp of the gifted and untrammeled individual, whatever may be the scale of resources, in knowledge, in tools, in human and material support, which he may require.

Bronowski has pointed out that perhaps the most fundamental discovery of the scientific age was that Nature was to be approached and won, not by attempting to outwit her by magic, as many a medieval alchemist had imagined reflecting a prevailing climate of his time, but rather by discovering the true quality of natural laws and taking care to work within them. It is easy to forget how tremendous was that change of view, how much of trial and vision was comprehended within Newton's simple admonition that "science must be kept free from occult influences." The atmosphere of true research is still as it was when that great advance of philosophy was made, still the atmosphere in which, as Lionel Trilling has recalled, Faraday refused to be called *physicist*, holding the term too narrowly imprisoning a chamber for his life's commitment. These are the dimensions, whatever be the nature of the structures in which they are embedded, which still evoke the great advances of today.

In the central context of discovery, it seems clear that the magnitude and organization of a research effort may be the least meaningful of parameters in any fundamental or enduring sense. One may indeed think of the large and the small research enterprises in our society as essentially symbiotic, each fulfilling its specific role—one more example of the rich diversity by which we live.

The relation, however, is actually more subtle. The responsibility that devolves upon small and mobile groups dedicated to the exploration of new frontiers is clearly greater in our own day than merely that of one component in a many-hued panoply of research. At least one aspect of the relation is far more serious, and wears a significance which must inevitably sharpen further in the coming years. It is not only important that the small and mobile research group be maintained and strengthened to ensure continuing advance along those remote boundaries of natural knowledge so vital to our spiritual as well as to our material well-being. It is not only important because, in such a massive and highly advanced technical and engineering society as our own is today and must even more become tomorrow, the scientific "leverage" of such pioneering groups must inevitably increase. It

is a further and a significant truth that, while climates that foster innovation can be maintained in the midst of complex and highly organized technical undertakings, preserving them intact is no common or easy achievement. It requires a particular determination, an extraordinary persistence of vision and pertinacity of will, an unusual sensitivity and skill, to sustain conditions favorable to original, exploratory research on remote and far-flung frontiers of the mind in massive working environments over considerable periods of time, undeflected by all the immediate demands that architecting to known ends in those environments inevitably imposes, in some multiple proportion of intensity to scale. Without the sustaining view that small and mobile groups attaining great discoveries can offer, without their inspiration, the task must become doubly difficult. These circumstances may define for the small and mobile group the most demanding and important of all its functions—the heavy responsibility of the keeper of a vision—the vision of the creating individual.

In the future that responsibility may well become not only wider but yet more challenging. For it is abundantly evident that science and technology, in the world as a whole as well as in our own nation, have entered phases of development in our day so different in scale and complexity from their beginnings—or from what, incidentally, the newly developing nations of the world may confront or may require in their own immediate futures—as to differ essentially in kind. As Pierre Teilhard de Chardin has written with sensitive perception, "The Earth is covering itself not merely by myriads of thinking units, but by a single continuum of thought, and finally forming a functionally single Unit of Thought of planetary dimensions." An important aspect of the qualitative growth of contemporary science, of course, inheres in its essentially additive nature, in the formidable integration of knowledge and of thought characteristic of a pursuit where discoveries in one field may in the span of a few months alter the entire basis against which thinking in very different areas must be projected. Another concerns almost the opposite situation. The significance of great research is largely measured by the impact of its results over a wide range of frontiers of inquiry, demanding the widest and swiftest communication possible and challenging human intellectual capacities for assimilation and generalization to their limits. But the *processes* of research bring heavy demands on quite opposite qualities—on extraordinarily detailed knowledge of a single field, on that supreme mastery of all its coordinates down to the most minute, developed over long periods of years, which so often is prerequisite to significant and sustained advance. In the past, science has been able to reconcile these two quite opposite requirements in tolerable fashion. With increase of scale the problem takes on new dimensions.

Science in the last decades has responded to the challenge with enormously increased sophistication, with vastly expanded organization and integration

of knowledge, with, indeed, quite a new development of recent years, the field of research on research itself. But as science has matured in its modes of cultivating the whole vast field of its thought, as its power has grown to enter and occupy new areas of research in force so soon as the first hint of them appears, these very qualities have brought novel and troubling consequences for the gifted individual, particularly for the gifted young research student just entering upon his life's work, upon whom so much of the future depends. As A. B. Pippard, among others, has pointed out dramatically, the legions of investigators can now be mobilized with such speed and effectiveness at a new and attractive breach in the frontier of knowledge that, particularly if the area offers a promise of practical benefit, a green and fertile intellectual valley can be reduced to aridity for the innovator within less than the working life of a generation of young scientists. The consequences incident to such swift and locustlike invasions, however effective and profitable they may be for a technical society in the large, can be discouraging to vulnerable individuals, and they bear at precisely the points of talent and dedication most precious to us. There can be no more urgent imperative than the creation of opportunity for individuals faced with this dilemma to address themselves once again to wholly new fields of inquiry. This too lies peculiarly in the domain of small and mobile and basically highly uncommitted research groups.

What, in final essence, is the deepest meaning of the scientific way? In the profoundest sense, what is the meaning of the individual human life dedicated to it? Within the scientific context, as well as outside it, what, at last, are people *for*? A generation, perhaps even a decade, ago such a question was all but unasked by most Americans. Certainly it was all but unasked in 1902. Even if put, in that day, it would have appeared to many not only irrelevant but quite possibly sinister. But in a world with a population estimated at nearly three billion and predicted by conservative demographers to reach almost four billion by-1980 and to attain nearly seven billion by the turn of the century, the question wears quite a different aspect. In our own nation, with a population now over one hundred and seventy million and destined perhaps to reach two hundred and twenty million by 1975, the revolutionary consequences of this flood tide upon every facet of the world we know demand no emphasis. It must profoundly affect every circumstance of our society, of its organization and its function. It must affect the individual's inner view of himself and his conception of his relation to his universe, his understanding and his reach in his own physical world, and much else besides.

The rate of growth of the scientific effort today considerably exceeds that of the population as a whole. Inevitably, it would seem, it must change after

two or three more periods of doubling. But in absolute terms it would seem beyond reasonable doubt that the legions of technically trained people in the future will vastly exceed in numbers those now active, even as these in turn so vastly exceed the numbers of only a few decades ago. Great technical and engineering efforts will be ready and available to confer rich meaning on the lives of many. In massive and compelling developmental undertakings opportunities will continue to be provided to great numbers of active minds to labor for ends not only dramatic, not only economically and socially adaptive, but as creative and as meaningful in our times as the tasks of the builders of Chartres or of the Parthenon must have been in theirs. Pippard has presciently pointed out that, if the field of technology is to prove sufficiently magnetic to attract first-class intellects to it, opportunities for the dramatic and the spectacular, outlets for the moral impulse to share in socially significant undertakings, the sheer intellectual quality of the undertakings themselves, must provide the motivations. Among the great and challenging technical and engineering undertakings of our time, all three motivations are presented on a scale the world may never have experienced before.

But there will be other scientific workers, too, of other and less specially identifiable tastes and talents, hostages to a more distant future. For them the requirements will be quite different. Perhaps the deepest question the times can pose for them, and as well the most poignant for all man's spiritual welfare, will be this. In a society as densely packed, as intricately organized, as highly urbanized, as our own must inevitably become in future years, can small and mobile enclaves of thoughtful and imaginative men and women continue to maintain integrity and distinctive freedom within the greater society? On their ability to do so in the broadest context will depend in no small measure the fate of the individual and of those goals and motivations through which in the past we have lived and taken our national being. In a very real sense their persistence alone can effectively preserve the priceless jewel of the opportunity for quietness and temporary solitude which in our past has been so vital a nursery for individual American greatness as well as for that of our society as a whole. For it is the gifted, unorthodox individual in the laboratory or the study or the walk by the river at twilight who has always brought to us, and must continue to bring to us, all the basic resources by which we live. His position must be guarded and honored and implemented with every resource that we can muster, now and in the future, for he is irreplaceable. This matter too, and all the circumstances attendant upon it, must be a central and abiding concern through all the coming years for the Carnegie Institution of Washington. As Chaucer said six hundred years ago, so may we today: "Out of the old fields cometh the new corn."

The Year in Review

It is fascinating to compare the Institution of approximately sixty years ago with that of today. There was, of course, very little to report from the first year or two of the Institution's existence, which was spent in a search for profitable lines of endeavor and experiments with organization toward that end. As early as 1904, however, the lines the Institution was to follow for some years were discernible, and the report for the year 1905 (*Year Book 4*) describes the nature of the Institution's work in nearly all of the broad fields in which it was active during 1961–1962. Some glimpses of these early activities, set alongside typical activities in our several fields for 1961–1962, give a most illuminating view of the progress of the Institution, and indeed of science in the United States.

In 1905 the resources and objectives of the Institution were much more widely dispersed than they are today. The total budget for that year was $586,000, a little more than half of which was allotted to ten "Departments of Investigations" which included the forerunners of all the Institution's present fields except embryology. Among the Departments were several that have since been terminated (Marine Biology, Economics and Sociology, History, Nutrition, and Horticulture). Half of the total budget for the Departments ($302,700) went to the Solar Observatory on Mount Wilson, which was under construction in that year. In addition, 43 individuals or organizations outside the Institution received grants to the sum of $130,625 in the fields of anthropology, archaeology, astronomy, bibliography, botany, chemistry, geology, history, paleontology, philology, phonetics and linguistics, physics, and zoology. The Institution also had in 1905 a program of subsidizing outside publications of "meritorious works which would not otherwise be readily printed." Nearly $30,000 was expended in 1905 for this purpose and for the publication of works written within the Institution itself.

By contrast the Institution's budget for 1961–1962 was $2,848,480, all of which was spent upon the six operating Departments that have been maintained in recent years. Except for departmental fellowships the Institution made no outside grants and did not subsidize publication for works written outside the Institution. While a great variety of subjects was

under investigation within the Institution in 1961–1962, research was undertaken in a better organized and more purposeful manner.

Four of the more promising lines of research, as viewed by the President and Trustees of the Institution in 1905, lay in the work of its Solar Observatory, in its Department of Terrestrial Magnetism, in geophysical research, and in biological investigations. With rather remarkable perception the importance of fundamental research in the physical and biological sciences is commented upon in the 1905 report. The Solar Observatory is described as ranking among Institution projects "first in order of cost for initial construction and equipment. This cost, however, is no more than commensurate with the magnitude of the problem attacked. . . ." Of the biological investigations, including those of the Station for Experimental Evolution and the Desert Botanical Laboratory, which was the predecessor of the Department of Plant Biology, the report noted that fundamental research in plant and animal biology "for a series of years can hardly fail to yield results of signal practical and theoretical value."

The Department of Genetics

1905

In our series of "then and now" snapshots it is appropriate to begin with the Department of Genetics, whose predecessor in 1905 was the Station for Experimental Evolution, one of the most active parts of the Institution in that year.

Even though the Station for Experimental Evolution at Cold Spring Harbor had been in existence for only a little more than a year, a year of very full activity was reported. Following the inspiration of Hugo de Vries, who had given the dedication lecture at the Station the year before, C. B. Davenport described the long-range objectives of the Station's work. "The factors of evolution are three—variation, inheritance, and adjustment. Studies may be made on any one of these factors or on all three together; as a matter of fact, they can hardly be studied wholly independently. . . . Since studies in inheritance have been relatively neglected. . . our first efforts have been directed primarily toward such studies."[1] Already five principal investigators and the Director, Dr. Davenport, had commenced their programs of research.

From a modern point of view the range of the work undertaken was astonishing. It was described as "investigations into inheritance and variability" of plants, insects, and other invertebrates; "investigations upon

[1] *Year Book 4*, p. 87.

aquatic vertebrates"; "studies on inheritance in domesticated animals"; and "investigations into the cytological basis of heredity." Experiments were in progress on eight beetle species, three species of moth, flies, aphids, crickets, bees, and snails (*Helix nemoralis*). The brown trout and several killifishes (*Fundulus* sp.) were studied, and the Station experimented with goats, sheep, and cats. During the year George H. Shull became well started on the research which led to his later valuable knowledge of maize reproduction. But in 1905 he was searching for suitable material for experiment, and had a garden of 81 different species of biennials, perennials, and annuals. Along with this search he conducted a variety of experiments, which included investigation of the inheritance of seed weights in beans (repeating W. Johannsen's experiments) and the vegetative habits of Russian sunflowers (*Helianthus annuus*) and other species. He had also begun his observation of the characteristics of maize. The particular character chosen for study in 1905 was the number of rows on the maize ear.

Although the importance of cytological research was recognized, the year's effort failed to devise even a suitable experiment. The report observed, "The results of the last three years confirm the belief in the importance of the chromatic material in inheritance. This chromatic material exhibits a bewildering complexity and diversity scarcely less than that of adult organisms."[2]

1961–1962

It is interesting to find in 1961–1962 two lines of investigation which were at a germinal stage in 1905. Experiments with maize are still productive of fundamental results, and cytological research using flies (now the familiar *Drosophila*) formed an important part of the departmental program. Thus in one way or another these lines have held some of the departmental attention for more than 56 years.

The approach of the Department in 1961–1962, however, was a vastly different enterprise. In a sense Barbara McClintock's methods of working with maize genes are lineal descendants of the variation and inheritance techniques that Shull was commencing to pioneer by counting rows of kernels on ears. But in Dr. McClintock's hands these methods have become highly sensitive and one of the sharpest tools in modern genetics. She has made them a match for other sharp new tools heavily dependent on chemistry and physics. For more than a dozen years she has been interested in the elements associated with genes that activate, control, suppress, or regulate genic action. Her work during these years has revealed the presence in maize of two controlling systems, an Activator (*Ac*) system, whose presence or absence is associated with the appearance or nonappearance of mutations of a particular gene, and the Suppressor-mutator system (*Spm*),

[2] *Year Book 4*, p. 94.

which causes a varied expression of the action of a single gene as observed in somatic cells. In her research this observation has been associated especially with the appearance of the reddish-blue pigment anthocyanin. Depending on its phase, the Suppressor-mutator element may either inhibit or activate the gene expression which results in the formation of anthocyanin in maize leaves or kernels.

A second theme of Dr. McClintock's work through these years has been a search for evidence that even the fine structure of inheritance is basically similar for all forms of life. In a much more general way Davenport and others started with the same hypothesis at the Station for Experimental Evolution, attempting to observe genetic expression in many forms of life. Dr. McClintock's first experimental evidence on the similarity of operation of genic control elements in different forms of life was reported in 1950.[3] In that year she observed, "Because the same types of mutability as those observed in maize have been described for a wide variety of organisms, it is probable that the same events, involving the same chromosome materials, may occur in all organisms.[4]

During the year 1961–1962 Dr. McClintock continued to examine the parallels between the gene-control systems in maize and bacteria. She observes in her report that both organisms have gene-control systems composed of an "operator" element directly controlling genic activity adjacent to the structural gene and a "regulator" element acting upon the operator element. Other investigators have shown that the position of the regulator element on the bacterial chromosome may differ for individual systems.[5] It may be near to or removed from the locus of the operator element. Dr. McClintock's work during the year confirmed her hypothesis that there is a high probability that genic control systems in maize and bacteria act in similar fashion. She concludes her report by stating that her findings "are sufficiently extensive to leave no doubt that a two-element system of control of gene action, composed of an operator element at the locus of the gene and a regulator element located elsewhere, may arise at a gene locus that initially carried the regulator of the system." It would appear that one more link has thus been added to the gradually extending chain of evidence on basic similarities for many forms of life at the cellular level.

A second field of departmental interest in 1905 survived to 1961–1962. This was the application of cytology to genetics, which was considered, but only futilely explored, in 1905. Indeed, successful development of this field

[3] *Proceedings of the National Academy of Sciences, 36,* 344–355, 1950.

[4] *Year Book 49,* p. 165, 1950.

[5] F. Jacob and J. Monod, On the regulation of gene activity, *Cold Spring Harbor Symposia on Quantitative Biology, 26,* 193–209, 394–395, 1961, presented completely for the first time evidence on the operator and regulator elements in bacteria.

actually was postponed for more than 15 years after 1905, when in the 1920's the work of John Belling finally laid the foundations for modern cytogenetics. This work was continued in 1961–1962 in the research of Berwind P. Kaufmann, Helen Gay, Margaret McDonald, and their associates. The general objectives of the group bore some resemblance to the crudely stated convictions about the importance of cytology in the 1905 report. The group continued its work of nearly two decades, charting the changes occurring in the organization of chromosomes and cytoplasmic organelles as cells in higher organisms grow and differentiate. Their methods, however, were a world apart from those of 1905, including as they did electron microscopy, fluorescent microscopy, enzyme chemistry, and biochemically specific stains. In addition, they had at their disposal the vast knowledge that has accumulated over 40 years on the genetic characteristics of *Drosophila* flies, which continued to be one of the objects of their observations. A second material for study has been the plant *Tradescantia* (spiderwort family), which offers a very favorable opportunity for cytoplasmic study during microsporogenesis.[6] Of particular interest has been the effort of this group to approach the problems of charting the submicroscopic organization of chromosomes by means of "enzymatic dissection."

All these techniques were employed during the year, adding to the results obtained in other years. Experiments were conducted on the mutagenic properties of deoxyribonuclease when introduced into *Drosophila*. An enzyme analogue, 5-bromodeoxyuridine, was added to the list of mutagenic agents employed on both *Drosophila* and *Tradescantia*. Perhaps the most interesting results from this group's program during the year were two discoveries: (1) The finding that direct chromosomal breakage occurs in *Tradescantia* root tips in the presence of 5-bromodeoxyuridine. This enzyme analogue acts by modifying the base sequences in nucleic acid rather than the phosphate-sugar helices attacked by deoxyribonuclease. (2) The observation that Golgi bodies, one of the types of cytoplasmic organelle, exhibit different forms in the progression of microsporogenesis in *Tradescantia*.

A third activity important to the 1961–1962 Department was not even dreamed of in 1905. It is represented in the work of Alfred D. Hershey and his associates, who are gradually charting the molecular structure of the viral chromosome. Dr. Hershey's work illustrates, more than anything else in the Department, the observation made by M. Demerec as early as 1942 that "From the purely biological science of early days, genetics has developed into a science where cooperation with physics, chemistry, and mathematics is essential."[7] Hershey and his associates observe in their

[6] Microspore = pollen.
[7] *Year Book 41*, p. 171.

report of this year that methods have been devised in recent years to characterize and differentiate among different types of deoxyribonucleic acid (DNA) molecules. Among these methods are optical analysis of thermal denaturation, chromatographic analysis, measurement of fragility and buoyant density, and specific enzymatic tests. But these tests do not give information about molecular structure, which remains a more or less "plausible inference." Hershey's objective is to remove genetics' dependence on inference for its concepts of molecular structure of genetic material. To this end, he and his associates are experimenting with the DNA of several types of bacteriophage.[8] He considers these DNA's to be favorable material for experiment because: (1) they can be isolated in a molecularly homogeneous state, permitting correlation between structure and biological function; (2) their synthesis can be studied in infected cells that have been proved suitable for metabolic study in the past; and (3) present intensive study of the genetics of a few bacteriophage species gives valuable reference points for physical and chemical findings. He considers his current work at least in part "exploratory."

Several interesting results ensued from Dr. Hershey's exploration of physical techniques in measuring molecular weight during the year. In one he established the molecular weight of the DNA of a bacteriophage known as T5 by first establishing an ingenious pair of "scales" by analyzing DNA fragments of another phage (T2). One scale is established by determining sedimentation constants[9] of fragments of labeled T2 DNA as separated by column chromatography.[10] The other was obtained from fragility tests that measured the rate of breakage of T2 DNA fragments of a given sedimentation coefficient when stirred in a mixer at a given speed. The sedimentation coefficient[9] and the fragility index of T5 DNA were then determined. By comparison with the T2 "scales" a molecular weight of 84 million was determined. The T5 DNA matched very closely fragments of T2 DNA in one sedimentation coefficient range (48.5–49.5).

By similar techniques Dr. Hershey also brought to light during the year some interesting molecular characteristics of the DNA of phage lambda, which was found to have astonishingly different molecular properties from other well known DNA's. Of particular interest was a broad range of denaturation temperatures, like that of bacterial DNA's and contrasting with an exceedingly narrow range typical of other phage DNA's. On one hand these and other properties suggest a marked tendency of the molecules to interact with each other, and on the other, a remarkable differentiation in structure along their lengths. These exceptional properties may be

[8] Bacteriophage—any of a number of intracellular virus parasites of bacteria.

[9] Measure of the rate of precipitation of particles in suspension in a solution when centrifuged.

[10] Chromatography—a method of separating and analyzing chemical substances by inducing differential migration and adsorption from solution in a porous, insoluble, sorptive medium.

related to each other and to some of the well known biological peculiarities of phage lambda.

By infecting bacteria with isotopically labeled phage particles and by labeling DNA synthesized in the bacteria after infection, Dr. Hershey and Dr. F. R. Frankel have determined that cells subjected to such infection always contain a considerable fraction of their total DNA in a form indistinguishable from that found in finished phage particles. They note that this points to a mechanism for the preservation and determination of molecular length that operates continuously during DNA replication, not only at some terminal stage in the formation of the phage particle. This conclusion is considered significant evidence bearing upon several hypotheses about genetic mechanisms.

The Department of Plant Biology

1905

The Department of Plant Biology also has developed from an operation under way in 1905. The Desert Botanical Laboratory was active that year, located at Tucson, Arizona. The program in 1905 was not as varied as that of the Station for Experimental Evolution. Twelve investigators were associated with the Laboratory in that year, most of them as recipients of grants. As might be expected, their investigations were heavily weighted toward the characteristics of arid-region plants, especially transpiration[11] and water-conducting mechanisms. A substantial amount of attention was paid to the character of plant environment, as in D. T. MacDougal's observations of soil temperature and B. E. Livingston's study of the relations of desert plants to soil moisture and evaporation. More typical, however, was F. E. Lloyd's study of correlation between stomatal[12] action and transpiration in certain types of desert plants. (No positive correlation was observed.) But along with these was displayed at least a secondary interest in what later became biochemistry and biophysics. For example, A. L. Dean conducted an "Investigation of the proteolytic enzymes of plants" and W. T. Swingle received a grant for an "Investigation of electromagnetic and electrostatic effects on lines of force found in living plant cells." No conclusive results were reported from the latter study, but Dean reported finding an ereptic enzyme[13] in all tissues of a species of bean (*Phaseolus vulgaris*).

[11] Transpiration—the escape of water vapor from living plants.

[12] Stomata—minute pores in the epidermis of plants, through which gases and water enter or escape from the plant.

[13] A type of enzyme that breaks down proteoses and peptones, as in the intestinal tract of animals.

Most interesting about the program of the Desert Botanical Laboratory in 1905 was the complete absence of any attention to the problems of photosynthesis, which have since become a major preoccupation of the Department of Plant Biology. Although the basic physical-chemical relations of photosynthesis[14] had been suggested sixty years before, there was no hint of the importance of these problems in the 1905 program. Th. W. Engelmann in 1887 discovered that light absorbed by pigments other than chlorophyll also produced photosynthesis, more than fifteen years before the establishment of the Laboratory. Even during the year of the 1905 report, the English plant physiologist, F. F. Blackman, demonstrated that photosynthesis includes at least one "dark" reaction not initiated by light.

The interest of the Institution in photosynthesis actually began six years later, in 1911, when H. A. Spoehr came to the Department of Botanical Research at Tucson, which succeeded the Desert Botanical Laboratory. Spoehr first came to the Institution to study the "chemical physiology" of plants but very soon became immersed in the problems of photosynthesis, an interest he maintained actively until his retirement in 1950. Just as intensively as in Spoehr's time the Department of Plant Biology today applies its research efforts to the great problem of unraveling the complexities of photosynthesis.

1961–1962

The work of the Department in 1961–1962 on photosynthesis still centers on a problem the general outlines of which emerged in Engelmann's time: the exact function of the two sets of pigments, chlorophyll and the accessory pigments, both of which induce photosynthesis. It is now supposed that photosynthesis comprises at least two photochemical events, one driven by chlorophyll *a*, the other by the accessory pigments. Two discoveries made about 1955 provided some evidence for this hypothesis. One discovery was Blinks' chromatic transient effect, a momentary change in photosynthetic rate observed when light absorbed by chlorophyll is changed to a color absorbed by accessory pigments. The other was Emerson's enhancement effect. In this effect photosynthesis resulting from wavelengths absorbed by chlorophyll *a* alone, when augmented by wavelengths absorbed through accessory pigments, is increased more than would be predicted from the simple sum of the effects from both radiations presented separately.

A major effort is now being made in the world of research to define the

[14] Joseph Priestley demonstrated the production of "good air" (oxygen) by plants in 1772; Jan Ingenhousz in 1778 showed that the effect noted by Priestley resulted from the influence of sunlight; Jean Senebier noted in 1782 that "bad air" (carbon dioxide) was a necessary input; Lavoisier determined the composition of carbon dioxide in 1784; Nicolas de Saussure showed precisely in 1804 that water, light, and carbon dioxide were inputs, and oxygen plus organic matter outputs; Julius Mayer, through his concepts of the conservation of energy, in 1845 suggested the place of sunlight and vegetative organisms in chemical action taking place on a global basis at the earth's surface.

nature of these two essential photochemical reactions and relate them to the chain of events in photosynthesis that results in oxygen evolution and carbon dioxide reduction. As throughout the long history of research in photosynthesis, ingenious theories currently exist to explain in detail most of the known effects. Generally considered, each investigator has his own favored concept of the process, and the different hypotheses are not entirely compatible with one another. Further experiments and more comprehensive concepts are still needed for an adequate understanding of photosynthesis.

At the Department of Plant Biology, C. Stacy French and his associates continued their efforts to provide experimental evidence on the exact functions of the different plant pigments.

A year ago they found in a red alga (*Porphyridium cruentum*) that chlorophyll *a* but not the accessory pigment, phycoerythrin, produces a chemically unidentified substance that rapidly consumes oxygen. Some of it is left over after a light exposure, as is demonstrated by the temporarily accelerated rate of oxygen uptake after an exposure to light absorbed by chlorophyll *a*. This material is also believed to be an intermediate in the process of photosynthesis.

This year the persistence of the chemically unidentified material previously formed by illumination of chlorophyll *a* was measured by French and Jeanette Brown. This was done by observing the increased oxygen production of the algae upon exposure to individual flashes of light at the wavelength absorbed by phycoerythrin. The presence of the material enhances the oxygen evolution by a light flash that activates phycoerythrin. The half-life of the material measured in this way was found to be about 18 seconds under certain conditions. By contrast, preillumination by phycoerythrin-absorbed light did not enhance oxygen production when chlorophyll *a* was subsequently activated.

Another series of experiments, made this year, shows even more complex relations between the effects of different pigments of green leaves. The story began about eighty years ago, when Engelmann found traces of oxygen evolution from isolated chloroplasts. This effect was further investigated by Molish early in this century, but since then the reaction has had very little attention until recently, no doubt owing to R. Hill's discovery in 1937 that the addition of oxidants such as ferricyanide greatly increases the amount of oxygen produced. An avalanche of papers on the Hill reaction followed, and experiments with the evolution of oxygen from within chloroplasts without added substances have been all but abandoned.

In the past year, however, Y. de Kouchkovsky of the Centre National de la Recherche Scientifique, Gif-sur-Yvette, France, and David C. Fork of the Department of Plant Biology, have reexamined this effect with greatly improved methods. The work, started independently at the two laboratories,

was continued as a collaborative effort during Dr. Fork's visit to Gif-sur-Yvette in March 1962.

By measuring oxygen exchange of Swiss chard chloroplasts Fork showed that it is possible to distinguish four separate effects of light, each with its characteristic action spectrum. They are:

1. The evolution of oxygen from chloroplasts without added oxidants is driven most effectively by light having a wavelength of 650 millimicrons (red).[15] This corresponds to the absorption peak of chlorophyll *b* in chloroplasts, thereby showing that chlorophyll *b* is more effective than chlorophyll *a* in this reaction. A shoulder on the curve of the action spectrum, however, shows that at least one of the three forms of chlorophyll *a* is also active. This oxygen production within the chloroplast goes rapidly for only a few seconds, then its rate drops to a very low value. Storage in the dark revives the ability to evolve oxygen. Apparently light consumes some material found in chloroplasts which is restored in darkness.

2. Dr. Fork found the recovery process to be strongly accelerated by exposure to far-red light. A wavelength of about 730 millimicrons was most effective for this purpose. This wavelength suggests identity with phytochrome, a substance which, though present in very small amounts, controls many plant responses. In addition to the 730-millimicron peak, however, the action spectrum for the regeneration of the chloroplasts' ability to evolve oxygen also has a peak in the blue wavelengths which does not activate phytochrome.

3. Ferricyanide [$K_3Fe(CN)_6$], when added to chloroplasts, substitutes for the natural oxidant substance responsible for photoproduction of oxygen. The rate of oxygen evolution remains for long light exposures, and the action spectrum, which peaks at 678 millimicrons, shows that chlorophyll *a* is more effective than chlorophyll *b* when ferricyanide is present.

4. A very specific inhibitor for oxygen production by chloroplasts is the herbicide DCMU.[16] When this poison is added to chloroplasts the photoconsumption of oxygen can be measured without interference by oxygen evolution and shows a maximum efficiency at wavelength 690 millimicrons (red).

Four different action spectra have thus been measured for oxygen exchange in isolated chloroplasts. French raises the question of the exact function of each pigment in these various photoprocesses. He says that the answer is clear for chlorophylls *a* and *b* (678- and 650-millimicron peaks): they are concerned with oxygen evolution. But it is not yet known why chlorophyll *b* is more effective than chlorophyll *a* for the reaction within the

[15] One millimicron = 10^{-6} millimeter.

[16] 3-(3,4-Dichlorophenyl)-1,1-dimethylurea; manufactured by E. I. du Pont de Nemours and Company.

natural chloroplast whereas the reverse is true when ferricyanide is added.

The two action spectra with peaks at 730 and 690 millimicrons are more obscure. They do not necessarily indicate that there are active pigments with absorption maxima at either wavelength. Instead, spectra may result from the activation of two pigments whose reactions either reinforce or counteract each other. In both cases the action spectra maxima may differ greatly from the absorption maxima of the reacting pigments. These are interesting subjects for further investigation.

Ellen C. Weaver started an attack on the problems of photosynthesis with an intriguing and promising new technique, that of electron paramagnetic[17] resonance (EPR) spectroscopy. She notes in her report the well established fact that illuminated chlorophyll-containing material has a higher level of unpaired electrons than material in the dark, suggesting that some phase of photosynthesis proceeds by single-electron transfers. Even though several research groups outside the Institution had employed this new technique (about six years old) in studying photosynthesis, no rigorous demonstration had yet been made that electron resonance[18] had a direct connection with photosynthesis.

Dr. Weaver set out during the year first to determine whether or not the established resonance was associated with chlorophyll. She observed two distinctly different light-induced resonances. One is the *R* (rapid-decaying) signal, seen only when cells are illuminated. The other may persist for hours in the absence of light, and it is designated the *S* (slow-decaying) signal. Using a yellow mutant (no chlorophyll) of the fresh-water alga *Chlamydomonas reinhardi*, Dr. Weaver obtained no *R* signals in EPR observation, suggesting that the *R* signal is ascribable to chlorophyll. She also discovered by using dilute cell suspensions that 680-millimicron light (near the absorption peak for chlorophyll *a*) was the most effective for producing the *R* signals. Another interesting result is her discovery that the amplitude of the *R* signal has a strictly linear (proportional) relation to light intensity for the wavelengths least absorbed by chlorophyll, whereas wavelengths most strongly absorbed by chlorophyll have no linear relation to light intensity (assuming low light levels in both cases). Dr. Weaver's tentative conclusion from these observations is that the *R* signal is associated with chlorophyll and arises from the "primary" act of photosynthesis.

Dr. Weaver also discovered that any inhibition of oxygen evolution, as by DCMU or by limiting the manganese-ion concentration in the growing medium, will produce an enhanced *R* signal. This suggests that if the

[17] Paramagnetic—atoms having spin systems with magnetic moment (or materials containing those atoms) are paramagnetic.

[18] Electron resonance—a property of unpaired electrons, whereby precession of the spinning electron may be inferred when it is subjected to an electromagnetic field at a specific frequency, as in EPR spectroscopy.

pathway of the electrons is in any way obstructed the net level of unpaired spins rises. The result indicates further that the alteration of photosynthetic processes other than oxygen evolution may provide a fruitful field for experiment using the EPR spectroscopic technique. Interestingly, the *S* (slow-decaying) signal is not altered by blocking the oxygen evolution pathway with DCMU, but manganese starvation reduces that signal to an extremely low level. It is thought that this result may be correlated with a lack of plastoquinone,[19] previously determined elsewhere to be a necessary and apparently universal factor in the oxygen evolution of green plants.

Dr. Weaver has thus presented evidence that chlorophyll is the source of one type of free electrons in an intact photosynthetic organism and that plastoquinone is the site of another type. She has also demonstrated the correlation of the two types of signals with the evolution of photosynthetic oxygen. The method and her results are of more than usual interest, because photosynthesis is essentially a photoreduction process when viewed in a highly general way, that is, the transfer of electrons from one substance to another.

Although photosynthesis still presents an awesome complexity to those investigating it, studies like those of French, Fork, Brown, and Weaver examining the effects of light on metabolic reactions are continually changing concepts of how synthesis takes place and, step by step, are building a more complete understanding of this vastly important phenomenon.

Another field in plant biology, experimental taxonomy, can trace its origin to the activities of the 1905 Desert Laboratory. Again, however, the diffuse approach of 1905 is gone. William M. Hiesey and his associates note in the 1961–1962 report that current developments in precise techniques have greatly extended the horizon of this field. Instead of the compartmentalizing of botanical study, which was commencing in 1905, they see "a truly integrated plant science whereby contributions from the various specialized fields, including taxonomy, ecology, cytology, genetics, physiology, developmental morphology, and biochemistry, can be incorporated in a panoramic view of plant relationships and evolution." Their goal is an integrated understanding of the chain of mechanisms that determine plant evolution, including the genetic and the biochemical. For a number of years plants of the genus *Mimulus*[20] had been used for comparative growth studies of altitudinal effects at the Stanford, Mather, and Timberline stations. More recently the races of one species, *Mimulus cardinalis*, have been subjected to controlled growth chamber experiments.

During the year Harold W. Milner made some particularly interesting

[19] Plastoquinone—quinone found in chloroplasts; the structure of this compound is given in figure 31 of the report of the Department of Plant Biology.

[20] The garden "monkey flower" belongs to the *Mimulus* genus.

studies of the photosynthetic rates of six races of the species originating in diverse climates and altitudes. Among the variations observed were a 60 per cent difference among the races in the light intensity required to saturate photosynthesis at high temperature, and a 100 per cent difference at a very low temperature (0°C). Significant variance in photosynthetic rate at extreme temperatures also was observed, as well as disparate abilities to maintain a high rate of photosynthesis over a long period. From these and other results one may conclude that climatic races within the same species may show differential patterns of response undoubtedly linked with variations in internal physiology.

During the year an important step was taken toward establishing tissue cultures from *Mimulus* plants, so as to make quantitative measurements of growth and photosynthetic rates in tissue cultures similar to those for whole plants. By examining the physiological requirements of tissue from various plant organs, it should be possible to localize the site of physiological differences within the plant.

In addition, the group extended its work during the year to species of *Solidago* (goldenrod), particularly in the collaborative work of Malcolm Nobs of the Department working at the Institute of Plant Systematics and Genetics at Uppsala, Sweden. The same type of difference in response to light intensity was observed between two races of *Solidago virgaurea:* one a shade-loving race from Sweden and the other an alpine race from Norway. The alpine race has a much higher requirement for light saturation than the shade race, and its chloroplasts remain normal at light intensities that cause the disintegration of those from the shade race.

The Department of Terrestrial Magnetism

1905

The Department of Terrestrial Magnetism was also among the active Departments of the Institution in the year 1905. The work of the Department in 1905 faithfully followed its name, although a wide range of projects was reported, with activity on an almost worldwide basis. A major preoccupation of the Department during that year was an effort to start a systematic series of magnetic observations on most parts of the globe, which at that time were informational blanks. L. A. Bauer, Director of the Department in that year, stated, "our progress with regard to the great and principal facts of the earth's magnetism will be at a standstill unless a magnetic survey of the whole globe be undertaken immediately." Toward that end a wooden sailing vessel, the brig *Galilee*, had been manned and outfitted, and had undertaken trial runs. This was the beginning of a program that

continued for almost 25 years thereafter, in which the sailing vessels *Galilee* and *Carnegie* logged more than 400,000 miles to undertake magnetic and other scientific observations in every ocean area of the globe. It ended only with the accidental destruction by fire of the *Carnegie* at Samoa in November 1929.

A very extensive land survey program also was being initiated for magnetic observations in 1905. Many of the islands of the West Indies were covered in that year, and arrangements were being completed for observations on the South Pacific Islands and in Canada, Mexico, Central America, South America, and China. Cooperative arrangements for observations and research were maintained with several German scientific institutions and with the St. Petersburg Academy of Sciences in Russia.

Besides its primary program on the study of and basic data collection for terrestrial magnetism, the Department in 1905 organized and participated in the program of observing the solar eclipse of that year, and it began cooperating with the Institution's Solar Observatory in the study of several solar phenomena.

The Institution in 1905 also expressed a substantial interest in physics research, but entirely through a program of grants to fourteen American physicists. Among the grants were several for studies of emission spectra and a study of the theory of light.

1961–1962

Although the emphasis so prominent in the 1905 program of the Department of Terrestrial Magnetism was continued until the early 1930's with relatively slight changes, the program of 1961–1962 in the Department was a much different one. The principal activities reminiscent of the earlier days of the Department came in the research of Scott E. Forbush, but again in an environment strikingly different from that of the first twenty-five years of the Department. Forbush's principal investigations during the report year were devoted to the intensity of the charged particles in the Van Allen trapped-radiation belt adjacent to the earth, as recorded during the transits of the satellite Explorer VII through the belt between 1959 and 1960. He also had under way studies examining the southward shift of the auroral-zone current system during magnetic storms in its probable association with particles coming from the outer Van Allen belt.

The bulk of the Department's varied and imaginative research in 1961–1962, however, derived from applying the techniques of physics to a wide variety of geophysical and biological problems. They ranged from the examination of the interior of living cells to charting the hydrogen clouds of our Galaxy.

Perhaps the most significant results to emerge from the year's work were from a quarter that could hardly have been envisioned as associated with

the Department even twenty years ago. They came from the work of the Biophysics Section (E. T. Bolton, R. J. Britten, D. B. Cowie, B. J. McCarthy, J. E. Midgley, and R. B. Roberts) on the fine structure of, and biochemical processes taking place within, bacterial and other cells.

As the end of the report year approached, the Section was engrossed in some striking experiments involving "messenger" ribonucleic acid (RNA). This type of RNA contains nucleotide[21] sequences complementary to those in the appropriate DNA which provides the genetic information. In following a lead provided by E. K. F. Bautz and B. D. Hall at the University of Illinois it was discovered that single-stranded DNA could be immobilized in agar and complementary RNA could be caused to hybridize with it through the formation of hydrogen bonds. By washing, the immobilized hybrid DNA-RNA combination was freed of other contaminating RNA. The hybridized RNA could then be reclaimed, in a state of high purity, by dissociation of the hydrogen bonds, and could be chemically analyzed.

With this simple and effective new procedure it has been possible to demonstrate that the DNA-like RNA comprises about 1 per cent of the total RNA of bacterial cells and that it has a half-life during active synthesis of approximately 2 minutes. On the assumption that this RNA is in fact the active template for protein synthesis, the measurements of its quantity and half-life show that a single molecule acts catalytically for the synthesis of many polypeptide[22] chains.

Further work has revealed that the method can be used to exploit the specificity inherent in the hybridization process, which depends upon long regions of complementary nucleotide sequences in molecules of RNA and DNA. Thus, RNA from bacteriophage T2 will hybridize well with DNA of the genetically closely related phage T4 but not with the apparently unrelated T7 DNA. Several species of bacteria have also been tested, and cross reactions have been found to occur to a greater or lesser degree in accord with accepted taxonomic relationships. Thus, the method has made feasible a quantitative chemical analysis of the amount of genetic information held in common among species.

Since the method is a general one, applying to the DNA of all species and tissues, it can be used in studies of the transcription of genetic information and of differentiation, two of the key subjects of modern biology.

During the year the Biophysics Section also contributed a new hypothesis about the code associated with the role of nucleic acid in specifying the order of amino acids in protein. The prevailing hypothesis interprets experimental findings in terms of a "three-letter" or triplet code. The experi-

[21] Precursor of or decomposition product from nucleic acid, composed of a nitrogenous base, a ribose sugar, and phosphoric acid.

[22] Peptides are proteins linked by amide (RCO–NHR′), or "peptide," bond.

ments of the Section lead its members to believe that a two-letter or doublet code eliminates the major failing of the triplet code, which implies an unrealistically high uridylic acid[23] content for the "template" material of protein synthesis. The doublet code apparently provides a good correlation between the amino acid composition of the bacterially synthesized protein and the nucleotide composition of the RNA templates on which it is formed.

Other fields in which the techniques of physics are being applied by Staff Members of the Department are seismological exploration of the earth's crust, radioactive dating of rocks, radio astronomy, and the development of image tubes for use in astronomical studies.

It is of particular interest that all these programs in one respect or another are cooperative, carrying on the tradition of joint investigations or joint enterprise which was started and even widely used in the earliest days of the Department. As Merle Tuve, the Director of the Department, observes in the introduction of his 1961–1962 report, " 'cooperation' . . . has many very different aspects in the current work of the Department, but in each case it represents a situation where there is special usefulness in our freedom of initiative and recognition of the infectious characteristic of personal enthusiasm." To some extent, the same thing might have been said for the programs in biophysics and geomagnetic studies.

A good example of the Department's cooperative approach is shown in its radio astronomy program. With the support of the National Science Foundation a new Carnegie Radio Astronomy Station will soon be established in Argentina. Parts for a major instrument, a parabolic antenna nearly 100 feet (30 meters) in diameter, are now being manufactured in this country and will be shipped to Buenos Aires for assembly there during 1962–1963. The Argentinian National Council for Scientific and Technical Investigations and the Research Council of the State of Buenos Aires have created a new National Institute of Radio Astronomy to participate in the construction and operation of the station. Later operation will be a cooperative venture among the Carnegie Institution, the University of Buenos Aires, and the University of La Plata. Invitations will be extended to astronomers in other institutions in South America to participate in the research program. Some fellowships are being offered by the Institution to bring students and professional research men interested in radio astronomy to this country for training in the use of parabolic antennas and for acquiring educational background in radio astronomy.

The observational program in radio astronomy using the Department's

[23] Uridylic acid—a nucleotide; technically uracil (2,6-dioxypyrimidine) + D-ribose sugar + phosphoric acid.

instruments also continued during the year. Observations of the hydrogen gas content at the center of our Galaxy confirmed previous observations at Leiden, the Netherlands, and Sydney, Australia, that the motions of hydrogen close to the Galactic center are complex, and that the hydrogen gas not only is rotating about the center of the Galactic mass but also is expanding. Because of its latitudinal position, the Derwood, Maryland, Station of the Department was able to extend observations nearly 20° farther south along the Galactic plane than the Dutch station.

The Department also decided during the year, after considerable experiment, to begin construction of an interferometer array from parabolic dish antennas, to be able to obtain precise positions of radio noise sources in the sky. A 30-meter dish closely following the design of the Argentinian radio telescope is now being constructed at Derwood and will be used with the existing 60-foot parabolic antenna as a two-element interferometer. These two antennas will be employed in experiment to evaluate the potentialities of such a system in determining precise radio-star positions.

A second cooperative venture of the Department in the area of astronomical study has been the work of the Committee on Image Tubes for Telescopes, of which Merle Tuve is chairman. In this the Department has collaborated with the Mount Wilson and Palomar Observatories, the Lowell Observatory, the National Bureau of Standards, and the United States Naval Observatory to develop electronic image tubes for magnifying signals received on optical telescopes. This work has also been supported in large part by generous grants from the National Science Foundation.

During the year the Committee continued the testing of tubes manufactured experimentally upon its order by the International Telephone and Telegraph Corporation Laboratories and by the Radio Corporation of America. The tests conducted were largely undertaken by W. K. Ford, Jr., of the Department. The tubes proved to have better operating characteristics than the Committee had hoped for only three years ago. Telescope observations were made at the Lowell Observatory with the tubes to examine their reliability and effectiveness, and laboratory investigations were conducted to distinguish among the relative merits of the several tubes. On the basis of the spectrographic tests from telescope observations and the laboratory tests, the Committee believes that the two types of tubes recently examined (mica-window and cascaded) will have wide application in astronomy because of their advantages over conventional photography. Development will be continued, again with the support of the grant from the National Science Foundation.

A major project of the seismic studies group in the Earth's Crust Section of the Department (J. S. Steinhart, L. T. Aldrich, M. A. Tuve, and associates) was an intensive study of the earth's crust in Maine, in which col-

leagues from the University of Wisconsin, Princeton University, Pennsylvania State University, the University of Michigan, and the Woods Hole Oceanographic Institution participated, and the United States Coast Guard assisted in detonating explosions in the Gulf of Maine in July 1961.

The data obtained from the explosions have since been the subject of appraisal to determine the application of explosion seismology to designation of crustal structures. This is a very real geological problem, because the traditional conception of the earth's crust as one or more horizontal layers of constant seismic wave velocity has appeared inadequate for more than a decade. Efforts to find the proper reflections from the surfaces of the supposed layers have been unsuccessful; and laboratory measurements of seismic velocities in various rock types contradict the layer hypothesis. Field evidence suggests significant lateral as well as vertical differences in structure. Several models that might conform to the seismic results received from the explosions were therefore constructed.

On the basis of these models it seems fairly certain that in Maine the Mohorovičić discontinuity[24] lies at 36 ± 3 kilometers below the surface. The most likely models suggest that the upper 3 kilometers of the crust is granitic and that below the granite the percentage of gabbro[25] increases at a rate that maintains a steady gradient in seismic wave velocity change to a depth of about 20 kilometers. These findings are of interest geologically in that they postulate appreciably less granitic material than is customarily thought to be in a continental crust.

The radioactive dating group is not only interinstitutional but also interdepartmental (L. T. Aldrich and S. R. Hart of the Department of Terrestrial Magnetism, G. L. Davis, G. R. Tilton, and B. R. Doe of the Geophysical Laboratory and associates). During the year the Department of Terrestrial Magnetism members of the group participated in an exchange program with the Geological and Mineralogical Institute of the University of Kyoto.

Dr. I. Hayase, of the University of Kyoto, spent part of the year at the Department becoming familiar with its techniques of measuring mineral ages. In the course of his visit he analyzed samples collected in Japan. The data were of interest as the first measurement of the kind from Japan. They showed no contradictions between the isotopically determined ages and ages implied by geological structure. They also showed discordances between rubidium-strontium and potassium-argon age determinations commonly enough to indicate a complex geological history for the Islands.

As a second part of the exchange, L. T. Aldrich of the Department is now

[24] A phenomenon recorded in the changing speed of seismic waves at certain depths.

[25] A granitic rock formed of plagioclase (light-colored) feldspar and a monoclinic pyroxene like augite (dark-colored).

in Kyoto as a visiting professor at the University. He is assisting in the establishment of a complete laboratory for the measurement of mineral ages. To facilitate this work the Department constructed and shipped to the University a mass spectrometer[26] which Dr. Aldrich now has in operation at the Institute there. It is expected that the spectrometer will serve as a model for similar equipment to be built elsewhere in Japan. We hope that this particular interinstitutional collaboration will continue indefinitely.

The Geophysical Laboratory members of the group also worked with a staff member of the Geological Survey of Finland, O. Kouvo, on the dating in two orogenic (mountain-building) belts in Finland: the Karelian belt extending from southeastern Finland northwesterly to Finnish Lapland, and the Svecofennian extending east-west in southern Finland. It is generally believed by geologists that the Svecofennian belt is older than the Karelian. The radioactive dating work, however, gives strong evidence that the intrusion of igneous rocks occurred about 1.9 billion years ago in both orogenic belts, and the two orogenies therefore are approximately contemporary.

The radioactive dating group has also compiled a new map of age distribution in crystalline basement rocks of North America. This shows one belt of rocks, ranging from 0.9 to 1.2 billion years old, extending from Labrador to Texas; another, 1.2 to 1.55 billion years old, occupying a large part of the central and southwestern part of the country; a third, 2.0 to 2.8 billion years of age, from the Rocky Mountains northeastward over the Laurentian Shield to Quebec; and still another, 1.55 to 2 billion years old, in Alberta and northwestern Canada. A picture of the geographical differentiation of ancient rocks in North America is thus beginning to emerge.

In cooperation with the University of Basel, Switzerland, the Department completed the installation of a polarized ion source in the departmental accelerator during the year. It consists of a discharge tube for the production of atomic hydrogen, diaphragms and pumps for defining the atomic beam, a quadrupole magnet for selecting and focusing the atoms having the desired orientation, an ionizer for the atomic beam, and a device for preaccelerating and focusing the ionized atoms. The machine was operated successfully. It is planned to use the polarized deuteron beam in the study of a number of nuclear reactions, thus returning the Department more directly to the field of nuclear physics than at any time since the end of World War II. For more than fifteen years after the mid-1920's the Department maintained a pioneering effort in nuclear physics, operating one of the first accelerators in this country.

[26] Mass spectrometer—an instrument for determining the masses of atoms or molecules in a gas, liquid, or solid. In it a beam of ions is directed through electric and magnetic fields so as to produce a mass spectrum identifiable by an electrical detector.

The Geophysical Laboratory

1905

Other than the Terrestrial Magnetism program, geophysical research in 1905 was not carried on within the premises of the Institution but nonetheless was considered an important part of the total program. It was the type of project that President Woodward advocated continuing, in his "Suggestions Concerning Pending Problems of the Institution."[27] Indeed, a large part of the total geophysical program in that year was carried on in close collaboration with the United States Geological Survey in Washington, thus commencing a friendly professional relation that has continued ever since. The two principal investigators of that year, Arthur L. Day and G. F. Becker, held appointments in the Survey even though a substantial proportion of Dr. Day's time was spent on Institution projects. Included was a three-month visit by Dr. Day to Europe for the purpose of studying laboratory equipment for geophysical research and making an inventory of European research.

Becker's research was concerned entirely with an effort to determine experimentally the relation between stress and strain. The main part of his apparatus was a 3-inch tube 480 feet long erected in the Washington Monument, within which steel tapes were suspended. He made some observations by means of this equipment during the year. In another project, F. D. Adams of McGill University conducted experiments on the cubic compressibility, the modulus of shear, and the flow of rocks, in which hundred-ton pressures were used.

The heart of the 1905 program, however, lay in the work of Dr. Day. Much of his time was spent in setting up his newly designed laboratory equipment. It comprised, among other apparatus, a furnace capable of reaching 2100°C in oxidizing or reducing atmospheres, a large electric furnace in which pressures up to 500 pounds or a vacuum could be maintained, and a water-pressure plant capable of reaching 2000 atmospheres. A similar plant capable of reaching 3000 atmospheres was under construction. Dr. Day's research included the completion of a three-year investigation of the lime-soda feldspar[28] group of rocks. His results showed "that the lime-soda feldspars form a continuous series of mixed crystals capable of stable existence in any proportion of the two component minerals." Experimental proof of this isomorphism was established by correlating melting points with change in the mixes of the two components. Experiments also were conducted on wollastonite ($CaSiO_3$), determining for the first time the exact temperature of crystallization of this mineral as found in nature.

[27] *Year Book 4*, pp. 28–29.

[28] Feldspar is one component of granite.

Inspired by his thought on silicates, Dr. Day already was looking toward the future, as he mentioned two practical problems to which his laboratory later contributed most significantly. He notes that "the study of lime-silica mixtures is fundamental in the preparation of Portland cement. Questions of technical interest in glass manufacture reappear everywhere in handling silicate solutions."[29] He concluded in a satisfied vein, "grave doubts were entertained as to the feasibility of handling physical phenomena at high temperatures with anything like the certainty attained at ordinary temperatures, but the experience of this first year has justified the effort . . . ."

1961–1962

If Dr. Day could look in on the Geophysical Laboratory of today he should feel greatly gratified, both because his beginning work in 1905 accurately forecast a direction and method of research that continues to be highly productive after nearly sixty years and because of the enormously great range and resolving power of the methods now in use.

The techniques upon which the Laboratory depend have become enormously more powerful and more sensitive than in Dr. Day's time. The 3000-atmosphere pressures, which were tremendous to Dr. Day, have been succeeded in 1961–1962 by pressures of 100,000 atmospheres. Moreover, these elevated pressures can be employed in combination with almost any temperature needed in geophysical experiment. In Dr. Day's 1905 experiments, high temperatures could be accompanied by a pressure of only a few hundred pounds. The present-day Laboratory has firm grasp of these tools, and it applies them to the whole range of problems on the frontiers of modern geology. From the first explorations of the potentiality of these geophysical techniques it has arrived at the full power of applying them to revelation of the earth's interior and its history. Furthermore, the capacities of the Laboratory now include a wide variety of techniques—beyond those of high temperature and high pressure—taken from modern physics, chemistry, and mathematics. The Department of 1961–1962 included work in experimental petrology, statistical petrology, crystallography, ore minerals, meteorite analysis, geothermal calculations, the ages of rocks and minerals, and organic geochemistry.

Among the numerous investigations carried on in these fields in 1961–1962, three will be described briefly to illustrate more in detail the characteristics of research at the Geophysical Laboratory. These are experimental petrology, in which much of the work this year was focused on pyroxene minerals, and emphasized the study of phase equilibria[30] at higher pressures;

[29] Later work of the Laboratory made fundamental contributions to the technology of both industries.

[30] In chemical terms, any crystalline compound or liquid is a phase; hence, a mineral separated from a rock is also a phase. Assemblages of phases (or minerals) which do not melt or react at a particular temperature and pressure are said to be at equilibrium. Study of these mineral equilibria is a means of understanding the conditions of formation of rocks.

the mineralogy of meteorites; and organic geochemistry, including analysis of Precambrian carbonaceous materials.

The program of studying the mineralogical composition of meteorites, begun last year, continued to produce most interesting results. Particularly relevant as a preview of the solid matter to be found in the spatial environs of the earth, the meteorites studied are continuing to yield mineralogical surprises. P. Ramdohr and G. Kullerud examined more than a hundred stony meteorites during the year, finding in them fourteen new minerals thought to be observed for the first time anywhere. Only one of them has been given a name, the others being referred to simply by letters of the alphabet for the time being. Because they occur in amounts too small to permit performance of standard chemical analyses or X-ray powder diffraction studies, the component elements in only two have been identified. These were a nickel-iron sulfide [$(NiFe)_2S$] called the Henderson phase, and a colorless mineral of spinel[31] type (Mg_2TiO_4). Several of the remaining twelve minerals are thought to be sulfides, and one, having an hexagonal layered structure, seems to be a compound of iron, carbon, and sulfur. One is thought to contain arsenic. The electron probe is considered to have promising potentialities for assisting in the chemical identification of these minerals. Another method of identification of the new phases is synthesis, once the major constituents are surmised from deductions about the origin of the minerals. Ramdohr and Kullerud state that their efforts in this direction are increasingly successful.

Ramdohr and Kullerud also made a number of observations on distinctive structural and textural phenomena in meteorites. They include evidences of mechanical distortion and crystallization in many meteorites, evidence of spontaneous melting in the interior of many, and the effects of terrestrial weathering, which may yield products that may be mistaken for primary components. Magnetite (Fe_3O_4) frequently may be such a product. In another set of analyses on meteorites, S. P. Clark, Jr., has identified an unknown mineral in tektites (glassy bodies probably of meteoric origin) as schreibersite (Fe_3P). He concludes that the content of minor elements in meteoric bodies, like sulfur, phosphorus, or carbon, should be helpful in identifying the number of meteoric falls in complex fields like those of southeast Asia or Australia. Presumably the minor elements would be the same in each fall but would differ in separate falls.

Another development of 1961–1962 meriting special mention is the study of phase equilibria at high pressures. This study has extended over sev-

[31] Spinel is typically magnesium aluminate ($MgO \cdot Al_2O_3$), but it has a wide variety of forms containing ferrous iron, manganese, ferric iron, and chromium. It may be red, yellow, green, black, or some other color. A general formula is $R''O \cdot R'''_2O_3$, where R″ may be one of the bivalent metals, magnesium, zinc, manganese, iron, nickel, cobalt, or cadmium, and R‴ may be trivalent aluminum, cobalt, iron, chromium, or gallium.

eral years and has drawn increasing effort by Laboratory Staff Members.

Geochemical studies at pressures up to 100,000 atmospheres have permitted geologists to take a fresh approach to various problems that have been the subject of spirited theoretical discussion for decades. Is the Mohorovičić discontinuity a phase change from basalt to eclogite?[32] Is it the same under the continents as under the oceans? What is the mineralogy of the earth's mantle?[33] Can the various types of basaltic lava be related to variations in the melting of mantle rocks at different depths and pressures? What temperatures are present in the lower mantle and core?[34] As yet none of the questions can be fully answered, but the high-pressure studies of the last five years have contributed to an understanding of all and promise to contribute far more.

The Mohorovičić discontinuity continues to be one of the more absorbing geological problems. High-pressure high-temperature experiment with the synthesis of rocks expected at the depths of the discontinuity has given some indication of the rocks to be found there. Under the continents they are principally basalt and eclogite. Basalt is transformed by high pressure to the denser eclogite. Eclogite consists essentially of jadeite-bearing pyroxene and pyrope-bearing garnet.[35] Both jadeite [$NaAl(SiO_3)_2$] and pyrope ($Mg_3Al_2Si_3O_{12}$) are high-pressure phases, and their pressure-temperature fields of stability have been established in recent years at elevated temperatures. Significantly, both the reactions leading to the formation of jadeite and pyrope take place in a relatively narrow pressure-temperature range. The experimental results now indicate that in the depth range 50 to 100 kilometers in the mantle, where basaltic lava is believed to form, the mineral assemblage will be characteristic of eclogites. The experimental data for the transition fit reasonably well the hypothesis that the continental Mohorovičić discontinuity is a basalt-eclogite transition. The nature of the discontinuity under the oceans apparently is different from the continental, and is a challenging question for future thought and experiment.

Additional experimental data for constructing concepts of the earth's mantle and crust are being obtained in quantity at the Laboratory from an examination of the melting relations of silicates at high pressure. As a result the present-day conceptions of reactions by which basalts form in the partial fusion of mantle rocks are wholly different from those of earlier workers. The system of petrology developed by N. L. Bowen and others

[32] A dense rock equivalent in composition to basalt, found in association with Russian and South African diamond pipes, and occurring in rocks, elsewhere on the earth's surface, thought to originate from deep in the earth's mantle.

[33] That part of the earth's interior between the Mohorovičić discontinuity and the core.

[34] The core is thought to commence at a depth of about 2900 kilometers.

[35] Garnet has the general formula $R''R'''(SiO_4)_3$, where R″ may be bivalent iron, magnesium, manganese, or calcium, and R‴ may be trivalent iron, aluminum, or chromium.

earlier at this Laboratory from experiments at atmospheric pressure successfully explained many characteristics of igneous rocks. It now is clear, however, that pressures as low as 10,000 to 20,000 atmospheres produce very pronounced changes in crystal-liquid equilibria in silicate rock systems. Even though the data on phase relations at high pressures still do not permit the construction of a system of petrology for the lower crust and upper mantle of the earth, answers to some important questions are being obtained.

One of the intriguing questions concerned the formation of silica-saturated basalt rocks of the crust from silica-undersaturated mantle rocks. F. R. Boyd, Jr., and J. L. England experimented during the year with the melting of pyrope garnet, thought to be an important constituent of the mantle, at pressures prevailing where basalts are considered to be formed. They found that pyrope garnet melts incongruently at these pressures to spinel and liquid. This melting relationship could explain the formation of the silica-saturated basalts, such as are found in Hawaii, from the typical minerals assumed to be in the upper mantle.

Continuing the experiments reported in *Year Book 60*, H. S. Yoder, Jr., and C. E. Tilley examined the possible origin of alkali basalt and tholeiitic basalt, two groups of rocks that are very important components of the earth's crust. Their previous experiments with natural rocks and synthetic mineral systems established that the same magma (liquid rock), depending on pressure, could yield both types of basalt. They now have suggested mechanisms whereby both alkali and tholeiitic basalt may be generated from an eclogitic liquid deep within the earth's mantle.

Sydney P. Clark, Jr., J. F. Schairer, and John de Neufville have attacked the same problem with a different approach. They also believe that it is necessary to examine critically important systems of minerals in their entirety under pressure before inferences about melting and solidification within the mantle can be drawn with confidence. They chose to examine the important but complicated quaternary system[36] that includes among its phases the oxides spinel and corundum, forsterite (Mg_2SiO_4), diopside ($CaMgSi_2O_6$), pyrope garnet, various forms of silica, and still other minerals. Their experiments were conducted at atmospheric pressure and at a pressure of 20,000 atmospheres. Their observations showed a range of solid solution in pyroxene minerals (e.g., diopside and others) at high pressures that is far more extensive than in the same system at atmospheric pressure. Pressure therefore undoubtedly produces profound changes in the melting relations within at least this mineral system. For some of the compositions, the system at 20,000 atmospheres is not even qualitatively similar to the

[36] Quaternary system—a system of phase relations among minerals having four end members, schematically expressible in a tetrahedral diagram.

system at atmospheric pressure, as in the appearance of quartz on the liquidus above 1000°C. The experiments showed that this system is well suited to the study of the complex chemical equilibria at high pressures. Further study should yield important contributions to the petrology of the earth's rocks at depth.

Heat is the source of energy for most geological processes, and knowledge of the temperatures at the depth of the core-mantle boundary[37] in the earth is of fundamental importance. It is probable that the temperature at this depth is not far below the minimum melting temperature of rocks in the lower mantle and not far above the solidifying temperature of the iron-nickel alloy believed to comprise the outer core. Data obtained at low pressures showed that the slopes of silicate melting curves were two to five times greater than the slopes of the melting curves of most metals. However, recent results for diopside and a few other silicates at pressures up to 50,000 atmospheres yield diagrams with slopes having a pronounced curvature. Extrapolation of the diopside data to a pressure at the core-mantle boundary[38] indicates that a temperature of 3750°C would be required to melt diopside there. Similar extrapolation of data on the melting of iron indicates a temperature of 5200° at the boundary. Although the uncertainties in these extrapolations are very numerous, it is interesting that the estimates are close. They are furthermore in rough agreement with estimates made by other, equally uncertain, methods.

An elegant example of the application of the sensitive and powerful modern research techniques in geophysics to a problem that scarcely could have been touched even a decade ago is shown in the identification of compounds characteristic of life from ancient rocks. P. H. Abelson and P. L. Parker have isolated fatty acids from rocks as old as 500,000,000 years. This is the oldest known occurrence of these substances. Among the compounds identified were the saturated acids myristic [$CH_3(CH)_{12}CO_2H$], palmitic [$CH_3(CH)_{14}CO_2H$], and stearic [$CH_3(CH)_{16}CO_2H$], the last being the most abundant. Although the quantities found are minute (10 micrograms[39] per gram of organic carbon), gas-liquid chromatography permits isolation, identification, and quantitative measurement of the individual acids, even when major amounts of impurities are present.

The same fatty acids were isolated from recent sediments. Palmitic acid was the major component in the young rocks, being as much as ten times as abundant as stearic acid, the more abundant in the old rocks. Thus although the fatty acids in very young and in old rocks are qualitatively similar a puzzling quantitative difference has been noted.

[37] Postulated to be at a depth of about 2900 kilometers.
[38] 1,400,000 atmospheres.
[39] A microgram is 0.000001 gram.

T. C. Hoering has investigated two important aspects of the geochemical record of very early life on earth. He has studied some of the earth's oldest sedimentary rocks, which contain structures geologists consider related to algal activity. He has measured stable carbon isotope ratios, C^{13}/C^{12}, in coexisting carbonates and reduced carbon obtained from these specimens. He has found that the isotopes have been fractionated into a C^{13}-enriched carbonate phase and a C^{13}-depleted reduced carbon phase. The amount of this fractionation is nearly identical to that found in contemporaneous algal cells and their associated carbonates. The magnitude of the effect is also similar to that found between limestones and coals of all geological ages. Such isotope fractionation is caused by a slightly different rate of photosynthesis for molecules of carbon dioxide containing C^{12} as compared with those containing C^{13}.

The samples examined include a limestone from the Belt Series of Glacier Park, Montana, with a minimum age of 1.2 billion years, the Randville dolomite of Crystal Falls, Michigan, with a minimum age of 1.5 billion years, and the Bulawayan limestone of Southern Rhodesia with a minimum age of 2.7 billion years. In these rocks, which are among the oldest known sedimentary rocks, the isotopic evidence is consistent with the presence of photosynthetic algae in the very early Precambrian era.

The second study by Hoering was on the reduced carbon of Precambrian sedimentary rocks. A successful effort was made to extract and partially identify organic molecules from them. By means of a number of chemical degradations he was able to liberate soluble fractions from the insoluble "fabric" of the reduced carbon. The fractions were analyzed with the aid of ultraviolet spectroscopy and chromatography. The results indicate that the insoluble reduced carbon may be related to the kerogen of more recent rocks. Kerogen is produced by interactions of organic products of cells when deposited in sediments deprived of oxygen. Thus additional evidence has been produced pointing to the existence of life in very early Precambrian times, more than two billion years ago.

Mount Wilson and Palomar Observatories

1905

In 1905 the astronomical activities of the Institution were mainly those of the Solar Observatory, whose building and equipment were under construction during the year, with view to completion in 1906. Mount Wilson was considered an especially favorable site because "The unusually favorable atmospheric conditions which prevail day and night at the site of the observatory have attracted the attention of astronomers and astrophysi-

cists generally."[40] "It has been been found that the average night-seeing is exceedingly good, while the low wind-velocity, coupled with the transparency of the atmosphere, afford. . . advantages which should render Mount Wilson an ideal site for the 5-foot reflector." George E. Hale, the Director, defined his purposes as: "(1) The investigation of the sun (*a*) as a typical star, in connection with the study of stellar evolution: (*b*) as the central body of the solar system, with special reference to possible changes in the intensity of its heat radiation, such as might influence the conditions of life upon the earth. (2) The choice of an effective mode of attack, involving (*a*) the application of new methods in solar research; (*b*) the investigation of stellar and nebular phenomena, especially such as are not within the reach of existing instruments; and (*c*) the interpretation of these celestial phenomena by means of laboratory experiments." He was at this time already considering the design of "a large reflecting telescope and of new types of instruments." He also looked forward to "The furtherance of international cooperation in astrophysical research through the invitation to Mount Wilson, from time to time, of investigators especially qualified to take advantage of the opportunities afforded. . . ."

A large part of Dr. Hale's report in 1905 was necessarily devoted to a statement on the numerous construction projects that had absorbed his attention during the year. They included ten buildings on Mount Wilson, and the Pasadena office and shop, which were constructed on land given by citizens of Pasadena. Dr. Hale nonetheless found time not only for instrument testing but also for an observing program and planning a future research program. Daily direct photographs of the sun on a scale of 6.7 inches to the solar diameter were taken on the Snow telescope. Observations were made to test an hypothesis of Dr. Hale's about the relation of calcium vapor to the faculae and plages[41] of the sun. Some experimental study of the spectra of sunspots, plages, and the chromosphere was undertaken for instrument design. Photographs were also taken of bright stars with a long-focus grating spectrograph. They included a photograph of the blue region of the first-order spectrum of Arcturus, which required an exposure of 14 hours on three successive nights.

Visiting investigators had already found their way to Mount Wilson. E. E. Barnard of the Yerkes Observatory photographed the southern part of the Milky Way, described by Dr. Hale as "a most important contribution to our knowledge of the structure of the Milky Way and of the remarkable nebulae within it." The Smithsonian Institution also sent an expedition

[40] *Year Book 4*, p. 25.

[41] Faculae—small irregular bright patches in the photosphere (visible disk) of the sun, surrounding sunspots.

Plages—faculae of the chromosphere, which is the outer layer of the sun's "atmosphere," extending to a height of several thousand kilometers from the visible disk.

to the mountain for observing solar radiation, directed by C. G. Abbot.

Other astronomical work supported by the Institution in 1905 included the compilation, by Lewis Boss of the Dudley Observatory, Albany, New York, of a Preliminary General Catalogue of Stars for the 6000 stars visible to the naked eye. Also included were grants to Simon Newcomb of Washington, D. C., for an "Investigation of the mean motion of the moon," and a rather enigmatical grant "To aid investigations in mathematical astronomy, statistical methods, and economic science." The economic science part was never reported upon, either in 1905 or in the four succeeding years when Dr. Newcomb held sequel grants.

1961–1962

The program of 1961–1962 at the Observatory, as for each of the four preceding Departments, differed vastly from its ancestor of sixty years ago. The primary emphasis on solar studies gave way in 1918 to a more general astronomy program with the completion of the 100-inch telescope. Nonetheless, solar observation and solar study have continued to the present day, but with gradually decreasing emphasis. A major change came in 1958–1959, with a decision to drastically curtail routine observations. Since then solar studies have centered on the sun's magnetic fields, of which daily observations are made with the aid of the solar magnetograph originated and developed by H. D. and H. W. Babcock of the Observatories. Daily solar magnetograms have been made since 1957.

During the 1961–1962 year R. F. Howard commenced an extensive study of the accumulated magnetograms to classify magnetic regions, and correlated them with optical and radio phenomena. He has already obtained the very interesting finding that the unipolar magnetic (UM) regions of the sun correlate in their position with calcium absorption phenomena observed spectroscopically. It may thus now become possible to extend observation of UM regions backward for 50 years or more, using the Observatory's extensive collection of spectroheliograms showing the absorption lines of the elements.

Responding somewhat to the explosion of national interest in interplanetary space, the year was also marked at the Observatories by renewed attention to the planets, which have been subject to recurring study at the Observatories in the past. It has seemed important to press ground-based observations like those that can be undertaken at the Observatories to the limits made possible with new photometric and infrared techniques, because information about the planets can be acquired by these techniques at a cost of much less effort and money than by observations from rockets. G. Münch, with the collaboration of H. Spinrad of the Jet Propulsion Laboratory of the California Institute of Technology, and R. Younkin of the same laboratory, began studies of the spectra of the major planets. Two

lines of the hydrogen molecule were found in the spectrum of Saturn, providing the first firm evidence of the presence of hydrogen in the atmosphere of that planet. Spinrad also analyzed high-dispersion spectra of Venus, finding evidence of large changes in the temperature of the atmosphere of Venus. B. Murray of the California Institute of Technology continued studies of the photoelectric colorimetry of the moon with the Mount Wilson facilities.

A major part of the Observatories' program, however, has been devoted to a study of the masses, luminosities, surface temperatures, and chemical composition of stars, and the variation of luminosity and surface temperature with age. During recent decades these have been among the major problems in astronomy. Even though such research has become increasingly important with time, steps toward the modern understanding of these phenomena date back to the early years of this century. The first important step was taken by E. Hertzsprung and H. N. Russell, when they plotted a diagram of the absolute magnitude of stars in the solar neighborhood against their surface temperatures as indicated by spectral class or color. They found that most stars fall in a narrow diagonal band on their diagram, the very hot giants being at one end and the cool dwarfs at the other. Later theoretical investigations based on nuclear physics showed that the fusion of hydrogen into helium was an important source of energy for most stars[42] and that stars obtaining their energy from this reaction logically fall on the color-magnitude diagram in the narrow "main sequence" band noted by Hertzsprung and Russell.

During the second world war, Walter Baade of the Observatories made a detailed investigation of the stellar content of the Andromeda galaxy. He found that the brightest stars in its spiral arms are very hot giants similar to those in the solar neighborhood, which also is on a spiral arm. In the nucleus, however, the brightest stars were cool red giants. To differentiate these, Baade introduced the concept of Population I (younger) stars typically on the spiral arms and Population II (older) stars typically at galactic centers.

Theory then predicted that, as the hydrogen fuel approaches exhaustion in a stellar core, a star expands greatly but cools and thereby moves off the narrow main-sequence band in the color-magnitude diagram and becomes a red giant. Since the brightest stars use their fuel most rapidly this change starts at the upper end of the main sequence and moves down the sequence with time. Obviously, the hot giants in the solar neighborhood and in galactic spiral arms indicate a population of stars that have formed recently, whereas the red giants in a galactic nucleus represent a population of old stars. Theory permits one to go even further and fix the age of a group

[42] The hydrogen-helium reaction is now considered ancillary to the hydrogen-deuterium-helium reaction.

of stars by observing the magnitude at which stars are just beginning to move off the main sequence. Color-magnitude diagrams have been constructed for a large number of globular and galactic clusters[43] by A. R. Sandage, H. C. Arp, and W. A. Baum of the Observatories, and many others. Ages of a few million up to ten billion or more years have been found.

With the aid of high-dispersion spectra it has become possible recently to make detailed quantitative chemical analyses of stellar atmospheres. The first studies of the sun and of bright nearby stars indicated a surprising uniformity of chemical composition. When these measurements were extended to some of the distant older clusters, however, it was found that their stars were deficient in the heavier metallic elements, often by factors of 100 or more compared with the stars near the sun. Since most of the strong metallic lines fall in the ultraviolet (U) region of the spectrum, a star of high metallic content exhibits a depressed U region compared with the blue (B) or green-yellow (V, "visible") spectral regions. Within the past two years astronomers at the Observatories have found it possible to fix the metallic content from a comparison of the magnitudes of a star measured in the U, B, and V regions. This makes feasible the extension of abundance determinations to stars far too faint for detailed spectrum analysis.

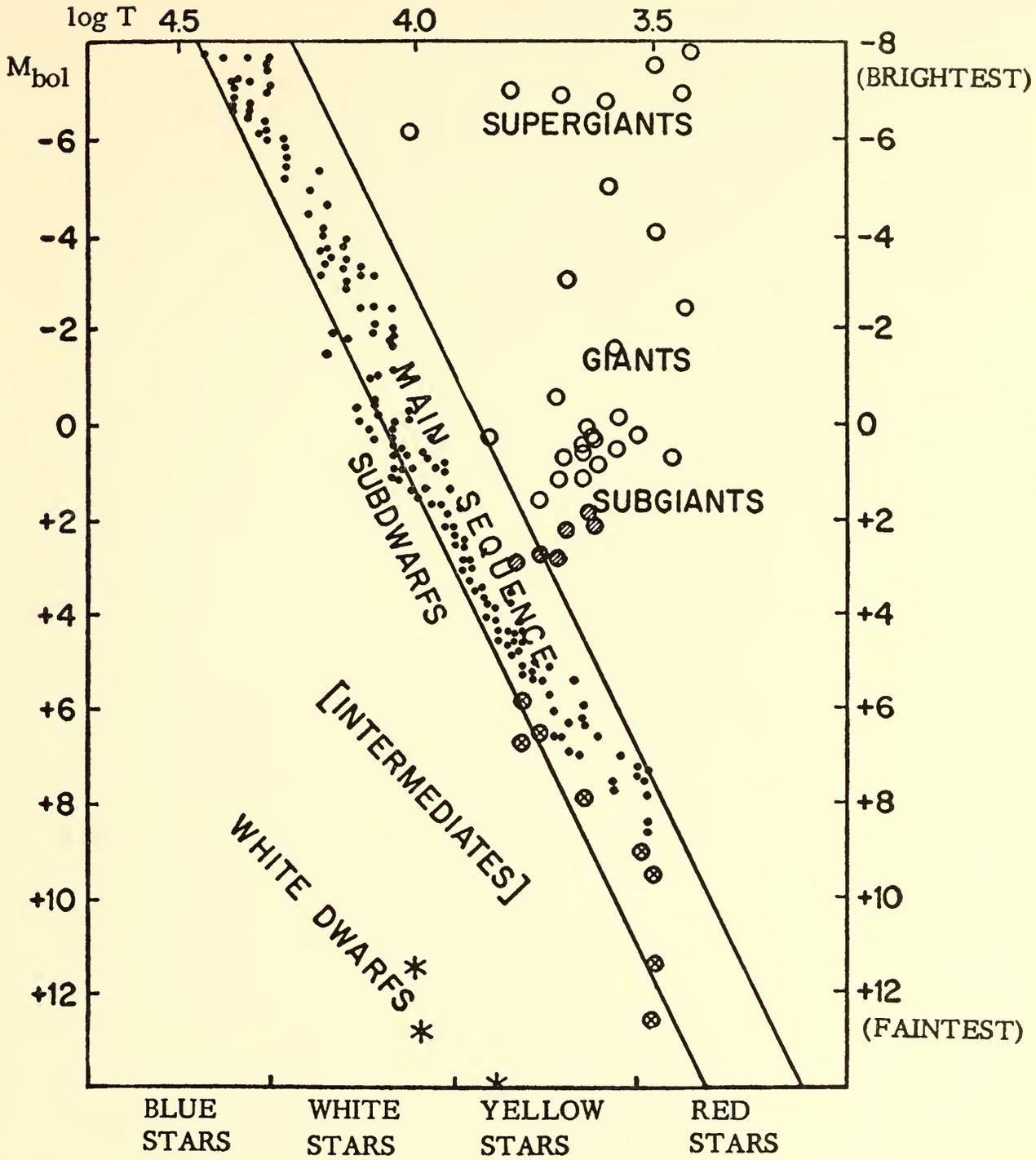
In general, old stars such as those in the globular clusters, or high-velocity stars,[44] which presumably were formed at the same time as those in the galactic nucleus, are metal deficient compared with the younger stars. Theory suggests that metals are formed late in the evolution of a star, after the hydrogen fuel has been exhausted in the stellar core and the central temperature has increased to many times that of stars on the main sequence. Therefore the metal-containing material in recently formed stars has gone through one or more earlier generations of stars in which the metals are formed and then blown off into space either in a gradual flow[45] or explosively in a nova or supernova outburst.

Obviously, a project to understand stars will require decades for completion as well as investigation by many astronomers at a number of observatories. The Institution can take pride not only in the participation of the Observatories in the grand conception of such a project but also in their preeminent position as contributor of observational data leading to widening views of the universe which these studies are providing. During the year 1961–1962 the staff of the Observatories skillfully exploited the wonderful instruments at their disposal to give us further insights on this frontier of

[43] A globular cluster is a group of many thousands of stars arranged in a regular form showing spherical symmetry. Many are located outside the plane of the Milky Way. A smaller group of stars always found near the plane of the Milky Way is known as a galactic cluster.

[44] A high-velocity star is a star that is moving about the galactic nucleus with a velocity markedly different from that of the sun.

[45] As studied by A. Deutsch of the Observatories. See *Year Book 59*, p. 8, and other year books.



Generalized Hertzsprung-Russell diagram of star color-magnitude relation. Log T = logarithm of temperature, degrees Kelvin; M_{bol} = bolometric magnitude, as measured from calculated total energy emission. (Adapted from Cecilia Payne-Gaposchkin, *Introduction to Astronomy*, Prentice-Hall, New York, 1954.)

the universe. Among the results were new knowledge about the differences in chemical composition among stars, the correlation of chemical composition and star movement, a determination of the time of formation of the Galaxy in which we are located, and new evidence on the expansion of the cosmos.

The staff of the Observatories participated in detailed chemical investigations of a number of stars. J. Greenstein and R. A. Parker of the Observatories have collaborated with G. Wallerstein of the University of California, H. L. Helfer of the University of Rochester, and L. Aller of the University of Michigan to study one group of three red giant stars. They found that the common metals were only 1/500 as abundant in this group as in the sun, and the heavy elements strontium, zirconium, barium, cerium, and europium were deficient by a factor of 25,000. Considering the deficiencies, they estimate that these stars, which are part of our Galaxy, probably condensed within a few hundred million years after the formation of the Galaxy. In investigating several dozen peculiar B and A stars,[46] J. Jugaku, W. L. W. Sargent, and L. T. Searle found that the abundances of individual elements vary erratically compared with neighboring elements in the periodic table, often fluctuating by factors of 100 or more. Elements found to have marked over- or underabundance in certain stars are beryllium, carbon, nitrogen, oxygen, silicon, phosphorus, and mercury.

For some years a group of stars have been recognized and designated as subdwarfs because they lie appreciably below the main sequence on the color-magnitude diagram. Early studies showed that they were metal-deficient, therefore old, stars. They have high velocities considered in relation to the sun. To learn more about these rather rare stars, A. Sandage and C. T. Kowal have started a program for the photoelectric observation of the ultraviolet-blue-visible magnitudes of the high-velocity stars given in the Giclas Proper Motion Catalogue. More than 100 new metal-deficient subdwarfs have been discovered among the 700 stars observed thus far. Spectroscopic studies by Greenstein and by Sandage of an enlarged sample of these subdwarfs confirmed the high velocity of all.

In a further effort to obtain information on the relation of the subdwarfs to other principal groups of stars, O. J. Eggen and Sandage studied the effect of "line blanketing"[47] on the position of a star in the color-magnitude diagram. The results were of special astronomical interest, for Eggen and Sandage found that if proper correction is made for line blanketing the subdwarf stars move into the same position as normal dwarf stars on the main

[46] The accepted spectral classification of stars designates them by arbitrary letters as O, B, A, F, G, K, and M. O and B stars have the highest temperatures, and M the lowest.

[47] Line blanketing refers to the situation in which the abundance in a star of the metallic elements having strong absorption bands in the ultraviolet is so great that it causes an appreciable deficiency in the spectral region compared with other parts of the spectrum of the star.

sequence of the color-magnitude diagram. Thus another addition was made to our understanding of the wonderful order which astronomers have been slowly illuminating with the aid of modern instruments.

Eggen, D. Lynden-Bell, and Sandage also studied the orbits around the nucleus of our Galaxy of a large number of dwarf stars, including both the normal and subdwarf types. They find a close correlation between metal deficiency and the eccentricity and angular momentum of the stellar orbit. They interpret this as indicating that metal-deficient stars were formed in an early period while our Galaxy was rapidly contracting. From the age of these stars they were able to fix the time of formation of our Galaxy out of the medium of the universe at about ten billion years ago.

Studies of stellar properties are important not only for understanding the characteristics of the stars themselves but also to provide a firm basis for the measurements on which the conceptions of the structure and origin of the entire universe depend. For example, nearly all determinations of large astronomical distances depend on the comparison of the apparent brightness of a nearby object with that of an identical object in a distant cluster or galaxy. Thus cepheid variables[48] were used by E. P. Hubble to fix the distance of the nearby Andromeda galaxy, and the galaxies themselves were used to estimate the distances of clusters of galaxies at the extreme range of telescopic penetration into space. However, the discovery of different stellar populations with major differences in age and chemical composition raised many doubts about the identity in absolute magnitude of a star in our own neighborhood with that of a star in a nearby galaxy, which might or might not have similar age or chemical composition.

One of the uncertainties in these extrapolations toward a picture of the universe has been the effect of light absorption by dust clouds along the path between star and observer. This is especially troublesome, since the shorter wavelengths of the spectrum are absorbed more strongly than the longer, producing a reddening effect. During the year H. C. Arp reexamined this problem in color-magnitude studies of globular clusters of stars. He found that the correction for absorption should be appreciably larger than had been allowed formerly. A substantial revision downward of previously determined globular cluster ages therefore must be made. This also eliminates a discrepancy existing between age determined from position on the color-magnitude diagram and age determined from models of cosmological expansion. They now become consistent.

Extrapolations to distant galaxies are also handicapped because most are too distant to permit observation of enough stars for the construction of a color-magnitude diagram. However, it is possible to analyze the integrated

[48] Stars whose light emission varies in a definite pattern over a relatively short period but longer than 24 hours.

light received from a galaxy and from it obtain information about the distribution in temperature, magnitude, and chemical composition of the component stars. W. A. Baum has studied some nearby galaxies of different types by photoelectric scanning methods in order to obtain information about the evolution of galaxies. His evidence indicates that some definitely are composed of true Population II (older) stars whereas others (large ellipticals) have mainly Population I stars. Such observations are important in the construction and interpretation of cosmological models. Present interpretation of the observable universe conceives it as having a radius of five billion or so light years,[49] expanding at its limits of observation at nearly half the speed of light. This interpretation depends on assumptions made about the magnitude-redshift relation, that is the reddening of the observed spectrum caused by recession of the distant galaxies in relation to the solar system. Distant galaxies, of course, are seen at an earlier age than nearby ones—billions of years of difference for the most distant. Since individual stars undergo large changes in luminosity and temperature with age, the observable integrated light of a galaxy also changes with time. How, for example, has the extremely distant galaxy 3C295 (now redesignated 1410+5224), mentioned in *Year Book 59*, changed in the five billion years since the observed light that fell on the Palomar photographic plate left the galaxy? Answers to questions like these will be obtained only from studies such as those undertaken by Baum and other Staff Members of the Observatories on stellar properties and evolution.

The Department of Embryology

1905

Although the Department of Embryology was not established until 1914, when it was organized by Franklin P. Mall, even its subject was not ignored among the activities of the Institution in 1905. In that year L. B. Mendel of Yale University was given a grant for "Study of physiology of growth, especially in its chemical processes." Professor Mendel's grant was renewed in each of several years thereafter. He reported that he was studying the "chemical composition of the developing animal body and the equipment of this organism for its nutrition, upon which growth essentially depends. Data are being collected at first hand regarding the composition of various embryonic tissues at different stages of embryonic growth. For the nervous system a correlation between morphological and

[49] Light year—the distance traveled by light in a vacuum during one year; about 5.88×10^{12} miles.

chemical development is already apparent. The chemistry of embryonic muscle is also already under investigation. "The purin content of the liver and muscles at various embryonic stages has been determined. . . . It is hoped . . . to ascertain whether the purin metabolism of the young is essentially different from that of the adult."[50] A grant to L. E. Griffin was also made in the same year "to secure material for a study of the embryology, histology, and physiology of the Nautilus." Studies supported at the Marine Biological Laboratory, Woods Hole, Massachusetts, included one on the "segmentation of certain fertilized eggs"; on "regenerative processes and structures"; and on "muscle-fibers of the fish heart."

These studies, however, were not in any sense an organized group. Nor did they command a major interest on the part of the Institution's administration, as was shown by the termination of this type of grant at the end of 1908. It remained for Dr. Mall to set in 1914 the lines on which the Department continued so long, an examination of the morphology and histology of the human embryo and the embryonic physiology of primates. Even at the beginning of the Department, however, other organisms were studied, as illustrated by the 1914 study of E. L. and E. R. Clark on the movements of the lymph heart in living chick embryos, and their report in that year "that the muscle of the lymph heart is derived from the myotomes."[51]

1961–1962

The year 1961–1962 was marked by the setting of an important milestone in the history of the Department. After several years of preparation the new Department of Embryology building, adjacent to the Homewood Campus of the Johns Hopkins University in Baltimore, Maryland, was completed. This building, specially designed for embryological research, should free the staff of the Department from the inconveniences that attended work in their former cramped quarters at the Johns Hopkins Medical School near the center of the city. The Department started to move on August 1, 1961, and was able to assume full operation by early November, in spite of a long delay in equipping the building because of an electricians' strike. The new building appears to have met with staff approval. Director J. D. Ebert describes it as having "an unusual combination of fine qualities, pleasing to both aesthetic and practical senses."

The Department in 1961–1962 is described by Dr. Ebert in the introduction of his report of this year as one holding to its "traditional organization of a group of independent investigators whose interests range widely from biochemistry and microbiology to anatomy and physiology, with substantial overlapping in experience and approach. . . . in developmental

[50] *Year Book 4*, pp. 259–260.
[51] *Year Book 13*, p. 112. A myotome is a muscle mass in a developing animal.

biology today it appears to favor the generation and interchange of ideas. . . ."

The multifaceted program Dr. Ebert describes included an interesting study of the physiologic aspect of frog-embryo growth from the stage of the fertilized egg onward by D. D. Brown and J. D. Caston, the nature of the testicular antigen in induced aspermatogenesis[52] by G. L. Carlson and D. W. Bishop, the role of deoxyribonuclease II during the metamorphosis of the tadpole by J. R. Coleman, a comprehensive study of the developing human eye by R. O'Rahilly, and still others.

Of particular interest among these was the Brown-Caston study of the embryonic development of the frog *Rana pipiens*. They found that the early embryos contain a measurable but small population of ribosomes in their cells. The early ribosomal content changes little until a stage near the end of morphogenesis,[53] when there is a very rapid appearance of more particles. This coincides with the time when the embryo has been shown to require magnesium ions from outside. In addition, the iron storage molecule, ferritin, was definitely identified in the egg. Also, although ribosomal synthesis was shown to begin after much of morphogenesis is completed, high-molecular-weight RNA, with a base composition identical to ribosomal-ion RNA, was found to be present in all stages of the embryo.

Perhaps the most striking progress to be reported from the Department during the year came in the studies of I. R. Konigsberg, who joined the Institution as a Staff Member on July 1, 1961. They will be described in some detail as an illustration of the methods and approach of the present-day Department.

From its beginning the Department of Embryology has numbered, among its Staff Members, investigators dedicated to the study of the development, structure, chemistry, and physiology of muscles. W. H. and M. R. Lewis (Department of Embryology, 1915–1940) pioneered in analyzing the origin of muscle fibrils in tissue culture; and Arpád Csapo (1949–1954) was among the first students of muscle chemistry to characterize the contractile proteins of the uterus and to examine their regulation under different physiological conditions. More recently J. D. Ebert and R. L. DeHaan and their associates have focused attention on the biochemistry of developing contractile proteins and on morphogenetic movements and relations of contractile and conductile cells in the heart. D. W. Bishop has contributed importantly to our understanding of mechanisms in primitive contractile systems like sperm tails.

To this roster the Department now adds Konigsberg's name. During the year he made substantial progress in a hitherto refractory subject, the

[52] Destruction of the power to produce sperm.

[53] The emergence of the specific structure of an animal during embryonic development.

investigation of the cytodifferentiation of embryonic skeletal muscle cells in dispersed cell culture. His system of culture is designed to offer greater opportunity for rigorous control of both the quantitative aspects of the cellular population and the extracellular environment than can be achieved either in vivo or in organ culture. Many years' experience by numerous previous investigators suggested that such culturing techniques could be expected to promote the loss of differentiative character and would not favor a progressive increase in the effects of cell specialization on morphology. No generally satisfactory explanation for this previously observed incompatibility has ever been given. However, Konigsberg's results with monolayer cultures of embryonic skeletal muscle cells are in striking disagreement with expectations from earlier experience.

Monolayer cultures prepared from suspensions of 11- to 12-day chick embryonic leg muscle pass through three recognizable phases. The period immediately following plating of the cells is marked by rapid proliferation with a mean generation time of 24 hours. During this period cultures consist exclusively of mononucleated cells and have the general appearance of cultures of "fibroblast-like"[54] cells such as might be derived from a great variety of tissues. The transition from the first to the second phase occurs in a matter of hours and is characterized by the formation of long multinuclear "ribbonlike" cells. Formation of these multinuclear cells coincides with the attainment of cell confluence in the culture. The effect of cell density is further suggested by experiments in which the inoculum size was varied. The smaller the inoculum, the greater the time of transition from phase one ("fibroblast-like" cell) to phase two (multinuclear "ribbon"), and vice versa. The abrupt appearance of multinucleated myotubes[55] in this second phase is paralleled by an equally abrupt break downward in the rate of proliferation. Again, the time required for this development can be shifted by varying the inoculum size.

Differentiation beyond the stage of the mononucleated myoblast[56] occurs in culture after cells have ceased rapid multiplication. This observation is consistent with Konigsberg's earlier findings, as well as with those from several laboratories, that myotube nuclei are postmitotic[57] and that they form by cellular fusion. The third phase of muscle differentiation in culture is characterized by the progressive development of the cross-striated myofibrillar pattern and the initiation of the spontaneous contraction characteristic of muscular tissue.

All Konigsberg's studies before the past year had been restricted to mono-

[54] Fibroblasts—elongated mononuclear cells which develop into and are also part of connective tissue.

[55] Aggregated-cell constituent of muscle.

[56] Unassociated single "premuscle" cell.

[57] Mitosis is cell division.

layer cultures established with inocula of 1 million to 2.5 million cells each. Such cultures reach confluence between the second and fourth day of culture, depending on the size of the inoculum. To probe for the lower limit of inoculum size that would still permit differentiation to occur he turned to the single-cell plating technique developed by T. T. Puck and his associates. In this procedure small numbers of cells are dispersed over a relatively large area. During appropriate periods of incubation the individual cells give rise to discrete colonies visible to the naked eye. The technique has been applied most successfully to permanently established cell strains.

Using freshly isolated embryonic muscle cells Konigsberg observed a plating efficiency of approximately 10 per cent. In plates cultivated for 10 to 13 days approximately 1 in 10 colonies exhibited unmistakable signs of skeletal muscle cell differentiation. The proportion of differentiated cells ranged from colonies containing several elongated myotubes in colonies of predominantly mononucleated cells to colonies in which virtually every nucleus was in syncytial[58] association. Under polarized light or bright-field illumination after staining, the myotubes showed the presence of longitudinal fibrils, which frequently exhibited the pattern of cross striation typical of mature skeletal muscle cells. It is apparent that some myoblasts, at least, through a sequence of rapid multiplications, can produce a large number of progeny that retain the capacity for differentiation.

Two major questions emerged from these observations. First, what is the significance of the finding that only 1 in 10 colonies eventually differentiates? Second, what is the stimulus initiating myotube formation? Konigsberg is attacking the second problem by examining the relationship of cell density to myotube formation. Two general mechanisms by which cell density might affect myotube formation were considered. Since myotube formation is a result of cell fusion, high cell density might ensure that a sufficient number of effective cell-to-cell collisions occur. Another, and equally likely, possibility is that a high cell density may be either supplementing the culture medium with cell products or removing some components.

Konigsberg designed experiments to test that possibility. His first tests showed that the medium is altered by the metabolic activity of cells cultured in it. In cultures grown on a medium preconditioned by the presence of other cells, myotube formation commenced as much as 24 hours earlier than initial cultures of equal numbers of cells from the same cell suspension but cultured in fresh medium. Furthermore, the cells in conditioned medium attached to the glass more firmly, presenting a strikingly different appearance from the control cultures.

These results are impressive in themselves, and indeed they represent something of a technical breakthrough in the difficult task of cell culture.

[58] Referring to a multinucleated aggregate of imperfectly separated cells, or a multinuclear cell.

But as so often in science they are probably more important for the questions they raise than for the results they give. Already they have pointed the way to a number of additional experiments to probe the relation between conditioned media and cell differentiation. But in the hint given of a hitherto unsuspected closeness of relation between cell and environment we touch a problem of wide application and perhaps vast significance in understanding all higher forms of life.

Although a report describing work like Konigsberg's can give something of the sense of high adventure experienced by scientists within the Institution and elsewhere, there are dimensions to the scientific life of today that must always escape any progress report. Most of those who work within the Institution share a deep conviction about the humanity of their calling and about the community of fellowship that not only is vital to the progress of their work but also is a deeply felt reward in itself. Happily these convictions occasionally shine through more esoteric daily concerns. They are notable this year in the comments of James Ebert and Merle Tuve, each on a point of his philosophy.

Ebert has written particularly of his own deep attachment to the essential unity of living science. He quotes Frank R. Lillie's memorable words that "Scientific discovery is a truly epigenetic process in which the germs of thought develop in the total environment of knowledge." The life of the laboratory, where one must be quick to acknowledge what has gone before, alert to the current actions of others having similar interests, and mindful of the needs for others to know, can be a social experience almost beyond comparison. Dr. Ebert notes his pleasure at having visiting investigators from other institutions: "They do contribute vitally to the Department . . . but of far greater moment is the question whether such a visit adds measurably to the man's ability as an investigator and teacher when he returns to his home laboratory. Has he found new direction or meaning for his research? Has the opportunity for reflection. . . led to a searching reexamination of his program?" With pride, the Institution can record that its Departments provided literally hundreds of such opportunities during the year.

Tuve's comments touch upon the aesthetic experience of being a scientist, and upon what is perhaps one of the deepest motivations in "exact" science. Contrasting it with the disorder and transience he sees in the life of men in the mass, he expresses his admiration at "the beautiful regularity and systematic relatedness. . . in every aspect of the natural phenomena. . . from distant stars to living bacteria." He considers this a cause of the sense of very deep satisfaction in scientific studies. Through science, man, bit by bit,

is adding to his stature and to "his awe of the stupendous and beautifully intricate universe in which he finds himself." Tuve considers it a "great good fortune" for scientists to be able to devote their energy and talents to illuminating "the intricate and orderly patterns of the physical world around us." To him this is "a princely gift of our time and circumstances."

Such motivations lead to a dedication which is the wonder of all who have not experienced their attractions. It is a dedication measured only in part by a voluntary 70-hour week, by long nights on a mountaintop in below-freezing weather, by a hundred frustrations with equipment design, or by a willingness to work at the modest salaries that fundamental science is able to provide. We can hope that Andrew Carnegie, after sixty years, might be approving of both the dedication and the insights of these men and their predecessors as they have striven "to secure, if possible, for the United States of America leadership in the domain of discovery. . . of new forces."[59]

Losses . . .

I must report with great sorrow the loss of a devoted member of the Board of Trustees, the Honorable Robert Woods Bliss, and of a highly valued Staff Member of the Mount Wilson and Palomar Observatories, Don O. Hendrix.

Robert Woods Bliss, a Trustee of the Institution for twenty-six years, died in Washington, D. C., on April 19, 1962. Elected a Trustee in 1936, he became a member of the Executive Committee the following year. He was Secretary of the Board of Trustees from 1953 until his death. He also served continuously from 1939 on the Committee on Archaeology, from 1939 to 1945 on the Auditing Committee, and from 1950 to 1953 and 1958 to 1961 on the Nominating Committee.

Before his association with the Institution he had already had a distinguished career of 33 years in the diplomatic corps of the United States, where he held many important posts. He was especially concerned with efforts to bring about world security through arms control and international organization. In 1908 he was United States delegate to the International Conference to Consider Measures for the Revision of Arms and Ammunition Regulations in Brussels. As counselor to our embassy in Paris from 1916 to 1919 he assisted in preparations for the Versailles Peace Conference and in its work. Again, in 1921, he was a member of the United States delegation to the Washington Conference on the Limitation of Armaments. His beautiful estate, Dumbarton Oaks, in Washington, was the scene of the conference that laid plans for the United Nations.

[59] Andrew Carnegie, Trust Deed Creating a Trust for the benefit of the Carnegie Institution of Washington, D. C., January 28, 1902.

Just before his retirement in 1933 he had served for six years as ambassador to Argentina; and during World War II he was called back from his technical retirement to serve as special consultant and then special assistant to the Secretary of State.

Mr. Bliss will be remembered by the Washington community for his many philanthropic and cultural contributions. The Institution will remember his dedication to its welfare and his gentle but always penetrating counsel on every problem.

Another loss that is especially felt is that of Don O. Hendrix of the Mount Wilson and Palomar Observatories, who died on December 26, 1961, at the age of 57. Joining the staff of the Observatories in 1913, Hendrix became Superintendent of its optical shop in 1947, where he carried out such important projects as the optical design for the 48-inch schmidt telescope and the final figuring of the 200-inch mirror after it had been moved to Palomar. His extraordinary skill was largely responsible for the high efficiency of the present equipment of the Observatories.

With keen regret I also record the loss to the Institution of four retiring members of the staff. Dr. Berwind P. Kaufmann, Director of the Department of Genetics, Dr. Robert K. Burns, Staff Member of the Department of Embryology, Mrs. Ruth L. McCollum, Assistant to the President, and Wilbur A. Pestell, Administrative Assistant at the Department of Plant Biology, all retired on June 30, 1962.

Dr. Kaufmann came to the Department of Genetics in 1937 from the University of Alabama, where he had served for ten years as professor and department head. Since that time his professional interests have touched on many facets of the broad field of cytogenetics, with emphasis on the varying patterns of chromosome structure that influence gene action. These interests stemmed from experience in the area of descriptive cytology, gained in the early 1920's, when chromosomes were generally regarded as uniformly staining rod-shaped structures with no discernibly precise pattern of internal organization. By developing and applying ingenious techniques, Dr. Kaufmann demonstrated that chromosomes contain paired, helically disposed strands at all phases of somatic and meiotic mitoses.

Upon joining the Institution's staff, Dr. Kaufmann undertook an analysis of the types and frequencies of chromosomal rearrangements induced by ionizing radiations, using the giant chromosomes in the salivary glands of *Drosophila* for diagnostic purposes. His discovery and evaluation of the effects of near-infrared radiation on the frequencies of X-ray-induced rearrangements was an outstanding accomplishment of that period.

In 1960 Dr. Kaufmann succeeded Dr. M. Demerec, first as Acting Director and in 1961 as Director of the Department. During his twenty-five

years at Cold Spring Harbor he maintained a strong interest in science education and in the training of young biologists. He has now returned to a university environment, having been appointed Professor of Zoology and Senior Research Scientist at the University of Michigan, where his sincerity, dedication, and technical skill will be inspiring to those who have the good fortune to work with him.

Dr. Burns joined the Department of Embryology in 1940 from the University of Rochester, where he had been a member of the Department of Anatomy, of which Dr. George W. Corner was the head before his own move to the Institution. When he went to Baltimore, Dr. Burns rejoined Dr. Corner and another long-time Rochester colleague, B. H. Willier, who had assumed the direction of the Johns Hopkins Department of Biology. Burns, who held the title of Honorary Professor of Biology at the University, served as an important link between the two departments, pointing the way to the close association that exists today.

Dr. Burns has devoted his entire career to studying the mechanisms of sex differentiation. A student of Ross G. Harrison, he began by demonstrating sex reversal in amphibians, using the technique of embryonic parabiosis. His was the first convincing laboratory research following up Frank R. Lillie's analysis of the freemartin.[60] Later he turned his attention to mammals, and again produced the first convincing evidence of sex reversal by the use of purified sex hormones in his analysis of the effects of estradiol on the prospective male opossum.

Dr. Burns has returned to Bridgewater College, where he received his first degree. He is teaching embryology and continuing his research on sex differentiation.

A loss most keenly felt by the President and the Office of Administration was the retirement of Mrs. Ruth McCollum, Administrative Assistant to the President. Mrs. McCollum joined the Institution staff in the administrative office of the Department of Terrestrial Magnetism in 1942, where she gave distinguished assistance during the difficult period of the war. In 1946 she transferred to the Bursar's office in the Office of Administration, where she served for thirteen years, first as secretary to the Bursar and then as Accountant. During the latter part of this period Mrs. McCollum contributed part of her time and skill to general responsibilities of the Office of Administration. Early in 1959 she became Administrative Assistant to the President. Her management of arrangements for the Annual Meeting of the Board of Trustees was always a model of organization and good taste. Her artistic talent appeared in many ways in her work, much to the Institution's advantage, as in the annual departmental exhibits. No problem was too difficult to tax her good humor, and long hours only increased her devotion

[60] A modified female of bovine heterosexual twins.

to the Institution. Her many talents and fine spirit are much missed by all who worked with her.

Wilbur A. Pestell, Administrative Assistant at the Department of Plant Biology, also retired on June 30, 1962. He was actively associated with the Institution for 42 years, a period of dedicated service seldom equaled by past employees. He worked first in the Division of Publications in Washington, subsequently at the Desert Botanical Laboratory near Tucson, then at the Coastal Laboratory at Carmel, California, and finally as Secretary in the Department of Plant Biology at Stanford. His faithful work cleared routine tasks from the way of many others whose scientific results have been reported in these Year Books.

. . . *and Changes* . . .

The year 1962, in addition to signalizing the sixtieth anniversary of the Institution, also marked a significant change in its internal organization. Upon the retirement of Dr. Berwind P. Kaufmann, the fourth Director of the Department of Genetics, on June 30, 1962, the status of genetics research within the Institution was altered. As of July 1, the Department of Genetics became the Genetics Research Unit, with Alfred D. Hershey as Director. The work of the Unit will center on the research of Hershey, Barbara McClintock, and their associates at Cold Spring Harbor. In September 1962, Helen Gay, another Staff Member of the Unit, transferred her work to Ann Arbor, Michigan, where she will continue her association with Dr. Kaufmann.

The Department of Genetics was formed in 1921 from a merger of the former Department for Experimental Evolution and the Eugenics Records Office. The Department for Experimental Evolution, which was formed in 1906, had been preceded by the Station for Experimental Evolution, established at the present site in Cold Spring Harbor, New York, during 1904. The dominant traits of the Department of Genetics were clearly those it inherited from the Department for Experimental Evolution. For fifty-eight years the research groups that successively made Cold Spring Harbor their scientific home maintained a research tradition which in many ways has been the story of genetics progress in the United States. Originally conceived by its first Director, C. B. Davenport, and inspired by the preceding work of Hugo de Vries in the Netherlands, the Cold Spring Harbor laboratory has had an uncanny record of association with and stimulation of the main currents in genetic thought during the more than half century of its existence. Even twenty years ago Milislav Demerec, on the eve of his becoming the third Director of the Department, could say that the "backing

given to genetical research by the Institution undoubtedly accounts to a large degree for the fact that the United States now occupies the leading position in this branch of science."[61]

The Station's first work followed Dr. Davenport's lead. He had a consuming ambition to prove experimentally the broad application of Mendel's law as rediscovered in 1900 by de Vries in the Netherlands, Correns in Germany, and von Tschermak-Seysenegg in Austria. Davenport's early work on poultry, birds, and mammals did actually furnish classic experimental confirmation of the broad application of Mendelian inheritance.

Davenport's student and later colleague G. H. Shull provided one of the most unusual chapters in the Laboratory's history by laying the theoretical foundations of hybrid corn cultivation, described by Mangelsdorf as "the most far-reaching development in applied biology of this quarter century."[62] Shull's recognition and exploitation of heterosis (hybrid vigor), which he named, gave the basic principle "which underlies almost the entire hybrid corn enterprise."[63] More recently the same plant in the hands of Barbara McClintock has been a highly successful medium for discovering the mutational behavior of genes. Both may certainly be counted among the most significant achievements in genetics.

Illustrative of the range of interests to be found in the work of the Laboratory through its fifty-eight years of history are the pioneering experimental studies of C. C. Little on the inheritance of tumors, followed later by E. C. MacDowell's and J. S. Potter's studies of mouse leukemia; the foundation of cytogenetics by John Belling, followed by the productive cytogenetic research undertaken on *Datura* (of the potato family) by A. F. Blakeslee, the second Director of the Department, and his colleagues, and succeeded more recently by B. P. Kaufmann's cytogenetic studies on *Drosophila;* the painstaking studies of Milislav Demerec, mapping the gene loci of *Escherichia coli* and *Salmonella;* and the work of A. D. Hershey, exploring the molecular structure of the bacterial phage chromosome.

Through the years the Department has been no less favored by geneticists who have been associated with it on a part-time basis. Among the Research Associates and Guest Investigators who were connected with the Department at one time or another in its history were: W. E. Castle, E. B. Wilson, C. B. Bridges, H. E. Crampton, E. B. Badcock, L. C. Dunn, Th. Dobzhansky, M. Delbrück, A. Hollaender, D. G. Catcheside, M. Westergaard, C. Stern, and S. Brenner.

The Institution will continue support of genetics research, although at a

[61] *Carnegie Institution of Washington Year Book 41*, p. 170.

[62] Paul C. Mangelsdorf, "Hybrid corn: its genetic basis and its significance in human affairs," in *Genetics in the Twentieth Century*, edited by L. C. Dunn, The Macmillan Company, New York, 1951, p. 555.

[63] *Ibid.*, p. 653.

reduced scale by comparison with the Department's peak staff. The Genetics Research Unit will remain at Cold Spring Harbor. It is hoped that it will be joined by an interuniversity-sponsored research group investigating quantitative biology, the formation of which was being explored at the year's end.

. . . *and Gains*

Two new members were elected to the Board of Trustees of the Institution on May 11, 1962: William Walden Rubey and Carl Joyce Gilbert.

Dr. Rubey is one of the country's most distinguished geologists. From 1920 until 1960 he was associated with the United States Geological Survey, where his work received signal recognition in the Award of Excellence of the Department of the Interior in 1943 and the Distinguished Service award in 1950. His contributions have added significantly to scientific understanding in several fields of geology, notably in knowledge of the original formation of the oceans, the transport of particles and sediments by running water, and the mechanics of very large overthrust faults. He is a graduate of the University of Missouri and holds honorary doctoral degrees both from that University and from Yale. At present he is serving as a member of the National Science Board (National Science Foundation) and of the board of directors of the American Association for the Advancement of Science.

Mr. Gilbert is Chairman of the Board, Gillette Company, Boston. He is a graduate of the University of Virginia and the Harvard Law School. He is a member of the board of directors of several corporations, including the Raytheon Manufacturing Company, the Fiduciary Trust Company, and the Pepperell Manufacturing Company. Devoted to public service as well as to business, Mr. Gilbert is a member of the board of managers and past president of the Boston Dispensary, vice-chairman of the Massachusetts Port Authority, trustee of the New England Center Hospital, member of the administrative board of the New England Medical Center, vice-president of the New England Council, and trustee and member of the executive committee of Tufts College. Before he became chairman of the board of the Gillette Company in 1958 he had served as its president.

It is always a special pleasure to record the honors that have come to members of the Institution.

Presentation of the Kettering award for 1961 to Dr. Vannevar Bush, retired President of the Institution, was made at a conference in Washington, D. C., of the Patent, Trademark, and Copyright Foundation of George Washington University for outstanding work in the field of patents, trademarks, and related areas.

At the Mount Wilson and Palomar Observatories, Ira S. Bowen, the Director, was elected a member of the Royal Society of Sciences of Uppsala, Sweden. The Newcomb Cleveland prize of $1000 was awarded to Halton C. Arp, Staff Member, on December 29, 1961, by the American Association for the Advancement of Science for "a noteworthy paper, representing an outstanding contribution in science." Robert P. Kraft, Staff Member, received the Helen B. Warner prize of the American Astronomical Society for outstanding research by a young member of the Society. Guido Münch and Allan R. Sandage, Staff Members, were elected fellows of the American Academy of Arts and Sciences. Fritz Zwicky, Staff Member, was elected a member of the International Academy of Astronautics. This organization, which is only a year old, is the first international academy of scientists and engineers who have made contributions to space technology. It is limited to 165 active members in the life sciences, basic sciences, and engineering.

At the Geophysical Laboratory, Philip H. Abelson, the Director, received on April 6, 1962, the Washington State University Regents' Distinguished Alumnus award for the academic year 1961–1962.

Scott E. Forbush, Staff Member of the Department of Terrestrial Magnetism, was elected to membership in the National Academy of Sciences, April 24, 1962, and on June 14, 1962, he received an honorary doctor of science degree from Case Institute of Technology, Cleveland, Ohio, for his contributions to our understanding of cosmic-ray phenomena.

At the Department of Plant Biology, Jens Clausen, retired Staff Member, was made a Knight of the Order of Dannebrog by the King of Denmark in recognition of his contributions to botany and genetics. The Danish consul presented the decoration to Dr. Clausen on October 13, 1961, in San Francisco.

M. Demerec, retired Director of the Department of Genetics, was awarded the Kimber Genetics medal of the National Academy of Sciences on April 24, 1962, "in recognition of his many contributions to the understanding of the genetics of various plants, *Drosophila*, bacteria, and viruses, and especially for his leadership in the investigation of unstable genes, the mutation process, genetics of micro-organisms and the genetic fine structure of the gene."

J. E. S. Thompson, retired Staff Member of the Department of Archaeology, received the honorary degree of doctor of humane letters and the Drexel Medal for Archaeology from the University of Pennsylvania in February 1962.

Scientists and Scholars, 1902 - 1962

In essence, the whole quality of the Institution, and its history, lie with those who have been associated with it over the years. Following are the names of the senior scientific staff members of all departments of the Institution over the last fifteen years (or over the last fifteen years of the existence of terminated departments). Below them in each department are listed the names of eminent and representative scientists and scholars who have been members of the staff or otherwise affiliated with the Institution since it was founded in 1902. A list is also given of all Fellows of the Carnegie Institution of Washington since the beginning of its Fellowship Program in 1947, and a list of grantees and others affiliated with the Institution but not with any particular department.

DEPARTMENT OF PLANT BIOLOGY

Desert Laboratory, opened in 1903, became headquarters of Department of Botanical Research in 1905; name changed to Laboratory for Plant Physiology in 1923; reorganized in 1928 as Division of Plant Biology, including ecology; name changed to Department of Plant Biology in 1951.

Directors

Daniel T. MacDougal, 1906–1927

Herman A. Spoehr, 1927–September 1930, September 1931–1947 (*Chairman*); 1947–1950 (*Chairman Emeritus*)

C. Stacy French, 1947—

Staff Members

Jeanette S. Brown, 1958—
Jens C. Clausen, 1931–1956
David C. Fork, 1961—
Paul Grun, 1949–1954
William M. Hiesey, 1926—
David C. Keck, 1928–1951
Donald W. Kupke, 1955–1956
Harold W. Milner, 1927—
Malcolm A. Nobs, 1939–1941, 1951—
James H. C. Smith, 1925–1961
Harold H. Strain, 1927–1962
Ellen C. Weaver, 1961–1962
Violet (Koski) Young, 1949–1953

John Belling, 1921–1933
William A. Cannon, 1903–1924
Frederic E. Clements, 1917–1941
Waldo S. Glock, 1931–1938
Harvey M. Hall, 1918–1932
Garrett J. Hardin, 1942–1946
Burton E. Livingston, 1906–1909
Francis E. Lloyd, 1906
Winston M. Manning, 1941–1946
Forrest Shreve, 1908–1945
Godfrey G. Sykes, 1906–1929

Other Scientists and Scholars Associated with the Department

Leroy R. Abrams, 1932
(Stanford University)
Ernest Anderson, *Research Associate* 1932–1936 (University of Arizona)
William A. Arnold, *Research Associate* 1956–1961
(Oak Ridge National Laboratory)
Eric Ashby, 1930
(Clare College, Cambridge University)
Daniel I. Axelrod, 1937, 1939, 1944, 1950, 1959
(University of California)
Ernest B. Babcock, *Research Associate* 1926–1945 (University of California)
Irving W. Bailey, *Research Associate* 1928–1930, 1932–1939
(Harvard University)
Charles E. Bessey, 1914
(University of Nebraska)
Nathaniel L. Britton, *Research Associate* 1902, 1912–1916, 1918–1922
(New York Botanical Garden)
Ursula Brodführer, 1956
(University of Munich)
Douglas H. Campbell, 1911
(Stanford University)
Ralph W. Chaney, *Research Associate* 1923–1956
(University of California, Berkeley)
William S. Cooper, 1919–1925
(University of Minnesota)
Frederick V. Coville, 1902–1905
(U. S. Department of Agriculture; later, U. S. National Museum)
Pierre Dansereau, 1949
(University of Montreal; later, New York Botanical Garden)

John P. Decker, 1957
(U. S. Forest Service)
Lee R. Dice, *Research Associate*
1929–1930, 1932–1934–1938
(University of Michigan)
Erling Dorf, 1930, 1936, 1942
(Princeton University)
A. E. Douglass, *Research Associate*
1924–1938 (University of Arizona)
Newton B. Drury, *Research Associate*
1937–1942
(California State Parks Commission)
Benjamin M. Duggar, *Research Associate*
1920–1921 (Missouri Botanical Garden;
later, University of Wisconsin)
Friedrich Ehrendorfer, 1951–1952
(University of Vienna)
Robert Emerson, *Research Associate*
1937–1941
(California Institute of Technology)
G. E. Erdtmann, 1930
(University of Stockholm)
William G. Farlow, 1905
(Harvard University)
Edward E. Free, *Research Associate*, 1920
(U. S. Department of Agriculture)
Martin Gibbs, 1962 (Cornell University)
John W. E. Glattfeld, *Research Associate*
1920–1921 (University of Chicago)
Richard H. Goodwin, 1950
(Connecticut College)
Verne E. Grant, 1949–1950
(Rancho Santa Ana Botanic Garden)
Helen M. Habermann, 1959
(Goucher College)
Per Halldal, 1955–1957 (University of Oslo)
Francis T. Haxo, 1957
(Scripps Institution of Oceanography)
Robert Hill, 1952 (Cambridge University)
A. Stanley Holt, 1959
(National Research Council of Canada)
Ellsworth Huntington, *Research Associate in Geology*, 1903–1904, 1910–1912, 1915–1917,
1922–1923 (Yale University)
Donald A. Johansen, 1931–1932
(private research)
Ivan M. Johnston, 1942 (Harvard University)
Erik G. Jørgensen, 1959
(Royal Danish School of Pharmacy)
Robert W. Krauss, 1951–1955
(University of Maryland)
Elias Landolt, 1953–1955
(Swiss Federal Institute of Technology)
Charlton M. Lewis, *Research Associate*
1938–1941 (Patent Agent,
Barkelow and Lewis, Pasadena)
Harlan Lewis, 1954–1955
(University of California, Los Angeles)
Esmond R. Long, 1914–1915
(University of Chicago; later, Henry Phipps
Institute, University of Pennsylvania)
John M. Macfarlane, 1902
(University of Pennsylvania)
Axel Madsen, 1962 (Royal Veterinary
and Agricultural College, Copenhagen)
Herbert L. Mason, 1925
(University of California)
Max Milner, 1957 (UN Children's Fund,
Food Conservation Division)
George T. Moore, 1914
(Missouri Botanical Garden, St. Louis)
Vladimir Moravek, *Research Associate*, 1926
(University of Brno, Czechoslovakia)
Jack E. Myers, 1950–1951, 1959
(University of Texas)
Hedda Nordenshiöld, 1949
(Royal Agricultural College, Uppsala)
Axel Nygren, 1950
(Royal Agricultural College, Uppsala)
Winthrop J. V. Osterhout, *Research Associate*
1922–1924 (Harvard University; later,
Rockefeller Institute for Medical Research)
James B. Overton, *Research Associate*
1903, 1926–1927 (University of Wisconsin)
George J. Peirce, 1910–1912
(Stanford University)
Gifford Pinchot, 1902
(U. S. Department of Agriculture; later,
Yale University and Governor of
Pennsylvania)
Thomas R. Pray, 1960–1961
(University of Southern California)
Joseph N. Rose, *Research Associate*
1908, 1910–1923 (U. S. National Museum)
Gilbert M. Smith, *Research Associate*
1926–1927 (Stanford University)
Roger Y. Stanier, 1959
(University of California, Berkeley)
G. Ledyard Stebbins, Jr., 1934–1936, 1945
(University of California, Davis)
Bernard Strehler, 1955 (National Heart
Institute, Baltimore City Hospital)
Walter T. Swingle, 1904
(U. S. Department of Agriculture)
Hiroshi Tamiya, 1952–1953 (Tokugawa
Institute for Biological Research)

Edwin W. Tisdale, 1959 (University of Idaho)
Sam F. Trelease, 1914 (Columbia University)
Vladimir Úlehla, *Research Associate*, 1924 (University of Brno, Czechoslovakia)
Cornelius B. Van Niel, 1931–1932 (Stanford University)
Chakrauarti S. Venkatesh, 1955–1956 (Forest Research Institute, India)
Wolf Vishniac, 1957 (Yale University; later, University of Rochester)
Diter von Wettstein, 1959 (University of Copenhagen)
Heinrich Walter, 1929 (University of Stuttgart)
John E. Weaver, *Research Associate* 1922–1930 (University of Nebraska)
George R. Wieland, *Research Associate* 1903–1934, 1941 (Yale University)
Ira L. Wiggins, *Research Associate* 1932–1933, 1936 (Stanford University)
Paul C. Wilbur, 1926–1927 (Food Machinery and Chemical Corporation, San Jose)
S. W. Williston, 1904 (University of Chicago)
Frederick T. Wolf, 1960 (Vanderbilt University)

MOUNT WILSON AND PALOMAR OBSERVATORIES

Mount Wilson Observatory organized in 1904; unified operation with the Palomar Observatory of the California Institute of Technology began in 1948.

Directors

George E. Hale, 1904–1923; 1923–1936 (Honorary)
Walter S. Adams, 1924–1945
Ira S. Bowen, 1946—

Staff Members

Halton C. Arp, 1957—
Walter Baade, 1931–1958
Harold D. Babcock, 1909–1948
Horace W. Babcock, 1946—
William A. Baum, 1950—
Arthur D. Code, 1956–1958
Armin J. Deutsch, 1951—
Olin Eggen, 1961—
Jesse L. Greenstein, 1948—
Robert F. Howard, 1961—
Fred Hoyle, 1957–1962
Edwin P. Hubble, 1919–1953
Milton L. Humason, 1917–1957
Alfred H. Joy, 1915–1948
Robert B. King, 1938–1948
Robert P. Kraft, 1960—
Paul W. Merrill, 1919–1952

J. A. Anderson, 1916–1943
Theodore Dunham, Jr., 1930–1947
Arthur S. King, 1908–1943
F. G. Pease, 1904–1938
G. W. Ritchey, 1905–1919
Charles E. St. John, 1908–1930

Rudolph L. Minkowski, 1937–1960
Guido Münch, 1951—
Seth B. Nicholson, 1915–1957
J. Beverley Oke, 1958—
Donald E. Osterbrock, 1953–1958
Edison Pettit, 1920–1955
Alexander Pogo, 1950–1959
Robert S. Richardson, 1931–1958
Allan R. Sandage, 1952—
Roscoe F. Sanford, 1918–1949
Maarten Schmidt, 1959—
Otto Struve, 1962—
Henrietta H. Swope, 1952—
Albert G. Wilson, 1948–1953
Olin C. Wilson, 1931—
Ralph E. Wilson, 1938–1951
Fritz Zwicky, 1925—

Frederick H. Seares, 1909–1940
Harlow Shapley, 1914–1921
Sinclair Smith, 1923–1938
Gustaf Stromberg, 1917–1946
Adrian van Maanan, 1912–1946

Other Scientists and Scholars Associated with the Department

Charles G. Abbot, 1909–1948
(Smithsonian Institution)
Giorgio Abetti, 1909, 1930
(Observatorio di Arcetri)
Lawrence Aller, 1946–1961
(University of Indiana; later, University of Michigan and University of California, Los Angeles)
Edward E. Barnard, 1904–1905, 1912
(Yerkes Observatory)
W. Becker, 1962 (University of Basel)
Dirk Brouwer, *Research Associate*
1940–1944 (Yale University)
John A. Carroll, 1924 (Cambridge University)
William de Sitter, 1932
(Observatory of Leiden)
Albert Einstein, 1933 (Preussische Akademie der Wissenschaft, Berlin)
E. Freundlich, 1926
(Astrophysical Observatory, Potsdam)
Henry G. Gale, *Research Associate*
1909–1911 (University of Chicago)
Leo Goldberg, 1940
(Harvard College Observatory)
Guillermo Haro, 1958
(Tonantzintla Observatory)
George Herbig, 1948, 1950, 1954
(Lick Observatory)
Ejnar Hertzsprung, 1912
(Potsdam Observatory)
Erik Holmberg, 1940–1941, 1947, 1951, 1955
(Lund Observatory)
Sir James Hopwood Jeans, *Research Associate*
1922–1947 (Royal Society of London)
Jacobus C. Kapteyn, *Research Associate*
1908–1922 (University of Groningen)
Philip C. Keenan, 1953–1962
(Perkins Observatory)
Gerard P. Kuiper, 1942, 1950, 1954
(Yerkes Observatory; later, University of Arizona)
Robert B. Leighton, 1951–1962
(California Institute of Technology)
Abbe Le Maitre, 1933 (University of Louvain)
A. O. Leuschner, *Research Associate*
1906–1907, 1924 (University of California)
Bertil Lindblad, 1920–1921, 1950
(Stockholm Observatory)
Knut Lundmark, 1922–1923, 1930, 1933, 1938
(University of Uppsala)
W. J. Luyten, 1951, 1959
(University of Minnesota)
Robert R. McMath, 1950–1960
(McMath-Hulbert Observatory)
N. U. Mayall, 1951 (Lick Observatory; later, Kitt Peak National Observatory)
A. A. Michelson, *Research Associate*
1903–1904, 1919–1931
(University of Chicago)
Dayton C. Miller, 1921
(Case School of Applied Science)
S. A. Mitchell, *Research Associate*
1924–1927, 1934–1944
(University of Virginia)
W. W. Morgan, 1957–1962
(Yerkes Observatory)
Earnest F. Nichols, *Research Associate*
1908–1909 (Dartmouth College; later, Yale University and Massachusetts Institute of Technology)
Y. Öhman, 1934 (University of Uppsala)
Jan H. Oort, *Research Associate*
1924, 1939, 1952, 1958–1959, 1961
(Leiden Observatory)
P. Th. Oosterhoff, 1934, 1960
(Leiden Observatory)
L. Perek, 1959 (Astronomical Institute of Czechoslovak Academy of Sciences)
L. Plaut, 1956–1959
(Kapteyn Astronomical Laboratory)
Frank E. Ross, 1903–1909, 1927–1939
(Yerkes Observatory)
S. Rosseland, 1925–1927
(International Research Fellow; later, University of Oslo)
Henry N. Russell, *Research Associate*
1903–1905, 1921–1947
(Princeton University)
Edwin E. Salpeter, *Research Associate*, 1959
(Cornell University)
Jan Schilt, 1925–1926 (International Research Fellow; later, Yale University and Rutherfurd Observatory, Columbia University)
Martin Schwarzschild, 1946–1954
(Princeton University)
C. D. Shane, 1929–1930
(University of California)
Frederick Slocum, 1933–1934
(Van Vleck Observatory)
Lyman Spitzer, Jr., 1937–1940, 1948–1959
(Princeton University)
Joel Stebbins, *Research Associate*
1930, 1932–1948 (University of Wisconsin)

Carl Störmer, *Research Associate,* 1912–1915 (University of Christiania)
Bengt Strömgren, 1950, 1960 (Institute for Advanced Study)
Pol Swings, *Research Associate* 1944–1946, 1958–1959 (University of Liége)
A. D. Thackeray, 1935–1936 (Commonwealth Fellow; later, Radcliffe Observatory)
Albrecht Unsöld, 1929, 1957, 1961 (University of Kiel)
H. C. Van de Hulst, 1954 (Leiden Observatory)
Albert E. Whitford, 1933–1957 (University of Wisconsin; later, Lick Observatory)
Rupert Wildt, 1935–1936 (National Research Fellow; later, Yale University)
R. v. d. R. Woolley, *Research Associate* 1929–1931, 1958–1959, 1961 (Royal Greenwich Observatory)

DEPARTMENT OF TERRESTRIAL MAGNETISM

Organized as the Department of International Research in Terrestrial Magnetism on April 1, 1904. Name changed to Department of Terrestrial Magnetism in 1905.

Directors

Louis A. Bauer, 1904–1929
John A. Fleming, 1929–1934 (Acting); 1935–1946
Merle A. Tuve, 1946—

Staff Members

Philip H. Abelson, 1939–1953
L. T. Aldrich, 1950—
Lloyd V. Berkner, 1933–1951
Ellis T. Bolton, 1951—
Roy J. Britten, 1951—
Bernard F. Burke, 1953—
Dean B. Cowie, 1944—
John W. Firor, Jr., 1953–1961
Scott E. Forbush, 1927—
W. Kent Ford, Jr., 1957—
Oliver H. Gish, 1922–1948
John W. Graham, 1951–1958
Stanley R. Hart, 1961—
Norman P. Heydenburg, 1935—
Ellis A. Johnson, 1935–1956
Brian J. McCarthy, 1960—
W. C. Parkinson, 1913–1950
Richard B. Roberts, 1937—
William J. Rooney, 1924–1949
T. Jefferson Smith, 1962—
John S. Steinhart, 1961—
Howard E. Tatel, 1947–1957
Georges M. Temmer, 1953—
George R. Tilton, 1951–1956
Oscar W. Torreson, 1923–1952
Ernest H. Vestine, 1938–1957
George R. Wait, 1920–1951
Harry W. Wells, 1932—
George W. Wetherill, 1954–1960

J. P. Ault, 1905–1929
S. J. Barnett, 1917–1926
E. H. Bramhall, 1941–1944
G. Breit, 1924–1929
O. Dahl, 1926–1936
F. T. Davies, 1929–1939
H. M. W. Edmonds, 1906–1930
H. W. Fisk, 1905–1932
Lawrence R. Hafstad, 1928–1946
James A. Van Allen, 1939–1941

Other Scientists and Scholars Associated with the Department

Samuel J. McIntosh Allen, *Research Associate* 1924–1928 (University of Cincinnati)
E. Amaldi, 1936–1937 (Royal University of Rome)
J. Bartels, *Research Associate*, 1930–1940 (Fortsliche Hochschule, Eberswalde, Germany; later, Geophysikalisches Institut, Göttingen, Germany)
Carl Barus, *Research Associate* 1902, 1904–1923, 1926 (Brown University)
Jesse W. Beams, 1934–1935 (University of Virginia)
J. C. Beattie, *Research Associate*, 1908–1911 (South Africa College, Cape Town)
Ralph D. Bennett, *Research Associate* 1933–1940 (Massachusetts Institute of Technology; later, Vallecitos Atomic Laboratory, Pleasanton, California)
Hans A. Bethe, 1936–1941 (Cornell University)
Henry G. Booker, *Research Associate* 1938–1940 (Cornell University)
Edward L. Bowles, *Research Associate* 1939–1945 (Massachusetts Institute of Technology)
Joseph C. Boyce, *Research Associate*, 1939–1950 (Massachusetts Institute of Technology; later, New York University and Illinois Institute of Technology)
Robert B. Brode, *Research Associate* 1939–1941 (University of California, Berkeley)
Richard E. Byrd, 1931–1932 (U. S. Navy, Arctic explorer)
Sydney Chapman, *Research Associate* 1934–1940, 1951–1953 (Trinity College, Cambridge; later, Imperial College, London; High Altitude Observatory, Boulder, Colorado, and Geophysical Institute, College, Alaska)
Georges N. Cohen, *Research Associate* 1956–1959 (Institut Pasteur, Paris)
Arthur H. Compton, *Research Associate* 1931–1945 (University of Chicago; later, Washington University)
Karl T. Compton, *Research Associate* 1928–1934 (Princeton University; later, Massachusetts Institute of Technology)
T. G. Cowling, 1950–1951 (Princeton University)
Hugh H. Darby, *Research Associate* 1948–1950 (consultant in biochemistry, Mt. Airy, Maryland)
N. Ernest Dorsey, *Research Associate* 1912–1913 (National Bureau of Standards)
George Gamow, *Research Associate*, 1935–1944 (George Washington University; later, University of Colorado)
Enrique Gaviola, *Research Associate* 1928–1929 (Comisión de Astrofísica y Radioastronomía, Universidad de Buenos Aires)
Ross Gunn, *Research Associate*, 1938–1944 (Naval Research Laboratory; later, U. S. Weather Bureau and American University)
Anton L. Hales, *Research Associate*, 1960 (University of the Witwatersrand; later, Graduate Research Center, Inc., Dallas, Texas)
John S. Hall, 1954— (Lowell Observatory, Flagstaff, Arizona)
Raymond G. Herb, 1935 (University of Wisconsin)
Victor F. Hess, *Research Associate*, 1940–1946 (Fordham University)
Thomas H. Johnson, *Research Associate* 1933–1946 (Bartol Research Foundation; later, Brookhaven National Laboratory, Atomic Energy Commission, and Raytheon Manufacturing Company)
Arthur E. Kennelly, *Research Associate*, 1924–1935 (Harvard University and Massachusetts Institute of Technology)
Serge A. Korff, *Research Associate*, 1936–1945 (New York University)
D. la Cour, 1931–1932 (Danish Meteorological Survey, Copenhagen)
H. A. Lorentz, *Research Associate*, 1920 (University of Leiden)
Frank T. McClure, *Research Associate* 1955–1960 (Applied Physics Laboratory, Johns Hopkins University)
J. D. McGee, *Research Associate*, 1959–1960 (Imperial College)
Kenneth R. McQuillen, *Research Associate* 1951–1960 (University of Cambridge)
Robert A. Millikan, *Research Associate* 1921–1945 (California Institute of Technology)

B. Y. Mills, 1953–1954
(Radiophysics Laboratory, Commonwealth Industrial and Research Organization, Sydney, Australia)
S. K. Mitra, 1936–1937
(University of Calcutta)
T. Nagata, 1950–1951
(Geophysical Institute, Tokyo)
J. L. Pawsey, 1957–1958 (Commonwealth Industrial and Research Organization, Australia)
Greenleaf W. Pickard, *Research Associate* 1927–1935 (consultant in electrical engineering, Newton Centre, Massachusetts)
Wilson M. Powell, *Research Associate* 1942–1943 (Lawrence Radiation Laboratory, University of California)
A. T. Price, 1952
(The Royal Technical College, Glasgow)
Norman Ramsey, 1938–1939
(Harvard University)
J. A. Ratcliffe, 1950–1951
(Cavendish Laboratory, Cambridge)
Bruno Rossi, 1932–1933
(Massachusetts Institute of Technology)
Sir Arthur Rucker, 1904–1915
(Royal College of Science, South Kensington, London)
M. N. Saha, 1936–1937
(Allahabad University, India)
Marcel Schein, 1939–1944 (University of Chicago; later, University of California)
A. Schmidt, 1905–1907
(Potsdam Magnetic Observatory)
B. F. J. Schonland, 1952
(University of the Witwatersrand)
Frederick Slocum, *Research Associate*, 1920
(Brown University; later, Wesleyan University)
F. Graham Smith, 1952–1954
(Cavendish Laboratory, Cambridge)
J. C. Street, *Research Associate*, 1933–1934
(Harvard University)
H. U. Sverdrup, *Research Associate*, 1926–1939
(Geophysical Institute, Bergen)
W. F. G. Swann, 1916–1920
(Bartol Research Foundation)
John T. Tate, *Research Associate*, 1941–1945
(University of Minnesota)
Edward Teller, 1936–1937 (University of Chicago; later, Lawrence Radiation Laboratory, University of California)
Manuel S. Vallarta, *Research Associate* 1940–1941, 1948–1950
(National University of Mexico)
John von Neumann, 1948–1949, 1955–1957
(Institute for Advanced Study, Princeton)

DEPARTMENT OF GENETICS

Station for Experimental Evolution opened in 1904; name changed to Department of Experimental Evolution in 1906; combined with Eugenics Record Office in 1921 to form Department of Genetics.

Directors

Charles B. Davenport, 1904–1934
Albert F. Blakeslee, 1935–November 30, 1941
Milislav Demerec, December 1, 1941–1942 (Acting); 1943–June 30, 1960
Berwind P. Kaufmann, July 1, 1960–January 31, 1962 (Acting); February 1, 1962–June 30, 1962

Staff Members

Ernst Caspari, 1947–1949
Helen Gay, 1960–1962
Alfred D. Hershey, 1950—
Barbara McClintock, 1942—
Margaret R. McDonald, 1946—
E. C. MacDowell, 1914–1952
George Streisinger, 1956–1960
Bruce Wallace, 1947–1949
Evelyn M. Witkin, 1950–1955

Arthur M. Banta, 1909–1930
Robert W. Bates, 1931–1941
Barbara S. Burks, 1936–1941
John Belling, 1920–1929

Ugo Fano, 1940–1946
Ross A. Gortner, 1909–1914
J. Arthur Harris, 1907–1924
H. H. Laughlin, 1918–1940
S. E. Luria, 1945–1946
Frank E. Lutz, 1904–1909
Charles W. Metz, 1914–1930
Oscar Riddle, 1912–1945
Sophie Satina, 1924–1942
George H. Shull, 1904–1915
Morris Steggerda, 1930–1944
H. E. Warmke, 1938–1945
Benjamin B. Wells, 1941–1942

Other Scientists and Scholars Associated with the Department

Edgar Anderson, 1941, 1945
(Washington University; later, Missouri Botanical Garden)
Ernest Ball, 1942–1943
(University of North Carolina)
Hans Bauer, 1936 (Kaiser-Wilhelm Institut für Biologie, Berlin-Dahlem; later, Max-Planck Institut für Meeresbiologie, Wilhelmshaven, Germany)
George W. Beadle, 1935
(California Institute of Technology; later, University of Chicago)
John J. Biesele, *Research Associate*, 1944–1946
(University of Texas)
Dietrich Bodenstein, 1944
(University of Virginia)
Sydney Brenner, 1954
(University of the Witwatersrand; later, Cavendish Laboratory)
Vernon Bryson, *Research Associate*, 1942–1943
(Rutgers University)
John T. Buchholz, 1921–1941 (University of Arkansas; later, University of Illinois)
Sir Macfarlane Burnet, 1950
(The Walter and Eliza Hall Institute of Medical Research, Melbourne)
J. Gordon Carlson, 1937–1940
(University of Alabama; later, University of Tennessee)
J. Lincoln Cartledge, 1921–1924
(University of West Virginia)
William E. Castle, *Research Associate* 1904–1943 (Harvard University; later, University of California)
David G. Catcheside, *Research Associate* 1957–1959 (University of Birmingham, England)
Donald R. Charles, 1929–1930
(Sarah Lawrence College; later, University of Rochester)
Albert Claude, 1946
(Rockefeller Institute for Medical Research; later, The Free University of Brussels)
Marie E. Conklin, 1937–1941
(Adelphi College)
J. N. Couch, 1925–1926
M(University of North Carolina)
ax Delbrück, 1937
(California Institute of Technology)
Hugo de Vries, *Research Associate*, 1904–1918
(University of Amsterdam)
Th. Dobzhansky, *Research Associate* 1936–1949 (California Institute of Technology and Columbia University; later, Rockefeller Institute for Medical Research)
L. C. Dunn, 1929 (Columbia University)
Boris Ephrussi, 1937 (Institut de Biologie Physico-Chimique, Paris; later, Centre National de la Recherche Scientifique, Gif-sur-Yvette)
Harold D. Fish, *Research Associate*, 1919–1924
(Denison University; later, University of Pittsburgh)
Pierre Fredericq, 1958 (University of Liége)
Gabriel Gasić, 1945–1946 (University of Chile)
Norman H. Giles, Jr., 1941
(Yale University)
Joseph S. Gots, 1954, 1955, 1957
(University of Pennsylvania)
John W. Gowen, 1915, 1940
(Iowa State College)
Ludwig von Graf, 1906 (University of Graz)
C. C. Guthrie, 1909 (University of Pittsburgh)
Åke Gustafsson, 1937–1938 (Institute for Genetic Research, Svalof, Sweden; later, Forest Research Institute of Sweden, Stockholm)
Olli Halkka, 1959 (University of Helsinki)
Alexander Hollaender, *Research Associate* 1942–1944 (National Institutes of Health; later, Oak Ridge National Laboratory)
Sally Hughes-Schrader, 1934
(Sarah Lawrence College; later, Duke University)

C. Leonard Huskins, 1935, 1936 (McGill University; later, University of Wisconsin)
Fritz Kaudewitz, 1954 (Max-Planck Institut für Virusforschung, Tübingen)
Tage Kemp, 1932 (University of Copenhagen)
P. C. Koller, 1938 (University of Edinburgh; later, Chester Beatty Research Institute, London)
Jaroslav Krizenecky, 1928–1929 (Zootechnical Research Institute, Brno, Czechoslovakia)
Victor K. LaMer, 1916–1917 (Columbia University)
Raymond Latarjet, 1945–1946 (Institut Pasteur, Paris)
Albert Levan, 1951 (University of Lund)
Cyrus Levinthal, 1951 (University of Michigan; later, Massachusetts Institute of Technology)
C. C. Little, *Research Associate*, 1919–1925 (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)
Edward L. Mark, *Research Associate* 1904–1910 (Harvard University)
Horace N. Marvin, 1941–1942 (University of Arkansas)
William J. Moenkhaus, 1904–1906 (Indiana University)
G. Montalenti, 1951 (University of Naples; later, University of Rome)
H. J. Muller, 1921 (Indiana University)
Robert K. Nabours, *Research Associate* 1929–1930 (Kansas State College)
James V. Neel, 1940 (Dartmouth College; later, University of Michigan)
Howard B. Newcombe, *Research Associate* 1938, 1945–1947 (Atomic Energy Commission of Canada, Ltd.)
Theophilus S. Painter, 1923 (University of Texas)
Raymond Pearl, *Research Associate* 1904–1906 (University of Michigan; later, Johns Hopkins University)
Marcus M. Rhoades, 1941 (Columbia University; later, Indiana University)
Maurice N. Richter, 1930–1952 (Columbia University; later, New York University Medical Center)
Franz Schrader, 1934 (Columbia University; later, Duke University)
Edmund W. Sinnott, 1938 (Columbia University; later, Yale University)
B. M. Slizynski, 1936–1937 (University of Cracow; later, University of Edinburgh)
Evelyn E. B. Smith, *Research Associate*, 1958 (University of Glasgow)
Laurence H. Snyder, 1922 (University of Hawaii)
Arnold H. Sparrow, 1940, 1941 (Brookhaven National Laboratory)
Warren P. Spencer, 1935 (College of Wooster)
S. G. Stephens, *Research Associate*, 1945–1947 (North Carolina State College)
Curt Stern, 1933, 1938, 1944, 1946 (University of Rochester; later, University of California, Berkeley)
A. Tavcar, 1951 (University of Zabreb)
Howard J. Teas, 1942–1943 (Nuclear Center, Mayagüez, Puerto Rico)
René Thomas, 1957–1958 (University of Brussels)
N. Timofeeff-Ressovsky, 1932 (Kaiser-Wilhelm Institut für Hirnforschung; later, Academy of Sciences, Novosibirsk, U.S.S.R.)
Jun-ichi Tomizawa, *Research Associate* 1957–1959 (National Institute of Health, Tokyo)
William L. Tower, *Research Associate* 1904–1917 (University of Chicago)
J. van Overbeek, 1940, 1941 (California Institute of Technology; later, Agricultural Laboratory, Shell Development Company, Modesto, California)
C. H. Waddington, 1938 (Cambridge University; later, University of Edinburgh)
Mogens Westergaard, *Research Associate* 1957–1959 (Universitetets Genetiske Institut, Copenhagen)
Fritz von Wettstein, 1938 (Kaiser-Wilhelm Institut für Biologie, Berlin-Dahlem; later, University of Vienna)
M. J. D. White, 1947, 1950–1952 (University of London and University of Texas; later, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia)
P. W. Whiting, 1933–1935 (University of Pennsylvania)
Maurice Whittinghill, 1938 (Bennington College; later, University of North Carolina)
Edmund B. Wilson, *Research Associate* 1904–1909, 1936–1938 (Columbia University)
Charles Yanofsky, 1956 (Western Reserve University; later, Stanford University)

The following persons carried on genetic studies with Carnegie Institution support:

Calvin B. Bridges, *Research Associate* 1916–1938 (Columbia University; later, California Institute of Technology)
Thomas Hunt Morgan, *Research Associate* 1916–1945 (Columbia University; later, California Institute of Technology)
Jack Schultz, *Research Associate*, 1929–1941 (California Institute of Technology; later, Institute for Cancer Research, Philadelphia)
A. H. Sturtevant, *Research Associate* 1916–1931 (Columbia University; later, California Institute of Technology)

GEOPHYSICAL LABORATORY

Organized in 1906. Opened in 1907.

Directors

Arthur L. Day, 1909–1936
Leason H. Adams, 1936–1937 (Acting); 1938–July 31, 1952
George W. Morey, August 1, 1952–August 31, 1953 (Acting)
Philip H. Abelson, September 1, 1953—

Staff Members

Norman L. Bowen, 1910–1937, 1947–1953
Francis R. Boyd, Jr., 1953—
John S. Burlew, 1936–1952
Felix Chayes, 1947—
Sydney P. Clark, Jr., 1957–1962
Gordon L. Davis, 1941—
Gabrielle Donnay, 1955—
Joseph L. England, 1926—
Hans P. Eugster, 1952–1958
Roy W. Goranson, 1926–1951
Hugh J. Greenwood, 1960—
Joseph W. Greig, 1922–1960
Thomas C. Hoering, 1959—
Earl Ingerson, 1935–1947
Frank C. Kracek, 1923–1956
Gunnar Kullerud, 1954—
William S. MacKenzie, 1951–1957
Patrick L. Parker, 1961—
Charles S. Piggot, 1925–1947
Eugene Posnjak, 1913–1947
Howard S. Roberts, 1917–1947
J. Frank Schairer, 1927—
George R. Tilton, 1956—
George Tunell, 1925–1947
O. Frank Tuttle, 1947–1953
William D. Urry, 1938–1949
Hatten S. Yoder, Jr., 1948—
Emanuel G. Zies, 1913–1949

Eugene T. Allen, 1907–1933
Olaf Andersen, 1912–1918
Tom. F. W. Barth, 1929–1940
F. Russell von Bichowsky, 1916–1919
A. F. Buddington, 1919–1920
J. K. Clement, 1904–1907
Pentti Eskola, 1921–1922
Clarence N. Fenner, 1910–1937
Michael Fleischer, 1936–1938
Ralph E. Gibson, 1924–1946
Sterling B. Hendricks, 1926
James H. Hibben, 1928–1939
John C. Hostetter, 1912–1919
John Johnston, 1908–1916
Esper S. Larsen, Jr., 1907–1909
Robert H. Lombard, 1915–1927
Herbert E. Merwin, 1909–1959
Elbert F. Osborn, 1938–1945
George A. Rankin, 1907–1916
Earnest S. Shepherd, 1904–1946
Robert B. Sosman, 1908–1928
Henry S. Washington, 1912–1934
Walter P. White, 1904–1935
Erskine D. Williamson, 1914–1923
Fred E. Wright, 1906–1944
Ralph W. G. Wyckoff, 1919–1927

Other Scientists and Scholars Associated with the Department

Frank D. Adams, 1903–1911
(McGill University)
Samuel K. Allison, 1925–1926
(University of Chicago)
George F. Becker, 1903–1909
(U. S. Geological Survey)
J. C. Branner, 1906–1907
(Arkansas Geological Survey)
L. E. J. Brouwer, 1931–1932
(Royal Dutch Petroleum)
Thomas C. Chamberlin, *Research Associate*
1903–1927 (University of Chicago)
Hessel de Vries, *Research Associate*, 1958–1959
(University of Groningen, the Netherlands)
J. D. H. Donnay, 1953—
(Johns Hopkins University)
William H. Emmons, 1903
(University of Chicago)
Henry Faul, 1956–1957
(U. S. Geological Survey)
Grove Karl Gilbert, 1904, 1906–1907
(U. S. Geological Survey)
Harry H. Hess, *Research Associate*, 1940–1942
(Princeton University)
Joseph P. Iddings, 1905
(University of Chicago)
Emilie Jäger, 1958–1959
(University of Bern, Switzerland)
Willard F. Libby, *Research Associate*
1954–1959 (University of California at
Los Angeles)
George D. Louderback, 1903–1906
(University of Nevada)
Gordon J. F. MacDonald, 1955—
(Institute of Geophysics and Planetary
Physics, University of California at Los
Angeles)
Forest Ray Moulton, *Research Associate*
1903–1922 (University of Chicago)
Paul Niggli, 1913–1914 (Zurich)
C. C. Patterson, 1958
(California Institute of Technology)
Frank A. Perret, *Research Associate*
1938–1943 (Volcanological Museum,
St. Pierre, Martinique)
Hans Ramberg, *Research Associate*, 1955–1958
(University of Chicago)
Paul Ramdohr, *Research Associate*, 1960—
(University of Heidelberg)
C. S. Slichter, 1903, 1906
(University of Wisconsin)
David B. Stewart, 1954–1956
(U. S. Geological Survey)
C. E. Tilley, *Research Associate*, 1955—
(Cambridge University)
Johan August Udden, 1925, 1928
(University of Texas)
C. R. Van Hise, 1902–1903
(University of Wisconsin)
C. E. Van Orstrand, 1904, 1906–1910
(U. S. Geological Survey)

DEPARTMENT OF EMBRYOLOGY

Organized 1914.

Directors

Franklin P. Mall, 1914–1917
George L. Streeter, 1918–1940
George W. Corner, 1941–1955
James D. Ebert, 1956—

Staff Members

George W. Bartelmez, 1949–1960
David W. Bishop, 1952—
Bent G. Böving, 1951—
Donald D. Brown, 1962—
Robert K. Burns, 1940–1962
Arpad Csapo, 1951–1955
Robert L. DeHaan, 1956—
James F. Didusch, 1913–1940, 1945–1955

Louis B. Flexner, 1940–1951
Osborne O. Heard, 1913–1956
Chester H. Heuser, 1921–1950
I. R. Konigsberg, 1961—
Elizabeth M. Ramsey, 1949—

Herbert M. Evans, 1913–1915
Carl G. Hartman, 1925–1941
Margaret R. Lewis, 1915–1946

Mary E. Rawles, 1957—
Samuel R. M. Reynolds, 1941–1955
Royal F. Ruth, 1956–1961
David B. Tyler, 1947–1950
Walter S. Wilde, 1944–1947

Warren H. Lewis, 1914–1940
Charles W. Metz, 1930–1940
Adolph H. Schultz, 1916–1925

Other Scientists and Scholars Associated with the Department

William E. Adams, 1957–1958
(University of Otago, New Zealand)
Ines de Allende, 1941–1943
(University of Cordoba)
Howard D. Andervont, 1923–1926
(Johns Hopkins School of Hygiene and Public Health; later, National Cancer Institute)
T. S. and B. F. Argyris, 1961–1962
(Syracuse University)
Alexander Barry, 1947
(University of Michigan)
T. H. Bast, 1929–1930
(University of Wisconsin)
J. D. Boyd, 1934–1935
(Cambridge University)
E. A. Boyden, 1939–1940 (University of Minnesota; later, University of Washington)
Washington Buno, 1945–1946
(University of Montevideo)
Gerald L. Carlson, 1960–1962
(Massachusetts Institute of Technology)
Eliot R. Clark and Eleanor L. Clark, 1907–1914 (Johns Hopkins School of Medicine; later, University of Pennsylvania)
George W. Corner, Jr., 1943—
(Johns Hopkins School of Medicine)
E. V. Cowdry, 1913–1916
(Johns Hopkins School of Medicine; later, Washington University)
Maria Victoria de la Cruz, 1949–1950
(Institute of Cardiology, Mexico City)
F. Cuajunco, 1927–1928
(University of the Philippines)
Harold Cummins, 1927–1928
(Tulane University)
Vera Danchakoff, 1924
(Columbia University)
Carl Lawrence Davis, 1920
(University of Maryland)
Vincent J. De Feo, 1955–1957
(University of Illinois)
Anatole S. Dekaban, 1959—
(National Institutes of Health)
Charles A. Doan, 1923–1924
(Johns Hopkins School of Medicine; later, Ohio State University)
Jules Duesberg, *Research Associate*, 1915–1918
(University of Liége)
Robert K. Enders, 1930–1932
(Swarthmore College)
Thomas R. Forbes, 1937–1938
(Johns Hopkins School of Medicine; later, Yale University)
Fritz Fuchs, 1950–1951
(University of Copenhagen)
Ernest D. Gardner, 1955
(Wayne State University)
E. M. K. Geiling, 1935–1936
(Johns Hopkins School of Medicine; later, University of Chicago and U. S. Food and Drug Administration)
Isidore Gersh, 1934–1935
(Johns Hopkins School of Medicine; later, University of Chicago)
G. O. Gey, 1924–1930
(Johns Hopkins School of Medicine)
Joseph Gillman, *Research Associate*
1941–1942, 1946–1948
(University of the Witwatersrand)
G. Gitlin, 1950
(Hebrew University, Jerusalem)
Timothy Glover, 1961
(University of Liverpool)
Charles M. Goss, 1948 (Louisiana State University School of Medicine)
Donald J. Gray, 1955 (Stanford University)
Gilbert S. Greenwald, 1954–1956
(University of Washington; later, University of Kansas)
Paul W. Gregory, 1928–1929
(Harvard University; later, University of California, Davis)
E. Grodzinski, 1928–1929
(University of Cracow)

Alan F. Guttmacher, 1921–1922
(Johns Hopkins School of Medicine; later, Mt. Sinai Hospital, New York City)
Manfred S. Guttmacher, 1921–1922
(Johns Hopkins School of Medicine; later, private practice in psychiatry, Baltimore)
John W. S. Harris, 1961
(London Hospital Medical College)
Arthur T. Hertig, *Research Associate* 1933–1956 (Harvard Medical School)
Marion Hines, 1925–1947
(University of Chicago and Johns Hopkins University; later, Emory University)
A. St. G. Huggett, 1952–1953
(St. Mary's Hospital Medical School, London)
Irwin H. Kaiser, 1946–1947
(University of Minnesota)
Seymour Katsh, 1955–1958
(University of Colorado)
Franz Keibel, *Research Associate*, 1914–1918
(Anatomical Institute, Strassburg)
Benjamin F. Kingsbury, 1917–1918
(Cornell University)
Abraham Kulangara, 1959–1961
(University of California, Los Angeles; later, All India Institute of Medical Sciences, New Delhi)
Orthello R. Langworthy, 1924–1930
(Johns Hopkins University)
Hans Laufer, 1957–1959
(Johns Hopkins University)
John McKenzie, 1959
(University of Aberdeen, Scotland)
Joseph E. Markee, 1935–1936
(Stanford University; later, Duke University)
Arthur Meyer, 1917–1918
(Stanford University)
Tom Mori, 1960–1961 (Tohoku University)
Harland W. Mossman, 1934–1935
(University of Wisconsin)
William B. Muchmore, 1959
(University of Rochester)
Jacques Mulnard, 1957
(University of Brussels)
Alton M. Mun, 1959–1961
(Washington State College; later, University of Maine)
G. Muratori, 1934–1935
(University of Padua, Italy)
Roberto Narbaitz, 1959
(University of Buenos Aires)
Catherine Neill, 1952–1953 (London; later, Johns Hopkins Medical School)
Martin Nordmann, 1929–1930
(University of Tübingen)
Ronan O'Rahilly, 1961–1962
(St. Louis University)
F. Orts Llorca, 1959 (University of Madrid)
John Papaconstantinou, 1958–1960
(Johns Hopkins School of Medicine; later, University of Connecticut)
W. M. Paul, 1954 (University of Toronto)
Donald F. Poulson, 1936–1937
(Yale University)
Curt P. Richter, 1927–1930
(Johns Hopkins School of Medicine)
Eduardo de Robertis, 1941–1942
(University of Buenos Aires)
John Rock, 1938–1945
(Harvard Medical School)
Edward Roosen-Runge, 1957
(University of Washington)
Florence Sabin, 1914–1924
(Johns Hopkins University; later, Rockefeller Institute)
Jorgen U. Schlegel, 1948–1949
(University of Copenhagen)
Harold D. Senior, 1917–1918
(New York University)
Ronald Singer, 1951–1952
(University of Cape Town, South Africa; later, University of Chicago)
William L. Straus, Jr., 1925–1930
(Johns Hopkins School of Medicine)
Fritz Strauss, 1950 (University of Bern)
Somers H. Sturgis, 1942–1943
(Massachusetts General Hospital)
Francis H. Swett, 1924–1927
(Johns Hopkins School of Medicine; later, Duke University)
Pierre Tardent, 1959–1960
(Zoological Station, Naples)
M. H. Toosy, 1948–1949
(Lahore Medical School)
Theodore W. Torrey, 1952
(Indiana University)
U. U. Uotila, 1939–1940
(Harvard Medical School)
W. J. van Doorenmaalen, 1958–1959
(Municipal University, Amsterdam)
R. Walmsley, 1935–1936
(University of Edinburgh)
Lewis H. Weed, *Research Associate* 1914–1919, 1921–1935
(Johns Hopkins University)

Karl M. Wilson, 1913–1924
(Johns Hopkins School of Medicine; later, University of Rochester)
Milton C. Winternitz, 1914–1916
(Johns Hopkins School of Medicine; later, Yale University)
George Wislocki, 1916–1931
(Johns Hopkins School of Medicine; later, Harvard University)
Emil Witschi, 1941–1942
(University of Iowa)

DEPARTMENT OF HISTORICAL RESEARCH

Organized as a "bureau" in 1903; became a "department" in 1905; terminated as a department and incorporated as the Section of United States History in a new Division of Historical Research, 1930.

Directors

Andrew C. McLaughlin, 1903–1905
John F. Jameson, 1905–1928
None, 1928–1930

Staff Members

Edmund C. Burnett, 1907–1932
Frances G. Davenport, 1905–1927
Elizabeth Donnan, 1911–1919
Shirley Farr, 1921–1922
Mary F. Griffin, 1922–1925
Waldo G. Leland, 1903–1945
Marguerite M. McKee, 1925–1929
David W. Parker, 1909–1913, 1925–1928
Charles O. Paullin, 1912–1936
Leo F. Stock, 1910–1945

Other Scientists and Scholars Associated with the Department

Charles Francis Adams, 1902
(Massachusetts Historical Society)
Ephraim D. Adams, 1904
(Stanford University)
William H. Allison, 1906–1911 (Bryn Mawr College; later, Colgate University)
Charles M. Andrews, 1904–1918
(Yale University)
James C. Ballagh, 1907–1908
(Johns Hopkins University; later, University of Pennsylvania)
Adolf F. A. Bandelier, 1911–1914
(Columbia University)
Eugene C. Barker, 1906
(University of Texas)
John S. Bassett, 1921–1928 (Smith College)
Herbert C. F. Bell, 1916–1923
(Bowdoin College; later, Wesleyan University)
Samuel F. Bemis, 1923–1925
(George Washington University; later, Yale University)
Herbert E. Bolton, 1907–1913
(Stanford University; later, University of California)
Harold Martin Bowman, 1907–1908
(Boston University School of Law)
Julian P. Bretz, 1906
(later, Cornell University)
Helen T. Catterall, 1918–1933 (Boston Bar)
Isaac Joslin Cox, 1906–1908
(University of Cincinnati; later, Northwestern University)
Walter F. Dodd, 1908 (Library of Congress; later, Yale University)
Max Farrand, 1912–1913 (Yale University; later, Henry E. Huntington Library and Art Gallery)
Albert B. Faust, 1912–1916
(Cornell University)
William S. Ferguson, 1906–1908
(University of California; later, Harvard University)
Carl R. Fish, 1908–1911
(University of Wisconsin)
Worthington C. Ford, 1903–1906
(Library of Congress; later, Massachusetts Historical Society)
Dixon R. Fox, 1919–1920 (Columbia University; later, Union College)

Frank A. Golder, 1914–1922
(State College of Washington; later, Stanford University)
Evarts B. Greene, 1918 (University of Illinois; later, Columbia University)
Charles W. Hackett, 1918–1929
(University of Texas)
Charles H. Haskins, 1905–1908
(Harvard University)
Roscoe R. Hill, 1910–1917
(later, Nicaraguan High Commission; National Archives)
Frank H. Hodder, 1912 (University of Kansas)
William Wirt Howe, 1904 (Board of Trustees, Carnegie Institution of Washington)
William I. Hull, 1914 (Swarthmore College)
Herman G. James, 1923 (University of South Dakota; Ohio University)
Marcus W. Jernegan, 1907–1926
(University of Chicago)
Louise P. Kellogg, 1922
(Wisconsin State Historical Society)
Benjamin B. Kendrick, 1910 (Women's College of University of North Carolina)
Marion D. Learned, 1908–1912
(University of Pennsylvania)
Orin G. Libby, 1912
(University of North Dakota)
George W. Littlehales, 1915
(George Washington University; formerly with Carnegie Institution of Washington's Department of Terrestrial Magnetism)
Alfred T. Mahan, 1914–1915
(U. S. Navy, retired)
William R. Manning, 1908–1910
(George Washington University; later, Department of State)
John J. Meng, 1936–1954
(Catholic University of America; later, Hunter College)
Herbert L. Osgood, 1912–1918
(Columbia University)
Edwin W. Pahlow, 1926–1927
(Ohio State University)
Frederic L. Paxson, 1910–1914
(University of Wisconsin; later, University of California)
Francis S. Philbrick, 1914–1915
(University of Pennsylvania)
Jesse S. Reeves, 1912–1913
(University of Michigan)
James A. Robertson, 1909–1917, 1931–1932
(Stetson University; later, Archives of Maryland)
Robert W. Rogers, 1924–1927
(Drew Theological Seminary)
Joseph Schafer, 1918–1919
(University of Oregon)
George W. Scott, 1903–1905 (Library of Congress; Columbia University)
William R. Shepherd, 1905–1908
(Columbia University)
William A. Slade, 1904–1905
(Library of Congress)
Frederick J. Turner, 1916–1917
(Harvard University)
Arnold J. F. van Lear, 1919–1926
(New York State Library; later, New York State Education Department)
Claude H. Van Tyne, 1904–1908
(University of Michigan)
Ray H. Whitbeck, 1914–1915
(University of Wisconsin)
Irene A. Wright, 1925–1928 (Library of Congress; later, National Archives)

DIVISION OF HISTORICAL RESEARCH

Established 1930, superseding the Department of Historical Research, which became a section of United States History in the Division. The other two sections were the Section of Aboriginal American History, which continued the archaeological work already begun by Sylvanus G. Morley in Central America and by E. H. Morris in southwestern United States, and the Section of the History of Science. Became the Department of Archaeology, 1951.

Alfred V. Kidder, Chairman, 1930–1950
Harry E. D. Pollock, Director, 1951–1958

Staff Members

Eleanor B. Adams, 1934–1949
Robert S. Chamberlain, 1937–1947
Sylvanus G. Morley, 1914–1948
Earl H. Morris, 1925–1955
Alexander Pogo, 1929–1950
Tatiana A. Proskouriakoff, 1939—
Ralph L. Roys, 1930–1953
Karl Ruppert, 1925–1956
George A. L. Sarton, 1918–1949
France V. Scholes, 1931–1946
Anna O. Shepard, 1933—
Edwin M. Shook, 1933–1958
A. Ledyard Smith, 1929–1958
Robert E. Smith, 1931–1960
Gustav Strömsvik, 1926–1957
Sol Tax, 1938–1947
J. Eric S. Thompson, 1935–1959
Alfonso Villa Rojas, 1932–1947

Manuel J. Andrade, 1932, 1936–1940
Abraham M. Halpern, 1941–1942
J. Ignacio Rubio Mañé, 1936–1942
Oliver G. Ricketson, Jr., 1920–1940
Henry B. Roberts, 1926–1939

Other Scientists and Scholars Associated with the Division

Sophie D. Aberle, *Research Associate* 1933–1940 (United Pueblo Agency, Albuquerque, New Mexico; later, Chief Nutrition, Bernalillo County Indian Hospital)
Robert M. Adams, Jr., 1951–1952 (Oriental Institute, University of Chicago)
Monroe Amsden, 1923–1924, 1927 (southwestern archaeologist)
E. Wyllys Andrews, 1939–1940, 1941–1942, 1947–1948 (Tulane University)
Herman Beyer, 1937 (Tulane University)
Franz Blom, 1924–1925 (Tulane University)
Stephen F. de Borhegyi, 1949 (University of Oklahoma; later, Milwaukee Public Museum)
George W. Brainerd, 1939–1942, 1948–1949 (University of California, Los Angeles)
Kirk Bryan, 1945 (Harvard University)
W. R. Bullard, 1951–1953 (Peabody Museum, Harvard University)
Alfonso Caso y Andrade, *Research Associate* 1936–1939 (Instituto Nacional Indigenista, Mexico)
Kenneth M. Chapman, 1935 (University of New Mexico)
Jean Charlot, 1926–1931 (painter and teacher)
Ann Chowning, 1954–1955 (Bryn Mawr College; later, University of Pennsylvania)
I. Bernard Cohen, 1938–1941 (Harvard University)
Fay-Cooper Cole, 1931 (University of Chicago)
G. W. Collins, 1936–1937 (U. S. Department of Agriculture)
Frank H. Connell, 1931–1932 (Dartmouth College)
Luther S. Cressman, *Research Associate* 1936–1942 (University of Oregon)
John H. Denison, Jr., 1937–1938 (Big Horn, Wyoming)
Rollins A. Emerson, 1934–1935 (Cornell University)
F. W. Gaige, 1930–1931 (University of Michigan)
Rutherford J. Gettens, 1955 (Freer Gallery of Art, Washington, D. C.)
John P. Gillin, 1941–1943, 1945–1946 (Duke University; later, University of Pittsburgh)
Antonio Goubaud, 1944–1945 (Instituto de Antropología e Historia, Guatemala City)
Carl E. Guthe, *Research Associate*, 1921–1922 (New York State Museum, Albany)
Lewis U. Hanke, 1935–1939 (Library of Congress; later, University of Texas and Columbia University)
Mark R. Harrington, *Research Associate* 1930–1936 (Southwest Museum, Los Angeles)
William A. Heidel, 1928–1939 (Wesleyan University)
Edgar B. Howard, 1934–1942 (University of Pennsylvania)
William T. Howard, Jr., 1924 (Johns Hopkins University)
Jesse D. Jennings, 1936–1937 (University of Utah)

J. H. Kempton, 1934–1938
(U. S. Department of Agriculture)
J. Steward Lincoln, 1940–1941
(Guatemala City)
John M. Longyear, III, 1937–1939, 1941–1942, 1945–1947, 1949–1950
(Colgate University)
Samuel K. Lothrop, 1922–1933
(Peabody Museum, Harvard University)
Cyrus L. Lundell, *Research Associate* 1933–1941 (University of Michigan)
Maud Worcester Makemson, 1943
(Vassar College)
Norman A. McQuown, 1937–1949
(University of Chicago)
Paul S. Martin, 1926–1928
(Chicago Natural History Museum)
Ann Axtell Morris, 1926–1931
(Boulder, Colorado)
Lila M. O'Neale, 1935–1936
(University of California, Berkeley)
Arthur S. Pearse, 1928–1936
(Duke University)
Wilson Popenoe, 1935–1936
(United Fruit Company; later, Escuela Agrícola Panamericana, Tegucigalpa, Honduras)
Robert Redfield, *Research Associate* 1930–1949 (University of Chicago)
Ruth Reeves, *Research Associate*, 1934–1935
(New York City, New York)
Juan de Dios Rosales, 1944–1946
(Instituto Indigenista de Guatemala)
George M. Saunders, 1930–1932
(Harvard University)
Adolph H. Schultz, *Research Associate* 1916–1925, 1937–1938 (Johns Hopkins University; later, University of Zurich)
George C. Shattuck, 1929–1939
(Boston City Hospital; later, Massachusetts General Hospital)
Joseph L. Smith, 1941
(Boston Museum of Fine Arts)
Philip E. Smith, 1953–1954
(University of Toronto)
R. Stadelman, 1936–1938
(U. S. Department of Agriculture)
L. C. Stuart, 1932–1933
(University of Michigan)
John Teeple, 1928–1931
(consulting chemist, New York City)
Antonio Tejeda F., 1938–1939, 1944–1947
(Museo Nacional de Arqueología y Etnología, Guatemala City)
Donald E. Thompson, 1954–1955
(University of Wisconsin)
Aubrey S. Trik, 1935–1938 (University Museum, University of Pennsylvania)
Melvin Tumin, 1942–1944
(University of North Carolina)
George C. Vaillant, 1925–1940
(American Museum of Natural History; later, University Museum, University of Pennsylvania)
Robert Wauchope, 1933–1936
(Tulane University)
Howell Williams, 1949–1950
(University of California, Berkeley)
Clark Wissler, *Research Associate*, 1924–1933
(American Museum of Natural History; later, Yale University)

DEPARTMENT OF ECONOMICS AND SOCIOLOGY

Organized 1904; terminated 1916.

Board Members

Carroll D. Wright, Director, 1904–1909 (Clark College)
Henry W. Farnam, Chairman, 1909–1916 (Yale University)
Kenyon L. Butterfield, Agriculture and Forestry, 1904–1915 (Rhode Island College of Agriculture and Mechanic Arts)
Victor S. Clark, Manufactures, 1906–1916 (Census Bureau)
John R. Commons, The Labor Movement, 1909–1915 (University of Wisconsin)
Davis R. Dewey, Money and Banking, 1904–1914 (Institute of Technology, Boston)
Henry B. Gardner, Federal and State Finance, 1904–1914 (Brown University)
J. W. Jenks, Industrial Organization, 1904–1914 (Cornell University)

Emory R. Johnson, Domestic and Foreign Commerce, 1904–1915 (University of Pennsylvania)
B. H. Meyer, Transportation, 1904–1916 (University of Wisconsin)
S. N. D. North, Manufactures, 1904 (Census Bureau)
Edward W. Parker, Mining, 1904–1915 (U. S. Geological Survey)
W. Z. Ripley, Transportation, 1904 (Newton Centre, Massachusetts)
Alfred Holt Stone, The Negro in Slavery and Freedom, 1906–1914 (Dunleith, Mississippi)
Walter F. Willcox, Population and Immigration, 1904–1914 (Cornell University)

Other Scientists and Scholars Associated with the Department

Edith Abbott, 1905–1910
(University of Chicago)
Henry C. Adams, 1904
(University of Michigan)
Charles H. Ambler, 1910
(Randolph-Macon College; later,
(University of West Virginia)
John B. Andrews, 1913–1915
(numerous activities in labor economics)
Oliver Edwin Baker, 1912
(U. S. Department of Agriculture)
Emily Greene Balch, 1904–1907
(Wellesley College)
F. Spencer Baldwin, 1908–1909
(Boston University)
J. Lynn Barnard, 1905–1908
(Philadelphia School of Pedagogy)
Alvard Longley Bishop, 1907–1908
(Yale University)
Frank W. Blackmar, 1904–1914
(University of Kansas)
Ernest Ludlow Bogart, 1904–1912
(Oberlin College; later, Princeton
University and University of Illinois)
Beverley Waugh Bond, 1908–1909
(Purdue University; later,
University of Cincinnati)
William K. Boyd, 1910–1913
(Duke University)
James E. Boyle, 1905–1908
(University of North Dakota)
Solon J. Buck, 1906–1913
(University of Indiana; later,
Archivist of the United States)
Thomas N. Carver, 1904–1912
(Harvard University)
Robert E. Chaddock, 1909
(Columbia University)
John B. Clark, 1902 (Columbia University)
Frederick A. Cleveland, 1905–1913
(New York University; later, in charge of
President Taft's Commission on Economy
and Efficiency)
Thomas Conway, Jr., 1904–1913
(University of Pennsylvania)
Mary Roberts Coolidge, 1907–1910
(Mills College)
John Lee Coulter, 1908–1912
(University of Minnesota; later,
U. S. Tariff Commission)
James Walter Crook, 1908–1911
(Amherst College)
Ira Brown Cross, 1909–1913
(University of California)
Stuart Daggett, 1904–1913
(University of California)
Edgar M. Dawson, 1908–1913
(Princeton University; later,
Hunter College)
Clive Day, 1907–1909 (Yale University)
David T. Day, 1908–1911
(U. S. Geological Survey; later,
U. S. Bureau of Mines)
Robert N. Denham, Jr., 1908
(University of Michigan; later,
National Labor Relations Board)
Carroll W. Doten, 1906–1908
(Massachusetts Institute of Technology)
W. E. B. Dubois, 1908 (Atlanta University)
Edwin C. Eckel, 1904–1908
(U. S. Geological Survey)
Richard T. Ely, 1904–1909
(University of Wisconsin)
Fred Rogers Fairchild, 1904–1909
(Yale University)
Henry Pratt Fairchild, 1908–1909
(Bowdoin College; later,
New York University)
John I. Falconer, 1912–1913
(Ohio State University)
Albert B. Faust, 1907–1910
(Cornell University)
Walter L. Fleming, 1908–1911
(Louisiana State University and
Vanderbilt University)

Albert A. Giesecke, 1904–1910
(University of Pennsylvania; later, University of Cuzco, Peru)
Eugene A. Gilmore, 1910
(University of Wisconsin; later, State University of Iowa)
E. A. Goldenweiser, 1904–1908
(various economic posts in U. S. Government)
L. C. Graton, 1908–1913
(U. S. Geological Survey; later, Harvard University)
Elmer C. Griffith, 1908–1911
(Kalamazoo College)
George Gorham Groat, 1904–1908
(Ohio Wesleyan University; later, University of Vermont)
James Edward Hagerty, 1905–1909
(Ohio State University)
Robert M. Haig, 1910–1913
(Columbia University)
Matthew Brown Hammond, 1904–1909
(Ohio State University)
Glover D. Hancock, 1908–1911
(Amherst College; later, Washington and Lee University)
Lewis Henry Haney, 1906–1910
(New York University)
Hugh Sisson Hanna, 1905–1908
(U. S. Bureau of Labor Statistics)
Adelaide R. Hasse, 1905–1917
(New York Public Library; later, Brookings Institution)
Frank I. Herriott, 1905–1911
(Drake University)
Benjamin H. Hibbard, 1908–1914
(Iowa State College; later, University of Wisconsin)
Henry E. Hoagland, 1911–1913
(Ohio State University)
Roy Jay Holden, 1908–1915
(Virginia Polytechnic Institute)
Jacob H. Hollander, 1904–1907
(Johns Hopkins University)
Solomon S. Huebner, 1904–1911
(University of Pennsylvania)
Walter Renton Ingalls, 1904–1908
(construction engineer, New York City)
Theodore H. Jack, 1909–1911
(Emory University; later, Randolph-Macon College)
Edward D. Jones, 1908–1914
(University of Michigan)
T. J. Jones, 1908 (Hampton Institute; later, Phelps Stokes Fund)
Clyde L. King, 1911–1913
(University of Pennsylvania)
Julius Klein, 1909
(U. S. Department of Commerce)
Francis Baker Laney, 1904–1915
(U. S. National Museum; later, U. S. Geological Survey)
John Lapp, 1908 (Cornell University; later, Marquette University)
Laurence M. Larson, 1905–1909
(University of Illinois)
C. K. Leith, 1904–1915
(University of Wisconsin)
Isaac P. Lippincott, 1909
(Washington University)
Oliver C. Lockhart, 1908–1913
(Ohio State University)
Isaac A. Loos, 1905–1909
(State University of Iowa)
Gerald Francis Loughlin, 1915
(U. S. Geological Survey)
David A. McCabe, 1912
(Princeton University)
Charles McCarthy, 1904
(U. S. Commission on Industrial Relations; later, U. S. Food Administration)
James Farley McClelland, 1904–1905
(Columbia School of Mines; later, Yale University)
George McCutchen, 1908–1912
(University of South Carolina)
S. J. McLean, 1906–1910
(University of Toronto)
F. L. McVey, 1908–1911
(University of North Dakota; later, University of Kentucky)
E. T. Miller, 1905–1915 (University of Texas)
H. A. Millis, 1909–1912
(Stanford University; later, University of Chicago)
Wesley C. Mitchell, 1904–1908
(University of California; later, New School for Social Research)
Blaine F. Moore, 1908–1909
(U. S. Commission on Industrial Relations; later, University of Kansas)
Charles E. Munroe, 1904–1910
(George Washington University)
Henry R. Mussey, 1904 (Wellesley College)
W. T. Nardin, 1905 (Pet Milk Company)
Selig Perlman, 1911–1915
(University of Wisconsin)
Warren Milton Persons, 1908
(Colorado College; later, Harvard University)

John B. Phillips, 1908–1909
(University of Colorado; later, University of Indiana)
Ulrich B. Phillips, 1904–1910
(Tulane University; later, University of Michigan and Yale University)
Charles F. Pidgin, 1908
(Massachusetts Bureau of Statistics of Labor)
Carl C. Plehn, 1904–1911
(University of California)
Fred Wilbur Powell, 1909–1913
(Brookings Institution)
Joseph Hyde Pratt, 1904–1910
(University of North Carolina)
E. P. Puckett, 1908–1913 (Central College)
Charles Lee Raper, 1905–1909
(University of North Carolina; later, Syracuse University)
William A. Rawles, 1904–1911
(University of Indiana)
Heinrich Ries, 1904–1909
(Cornell University)
Thomas James Riley, 1908–1909
(University of Missouri; later, Washington University)
Clyde Orval Ruggles, 1908–1911
(Ohio State University; later, Harvard University)
Aaron M. Sakolski, 1906
(New York University)
David J. Saposs, 1911–1915
(various government posts in labor economics)
William O. Scroggs, 1905–1911
(Louisiana State University)
A. E. Sheldon, 1904–1905
(Nebraska Historical Society)
St. George L. Sioussat, 1904–1913
(University of the South; later, University of Pennsylvania)
J. Russell Smith, 1904–1908
(University of Pennsylvania; later, Columbia University)
Yates Snowden, 1910–1911
(University of South Carolina)
Don C. Sowers, 1910–1913
(University of Colorado)
Robert James Sprague, 1908–1909
(University of Maine; later, Rollins College)
Harry Harkness Stoek, 1904–1908
(editor, *Mining and Minerals;* later, University of Illinois)
Edgar M. Sydenstricker, 1908–1915
(U. S. Public Health Service)
Henry C. Taylor, 1908–1915
(University of Wisconsin; later, Farm Foundation)
D. Y. Thomas, 1907–1908
(University of Florida; later, University of Arkansas)
William H. Tolman, 1908
(Pawtucket, Rhode Island)
Walter Sheldon Tower, 1905–1908
(Bethlehem Steel Corporation; later, Iron and Steel Institute)
Robert James Usher, 1905
(Howard-Tilton Memorial Library, Tulane University)
Francis Walker, 1909–1910
(Federal Trade Commission)
Royal Brunson Way, 1906–1908
(Northwestern University; later, Beloit College)
Nathan Austin Weston, 1905–1910
(University of Illinois)
Horace L. Wilgus, 1905–1909
(University of Michigan)
C. C. Williamson, 1905–1908
(New York Public Library; later, Columbia University)
Calvin Dill Wilson, 1908–1912
(clergyman and author)
Edwin E. Witte, 1911–1912
(University of Wisconsin)
R. R. Wright, Jr., 1908–1910
(Georgia State Industrial College)
Allyn A. Young, 1905–1910
(Stanford University; later, Cornell University and Harvard University)
Frederic G. Young, 1905–1913
(University of Oregon)

DEPARTMENT OF MARINE BIOLOGY

Established in 1904. Name changed to Tortugas Laboratory in 1923. Activities terminated in 1939.

Directors

Alfred G. Mayer, 1904–1922
William Harding Longley, 1923–1927 (Administrative Officer); 1928–1937 (Executive Officer)
David Hilt Tennent, 1938–1939 (Executive Officer)

Staff Members

Paul S. Conger, 1924–1929, 1937–1938
Albert Mann, 1919–1933
John W. Mills, 1906–1939

Other Scientists and Scholars Associated with the Department

Percy L. Bailey, Jr., 1937
(College of the City of New York)
Stanley C. Ball, 1913–1914, 1917
(Massachusetts Agricultural College; later, Bishop Museum, Honolulu, and Peabody Museum, Yale University)
Paul Bartsch,
1912–1917, 1919, 1921–1927, 1930–1932
(U. S. National Museum; later, George Washington University)
Norman J. Berrill, 1937 (McGill University)
Lawrence R. Blinks, 1925–1928
(Rockefeller Institute; later, Stanford University and Hopkins Marine Station)
H. Boschma, 1924 (Rijksuniversiteit, Leiden)
Howard H. M. Bowman, 1915–1916
(University of Pennsylvania; later, University of Toledo)
Alan A. Boyden, 1931, 1933, 1935
(Rutgers University)
Charles M. Breder, Jr., 1928
(New York Aquarium and American Museum of Natural History)
Floyd J. Brinley, 1936–1937
(North Dakota Agricultural College; later, University of Toledo)
William K. Brooks, 1905–1907, 1909
(Johns Hopkins University)
Dugald E. S. Brown, 1934
(New York University Medical School; later, University of Michigan)
Walter E. Bullington, 1929–1930, 1934
(Randolph-Macon College)
Martin Burkenroad, 1928 (Tulane University; later, Marine Biological Station, National Museum of Panama)
Lewis R. Cary, *Research Associate*
1910–1918, 1920, 1929–1933, 1935
(Princeton University)
Edward L. Chambers, 1936
(Princeton University; later, University of Miami School of Medicine, Coral Gables)
Robert Chambers, 1936
(Washington Square College, New York University; later, Marine Biological Laboratory, Woods Hole)
Frank M. Chapman, 1907–1909
(American Museum of Natural History)
Hubert Lyman Clark, *Research Associate*
1912–1917, 1929–1930 (Museum of Comparative Zoology, Harvard University)
Leonard B. Clark, 1936–1937
(Union College)
Frank W. Clarke, 1919
(U. S. Geological Survey)
Leon J. Cole, 1906–1914 (Yale University; later, University of Wisconsin)
John Colman, 1930 (Cambridge University)
Edwin G. Conklin, 1905, 1907, 1909, 1915
(Princeton University)
Benjamin R. Coonfield, 1937
(Brooklyn College)
Rheinart P. Cowles, 1905–1909, 1914
(Johns Hopkins University)
Paul R. Cutright, 1936 (Beaver College)
Ulric Dahlgren, 1906, 1908, 1911–1922
(Princeton University)
Reginald A. Daly, 1919 (Harvard University)
John H. Davis, Jr., 1936–1937
(Southwestern College; later, University of Florida)

May W. de Laubenfels, 1926–1927, 1931, 1935
(Pasadena Junior College; later, Oregon State College)
George S. de Rényi, 1933
(University of Pennsylvania)
Richard B. Dole, 1913
(U. S. Geological Survey)
Henry H. Donaldson, 1916
(Wistar Institute of Anatomy)
William L. Doyle, 1933–1934
(Johns Hopkins University; later, University of Chicago)
George Harold Drew, 1911–1913
(Christ's College, Cambridge University)
Gilman A. Drew, 1912
(Marine Biological Laboratory, Woods Hole)
Charles H. Edmondson, 1906–1907
(Iowa Wesleyan; later, University of Hawaii and Bishop Museum, Honolulu)
Richard M. Field, 1919 (Museum of Comparative Zoology, Harvard University; later, Princeton University)
A. Haldane Gee, 1929
(Scripps Institution of Oceanography; later, Foster D. Snell, Inc., New York)
John H. Gerould, 1915, 1921–1922
(Dartmouth College)
Isidore I. Gersh, 1934
(Johns Hopkins University Medical School; later, University of Chicago School of Medicine)
Abraham J. Goldforb, 1912–1913, 1915–1916
(College of the City of New York)
Hubert B. Goodrich, 1934
(Wesleyan University)
Myron Gordon, 1927, 1932
(Cornell University; later, American Museum of Natural History and New York Zoological Society)
James N. Gowanlock, 1929
(Dalhousie University)
Caswell Grave,
1924, 1926–1929, 1932, 1934–1935
(Washington University)
George M. Gray, 1912
(Marine Biological Laboratory, Woods Hole)
Eugene W. Gudger, 1908, 1912–1915
(North Carolina College for Women; later, American Museum of Natural History)
George T. Hargitt, 1905
(Northwestern University; later, Syracuse University)
John E. Harris, 1933–1934, 1936
(Cambridge University)
J. A. Harrison, 1936 (University of London)
Robert Hartmeyer, 1907
(Berlin Zoological Museum)
E. Newton Harvey, *Research Associate* 1909–1925, 1929 (Princeton University)
Shinkishi Hatai, 1916–1917
(Wistar Institute of Anatomy)
Frederick R. Hayes, 1931
(Institute of Oceanography, Dalhousie University, Halifax)
Edwin R. Helwig, 1932
(University of Pennsylvania; later, University of Colorado)
Walter N. Hess, 1930, 1937
(Hamilton College)
Davenport Hooker, 1905, 1907–1909, 1914
(Yale University)
Dwight L. Hopkins, 1928–1930
(Duke University; later, Mundelein College, Chicago)
Robert Tracy Jackson, 1912
(Museum of Comparative Zoology, Harvard University)
Merkel H. Jacobs, 1911
(University of Pennsylvania)
Norris Jones, 1936–1937
(Swarthmore College)
Harvey E. Jordan, 1907, 1909, 1912–1914
(University of Virginia)
E. Jörgensen, 1910 (University of Bergen)
Carl Kellner, 1905–1907, 1909
(Yale University)
Milton J. Kopac, 1932–1934, 1936
(University of California; later, New York University)
Beverly W. Kunkel, 1930 (Lafayette College)
Karl S. Lashley, 1913–1915
(Johns Hopkins University; later, Harvard University and Yerkes Laboratories of Primate Biology)
Marius Le Compte, 1936
(Royal Museum of Natural History, Brussels)
James L. Leitch, 1931, 1933, 1935
(University of California; later, Armstrong College)
Ivey F. Lewis, 1927 (University of Virginia)
Frank R. Lillie, 1935–1936
(University of Chicago)
Edwin Linton, 1906–1909
(Washington and Jefferson College; later, University of Pennsylvania)

Charles B. Lipman, 1920, 1922–1923
(University of California)
Balduin Lucké, 1936–1937
(University of Pennsylvania Medical School)
Jesse F. McClendon,
1908–1910, 1916–1917, 1919
(University of Missouri; later, University of Minnesota and Einstein Medical Center, Philadelphia)
Oliver McCoy, 1927–1928
(Johns Hopkins University; later, University of Rochester and China Medical Board of New York)
Harold W. Manter, 1929–1931, 1933
(University of Nebraska)
Gordon Marsh, 1929, 1934–1937
(University of Iowa)
James C. Martin, 1933
(University of California)
Cloyd Heck Marvin, 1932
(George Washington University)
Samuel O. Mast, 1910 (Goucher College; later, Johns Hopkins University)
George Matthai, 1915 (Emmanuel College, Cambridge University)
Grace Medes, 1915 (Bryn Mawr College; later, Lankenau Hospital Research Center, Philadelphia)
Seth E. Meek, 1909
(Field Museum of Natural History, Chicago)
Charles W. Merriam, 1932
(University of California; later, Cornell University and U. S. Geological Survey)
Harry M. Miller, Jr., 1924–1926, 1928
(Washington University; later, Rockefeller Foundation, Paris)
Sergius Morgulis, 1923–1924
(Creighton University)
Charles E. Moritz, 1935
(University of California; later, Redlands College and Philip Morris and Company)
Theodor Mortensen, 1916
(University of Copenhagen)
Paul A. Nicoll, 1932, 1934–1935, 1937
(Washington University; later, Indiana School of Medicine)
Raymond C. Osburn, 1908, 1914
(New York Aquarium; later, Ohio State University)
Fernandus Payne, 1932, 1937
(University of Indiana)
Arthur S. Pearse, 1927, 1930
(Duke University)
Henry F. Perkins, 1903–1905
(University of Vermont)
Alexander Hamilton Phillips, 1915
(Princeton University)
Robert F. Pitts, 1935
(New York University; later, Cornell University)
Harold H. Plough, 1935–1937
(Amherst College)
Frank M. Potts, 1913–1915, 1920, 1922
(Cambridge University)
Philip B. A. Powers, 1932, 1936
(University of Pennsylvania)
Henry S. Pratt, 1909–1910, 1924
(Haverford College)
Jacob E. Reighard, 1905, 1907, 1909
(University of Michigan)
Edwin E. Reinke, *Research Associate*
1911–1915 (Vanderbilt University)
Oscar W. Richards, 1933, 1935
(Yale University; later, American Optical Company, Southbridge, Massachusetts)
Gordon A. Riley, 1937 (Yale University; later, Bingham Oceanographic Laboratory, Yale University)
Asa A. Schaeffer, *Research Associate*
1919, 1921–1927, 1929
(University of Tennessee; later, Temple University)
Waldo L. Schmitt, 1924, 1929–1931
(U. S. National Museum)
William A. Setchell, 1920, 1922–1923
(University of California)
Eugene W. Shaw, 1915
(U. S. Geological Survey)
Clarence R. Shoemaker, 1925
(U. S. National Museum)
Charles F. Silvester, 1915
(Princeton University; later, Captain, U. S. Army)
H. G. Smith, 1933 (University of Bristol)
Frederick C. Steward, 1932–1934, 1936
(University of London; later, Cornell University)
Charles R. Stockard, 1907–1910
(Cornell University Medical College)
Raymond G. Stone, 1930–1931, 1934
(University of Kansas City)
Frank A. Stromsten, 1907–1910
(University of Iowa)
Geoffrey Tandy, 1930, 1932
(British Museum of Natural History)
Vance Tartar, 1937 (Yale University)

Shiro Tashiro, 1914–1915
(University of Chicago; later, University of Cincinnati)
Charles V. Taylor, 1924–1925
(University of California; later, Stanford University)
William R. Taylor, 1924–1925
(University of Pennsylvania)
David M. Tennent, 1936 (Yale University; later, Merck Institute for Therapeutic Research and Hess and Clark Division of Richardson-Merrell, Inc.)
Harry Beal Torrey, *Research Associate* 1926–1927 (Cornell University Medical School; later, Stanford University)
Aaron L. Treadwell, 1904, 1909–1910, 1913–1916, 1918, 1920–1921 (Vassar College)
Joseph M. Valentine, 1925
(Yale University; later, Alabama Museum of Natural History)
Gilbert Van Ingen, 1915
(Princeton University)
T. Wayland Vaughan, *Research Associate* 1908–1917, 1919, 1922–1923
(U. S. Geological Survey; later, Scripps Institution of Oceanography)
J. Paul Visscher, 1929–1930
(Western Reserve University)
W. Seward Wallace, 1908
(University of Nevada)
John C. Waller, 1915
(King's College, Cambridge University)
William B. Wartman, 1928
(University of Pennsylvania Medical School; later, Northwestern University)
John B. Watson, 1907, 1909–1915
(University of Chicago; later, William Esty and Company, New York)
John W. Wells, 1931 (Cornell University)
Roger C. Wells, 1919
(U. S. Geological Survey)
E. I. Werber, 1915 (Yale University)
Douglas M. Whitaker, 1925
(Stanford University)
J. L. Williams, 1931
(University of California)
Benjamin H. Willier, 1935
(University of Rochester; later, Johns Hopkins University)
Henry V. Wilson, 1924
(University of North Carolina)
J. M. Wilson, 1932–1933
(Medical College of South Carolina)
C. M. Yonge, 1933 (University of Bristol)
Charles Zeleny, 1906–1909
(University of Indiana; later, University of Illinois)

DEPARTMENT OF MERIDIAN ASTROMETRY

1906–1936

COMMITTEE ON MERIDIAN ASTROMETRY

1936–1938

DUDLEY OBSERVATORY

Albany, New York

Directors

Lewis Boss, 1905–October 5, 1912
Benjamin Boss, 1912–1936; Chairman, Committee on Meridian Astrometry, 1936–1938

Staff Members

Sebastian Albrecht, 1913–1937
Heroy Jenkins, 1909–1937
Harry Raymond, 1905–1940
Arthur J. Roy, 1903–1936
William B. Varnum, 1903–1936

NUTRITION LABORATORY

Organized in 1907, opened in 1908. Activities terminated January 1, 1946.

Directors

Francis G. Benedict, 1907–1937
Thorne M. Carpenter, 1938–1942 (Acting); 1943–1945

Staff Members

V. Coropatchinsky, 1923–1946
Harold L. Higgins, 1908–1915
Robert C. Lee, 1929–1944
Walter R. Miles, 1914–1922
H. Monmouth Smith, 1913–1920

Other Scientists and Scholars Associated with the Department

Henry P. Armsby, 1919–1920
(Pennsylvania State College)
James E. Ash, 1915
(Harvard University Medical School; later, Army Medical Museum)
Cornelia Golay Benedict, 1918–1920, 1923, 1925–1926, 1929
Edward H. Bensley, 1935
(Montreal General Hospital)
C. C. Benson, 1912, 1928–1929
(University of Toronto)
Alice F. Blood, 1917–1918
(Simmons College)
Samuel Brody, 1927 (University of Missouri)
Ernest W. Brown, 1911
(U. S. Navy Medical Corps)
John M. Bruhn, 1932–1934
(Yale Anthropoid Experiment Station, Orange Park, Florida; later, University of Alabama School of Medicine)
M. Lucien Bull, 1914 (Institut Marey, Paris)
Walter G. Cady, 1912–1913
(Wesleyan University)
E. P. Cathcart, *Research Associate* 1912–1914 (University of Glasgow)
Elizabeth E. Crofts, 1924
(Mount Holyoke College)
G. H. de Paula Souza, 1920 (São Paulo, Brazil)
David B. Dill, 1935 (Harvard University)
Raymond Dodge, 1912–1913
(Wesleyan University; later, Yale University)
Eugene F. Du Bois, 1915, 1921, 1925–1927, 1930
(Russell Sage Institute of Pathology; later, Cornell University Medical College)
David L. Edsall, 1912
(Washington University Medical School; later, Harvard University)
H. T. Edwards, 1935 (Harvard University)
W. Falta, 1909
(First Medical Clinic, Vienna)
Gertrude A. Farr, 1925–1929
(University of New Hampshire)
John M. Fuller, 1925–1926
(New Hampshire Agricultural Experiment Station)
James L. Gamble, 1913
(Harvard University Medical School)
H. S. D. Garven, 1927–1932
(Moukden Medical College, Manchuria)
Florence Gustafson, 1925–1927
(Wellesley College)
Tom S. Hamilton, 1925 (University of Illinois Agricultural Experiment Station)
C. S. Hicks, 1927–1930
(University of Adelaide, South Australia)
Fred A. Hitchcock, 1932
(Ohio State University)
John Homans, 1910–1912
(Harvard University Medical School)
Roy G. Hoskins, 1933
(Harvard University Medical School; later, Tufts College)
Elliott P. Joslin, 1909–1925, 1930, 1941–1943
(New England Deaconess Hospital, Boston; later, Harvard University Medical School)
Howard T. Karsner, 1914–1915
(Harvard University Medical School; later, Bureau of Medicine and Surgery, Navy Department)

Leslie G. Kilborn, 1927–1932
(West China Union University; later, University of Hong Kong)
Zing Yang Kuo, 1938 (Hangchow, China)
Walter Landauer, 1931
(Storrs Agricultural Experiment Station)
Milton O. Lee, 1935–1936
(Harvard University Medical School)
Helge Lundholm, 1929
(McLean Hospital, Waverley, Massachusetts; later, Duke University)
Grace MacLeod, 1922–1927
(Teachers College, Columbia University)
Eleanor D. Mason, 1927–1933
(Women's Christian College, Madras)
James H. Means, 1913–1915
(Massachusetts General Hospital, Boston; later, Massachusetts Institute of Technology)
Mary Henderson Meyer, 1931
(Massachusetts Home, Boston)
Carey D. Miller, 1928–1935
(University of Hawaii Experiment Station)
Sergius Morgulis, 1913 (Creighton University; later, University of Nebraska College of Medicine)
John R. Murlin, 1909
(Cornell University Medical College; later, University of Rochester College of Medicine)
Hans Murschhauser, *Research Associate* 1914 (Düsseldorf, Germany)
Julius Nitzulescu, 1928
(Faculty of Medicine, Jassy, Roumania)
Francis W. Peabody, 1915
(Peter Bent Brigham Hospital, Boston)
Josef M. Petrik, 1929
(Masaryk University, Brno, Czechoslovakia)
Joseph H. Pratt, 1911–1913
(New England Medical Center, Boston)
E. G. Ritzman, *Research Associate*, 1933–1939
(University of New Hampshire)
F. W. Rolph, 1918 (University of Toronto)
Howard F. Root, 1921–1927, 1930, 1933–1934, 1936, 1939–1943
(New England Deaconess Hospital, Boston)
Paul Roth, 1911–1914, 1917–1921, 1923
(Battle Creek Sanitarium, Michigan)
George C. Shattuck, 1929–1930
(Harvard University Medical School)
Henry C. Sherman, 1934–1936
(Columbia University)
Hazeltine L. Stedman-Parmenter, 1925–1927
(Mount Holyoke College)
Nils Stenström, 1920 (Stockholm, Sweden)
F. Strieck, 1928
(University of Würzburg, Germany)
Fritz B. Talbot, 1911–1922, 1924
(Harvard University Medical School)
Carl Tigerstedt, *Research Associate*, 1913–1914
(University of Helsingfors)
Harry C. Trimble, 1939–1940
(Harvard University Medical School)
Abby H. Turner, 1924, 1927–1929
(Mount Holyoke College)
E. C. van Leersum, 1920
(Institute for Human Nutrition, Amsterdam)
H. S. Halcro Wardlaw, 1931 (Australia)
Laurence G. Wesson, 1938
(Veader Leonard Laboratory of Experimental Therapeutics, Baltimore; later, Massachusetts Institute of Technology)
Paul Dudley White, 1937
(Massachusetts General Hospital, Boston)
Priscilla White, 1936, 1939
(New England Deaconess Hospital, Boston)
John C. Whitehorn, 1929
(McLean Hospital, Waverley, Massachusetts; later, Johns Hopkins Hospital)
Francis H. Williams, 1912
(Boston City Hospital)
G. D. Williams, 1926–1927
(Washington University Medical School)
Stanley D. Wilson, 1930–1935
(Yenching University, Peiping)
Robert M. Yerkes, 1932–1934
(Yale Anthropoid Experiment Station, Orange Park, Florida)

FELLOWS OF THE CARNEGIE INSTITUTION OF WASHINGTON

Office of Administration

Horace B. Barlow, 1961 (King's College, Cambridge University)

Department of Plant Biology

Herbert G. Baker, 1948–1949
(University of California)
Shao-lin Chen, 1949–1950
(Red Star Yeast Company)
Edwin A. Davis, 1949–1950
(U. S. Department of Agriculture)
L. N. M. Duysens, 1952–1953
(University of Leiden)
Fulton J. F. Fisher, 1956–1957
(University of Melbourne)
Joop C. Goedheer, 1957–1958
(University of Utrecht)
Bessel Kok, 1951–1952
(Research Institute for Advanced Studies, Baltimore)
Paul H. Latimer, 1956–1957
(Auburn University)
Josef E. Loeffler, 1954–1955
(Shell Development Company)
Fergus D. H. Macdowall, 1947–1949
(Canadian National Research Council)
Guy C. McLeod, 1959–1960
(SIAS Institute, Brooks Hospital, Brookline, Massachusetts)
Ruth Sager, 1961 (Columbia University)
Jerome A. Schiff, 1962 (Brandeis University)
Kazuo Shibata, 1956
(Tokugawa Institute for Biological Research)
Hemming I. Virgin, 1954
(University of Gothenburg)

Mount Wilson and Palomar Observatories

M. K. Vainu Bappu, 1951–1952
(Astrophysical Observatory, Kodaikanal, India)
Geoffrey R. Burbidge, 1955–1957
(University of California, La Jolla)
William A. Buscombe, 1950–1952
(Mount Stromlo Observatory, Australian National University, Canberra, Australia)
Edward R. Dyer, Jr., 1949–1950
(National Academy of Sciences)
Roger F. Griffin, 1960–1961
(St. John's College, Cambridge University)
Colin S. Gum, 1959–1960
(Radiophysics Laboratory, Commonwealth Scientific and Industrial Research Organization, Sydney, Australia)
Karl G. Henize, 1955–1957
(Dearborn Observatory, Northwestern University)
Léo Houziaux, 1960–1962
(Institut d'Astrophysique, University of Liége)
Thomas A. Matthews, 1956–1958
(California Institute of Technology)
Charles Robert O'Dell, 1962—
(Mount Wilson and Palomar Observatories)
George W. Preston, III, 1959–1961
(Lick Observatory, Mount Hamilton)
Alexander W. Rodgers, 1959–1960
(Mount Stromlo Observatory, Australian National University, Canberra, Australia)
John B. Rogerson, Jr., 1954–1956
(Princeton University Observatory)
Stewart L. Sharpless, 1952–1953
(U. S. Naval Observatory)
Carlos M. Varsavsky, 1959
(Comisión de Astrofísica y Radioastronomía, Buenos Aires)
Merle F. Walker, 1952–1954
(Lick Observatory, Mount Hamilton)

Department of Terrestrial Magnetism

Arthur I. Aronson, 1959–1960
(Purdue University)
Toshi Asada, 1960–1962
(Geophysical Institute, Tokyo)
Manuel N. Bass, 1958–1959
(Northwestern University)
Prabhat K. Bhattacharya, 1948–1950
(California Institute of Technology)
Louis Brown, 1961— (University of Basel)
Mateo Casaverde, 1948
(Instituto Geofísico del Peru)
William Compston, 1958
(Australian National University)
E. H. Creaser, 1955–1956
(University of Cambridge)
J. D. Duerksen, 1959–1960
(National Institute for Medical Research, London)

William C. Erickson, 1956–1957
(Leiden Observatory)
Gonzalo Fernandez, 1948–1949
(Instituto Geofísico del Peru)
George B. Field, 1953
(Princeton University Observatory)
J. W. Findlay, 1952
(National Radio Astronomy Observatory, Green Bank)
Kenneth L. Franklin, 1954–1956
(Hayden Planetarium)
John W. Graham, 1947–1949
(Woods Hole Oceanographic Institution)
Ronald Green, 1961–1962
(University of Tasmania, Hobart)
Richard Hall, 1962— (Indiana University)
Pembroke Jones Hart, 1952–1954
(National Science Foundation)
H. Lawrence Helfer, 1953–1957
(University of Rochester)
Ellis S. Kempner, 1958
(National Institutes of Health)
John J. Leahy, 1956–1957
(City of Hope Hospital, California)
Howard M. Lenhoff, 1958
(Howard Hughes Medical Institute, Miami)
Sören Lovtrup, 1951–1952
(Carlsberg Laboratories, Copenhagen)
John E. Midgley, 1960–1962
(Oxford University)
Thomas Murphy, 1947–1948
(National University of Ireland)
Jatinder Nath Nanda, 1949–1951
(Indian Naval Physical Laboratory, New Delhi)
Leif Owren, 1953–1954
(Geophysical Institute, College, Alaska)
W. D. Parkinson, 1947–1948
(Bureau of Mineral Resources, Melbourne, Australia)
Gerald C. Phillips, 1950–1952 (Rice Institute)
George F. Pieper, 1956–1957
(Applied Physics Laboratory, Johns Hopkins University)
Hector Rojas, 1961–1962
(Pan American College Observatory, Edinburgh, Texas)
Hermann Rudin, 1962— (University of Basel)
Jorma J. Rühimas, 1959–1960
(University of Helsinki)
George C. Sponsler, 1950
(U. S. Department of the Navy)
M. Sugiura, 1955
(Geophysical Institute, College, Alaska)
Harold Weaver, 1956–1957
(Lick Observatory, Mount Hamilton)
James A. Weinman, 1958–1960
(University of Wisconsin)
Dexter Whitehead, 1947–1948
(University of Virginia)
Francis Waverly Wood, 1949–1951
(Bureau of Mineral Resources, Melbourne, Australia)

Department of Genetics

Guiseppe Bertani, 1948–1949
(Karolinska Institutet, Stockholm)
Katherine S. Brehme (Warren), 1939–1941
(National Institutes of Health)
Hugh J. Cairns, 1960–1961
(Australian National University, Canberra)
H. Clark Dalton, 1948–1950
(Washington Square College, New York University)
Berthe Delaporte, 1948–1949
(École Pratique des Hautes Études, Paris)
A. H. Doermann, 1947–1949
(Vanderbilt University)
Kazuo Hashimoto, 1957–1958
(Keio University School of Medicine, Tokyo)
Etta Käfer (Boothroyd), 1956–1957
(McGill University)
Joseph D. Mandell, 1955–1957
(Palo Alto Medical Research Foundation)
Hermann Moser, 1953–1956
(Frances Delafield Hospital, New York City)
Frank H. Mukai, 1959
(Biological Laboratory, Long Island Biological Association)
Kenneth Paigen, 1950–1952
(Roswell Park Memorial Institute, Buffalo)
Catherine Roesel, 1950–1951
(University of Georgia School of Medicine)
Janine Séchaud, 1960 (University of Oregon)
Atif Sengün, 1957
(University of Istanbul, Turkey)
Robert C. von Borstel, 1952–1953
(Oak Ridge National Laboratory)

1947-1962

Geophysical Laboratory

Ralph Arnold, 1956–1959
(Princeton University; later, Saskatchewan Research Council, University of Saskatchewan)
D. Kenneth Bailey, 1962—
(Trinity College, Dublin, Ireland)
Hubert L. Barnes, 1956–1959
(Columbia University; later, Pennsylvania State University)
Robin Brett, 1961— (Department of Geological Sciences, Harvard University)
Charles W. Burnham, 1961—
(Massachusetts Institute of Technology)
Peter R. Buseck, 1961—
(Department of Geology, Columbia University)
G. A. Chinner, 1958–1960
(University of Cambridge)
John de Neufville, 1961–1962
(Yale University; later, Harvard University)
Bruce R. Doe, 1960–1962
(California Institute of Technology; later, U. S. Geological Survey)
W. Gary Ernst, 1955–1958
(Johns Hopkins University; later, University of California, Los Angeles)
Jeff J. Fawcett, 1961—
(University of Manchester)
S. B. Halferdahl, 1954–1958
(Johns Hopkins University; later, Research Council of Alberta, Edmonton, Alberta, Canada)
Kai Hytönen, 1959–1961
(University of Helsinki; later, Geological Survey of Finland, Otaniemi)
Mackenzie L. Keith, 1947–1950
(Pennsylvania State University)
Donald H. Lindsley, 1960–1962
(Johns Hopkins University)
Günter Moh, 1962 (Heidelberg University)
Nobuo Morimoto, 1957–1959, 1962
(Mineralogical Institute, University of Tokyo)
Kaarlo J. Neuvonen, 1948–1950
(Geological Survey of Finland; later, University of Turku, Finland)
Louis Otto Nicolaysen, 1951–1954
(Massachusetts Institute of Technology; later, Bernard Price Institute of Geophysical Research, Johannesburg, South Africa)
Philip M. Orville, 1957–1958
(Yale University; later, Cornell University)
Edwin W. Roedder, 1947–1948
(Columbia University; later, U. S. Geological Survey)
Eugene H. Roseboom, 1956–1959
(Harvard University; later, U. S. Geological Survey)
Bruno Sabels, 1962 (University of Nevada)
Th. G. Sahama, 1947–1949
(University of Helsinki)
Werner F. Schreyer, 1958–1959, 1962—
(University of Kiel)
James R. Smith, 1954–1957
(Princeton University; later, Saskatchewan Research Council, University of Saskatchewan)
Joseph Victor Smith, 1951–1954
(Cavendish Laboratory, University of Cambridge; later, Pennsylvania State University and University of Chicago)
Yoshio Suzuki, 1960–1962
(Hakkaido University, Japan; later, Geological Survey of Japan)
Per-Fredrick Tröften, 1960
(Norwegian Geological Survey; later, Geofysisk Malmleting, Trondheim, Norway)
Allan C. Turnock, 1958–1960
(University of Manitoba; later, Department of Mines and Technical Surveys, Ottawa)
J. R. Vallentyne, 1956–1957
(Queen's University, Ontario; later, Cornell University)
Bruce Velde, 1962—
(Montana State University)
David R. Wones, 1957–1959
(Massachusetts Institute of Technology; later, U. S. Geological Survey)
Kenzo Yagi, 1950–1951, 1960–1961
(Tohoko University, Japan)
Richard A. Yund, 1959–1961
(University of Illinois; later, Brown University)

MacKenzie, W. S., 1951-1952
(Cambridge U., later U. of Manchester)

Department of Embryology

Michael Abercrombie, 1962
(University College, London)
Vittorio Danesino, 1953–1954
(University of Naples)
L. E. DeLanney, 1957 (Wabash College)
Christine Gilbert, 1950–1951
(University of the Witwatersrand)
Perry W. Gilbert, 1949–1950
(Cornell University)
E. Clark Gillespie, 1948
(Johns Hopkins University; later,
University of Arkansas)
Richard J. Goss, 1960–1961
(Brown University)
Jerome S. Harris, 1948–1949
(Johns Hopkins University; later,
private practice in obstetrics in Denver)
Beni Horvath, 1952–1953
(Columbia University; later,
National Institutes of Health)
Yoshihiro Kato, 1959–1961
(Tokyo University; later,
University of Nagoya)
Efstathios J. Kokrikos, 1953–1954
(Red Cross Hospital, Athens)
Ben C. Moffett, Jr., 1954
(University of Alabama; later,
Armed Forces Institute of Pathology)
Brenda Schofield, 1953–1954
(Oxford University)
E. Carl Sensenig, 1945—
(University of Alabama)
Peter H. S. Silver, 1961–1962
(Middlesex Hospital Medical School,
London)
Malcolm S. Steinberg, 1956–1958
(Johns Hopkins University)
Ikuo Takeuchi, 1959–1961
(Princeton University; later,
University of Osaka)
L. J. Wells, 1948 (University of Minnesota)
Douglas R. Wilkie, 1955
(University of London)
Ian B. Wilson, 1961–1962
(University College of North Wales)
Fred H. Wilt, 1958–1960 (Purdue University)

Department of Archaeology

Robert H. Barlow, 1949–1950
(Mexico City College)
Heinrich Berlin, 1952–1955
(Instituto de Antropología e Historia de
Guatemala)
Joseph A. Hester, Jr., 1952–1954
(University of California, Los Angeles)
William T. Sanders, 1954–1955
(Pennsylvania State University)
Raymond H. Thompson, 1950–1952
(University of Arizona)

GRANTEES AND OTHERS AFFILIATED WITH THE CARNEGIE INSTITUTION BUT NOT WITH PARTICULAR DEPARTMENTS

Chemistry

Solomon F. Acree, 1904–1913
(Johns Hopkins University; later,
National Bureau of Standards)
Charles Baskerville, 1903–1905
(College of the City of New York)
Gregory P. Baxter, *Research Associate*
1904–1914, 1924 (Harvard University)
Gustavus E. Behr, 1906 (Harvard University)
Amos P. Brown, 1904–1908
(University of Pennsylvania)
Paul B. Davis, *Research Associate*, 1916–1917
(Johns Hopkins University; later,
Davison Chemical Corporation, Baltimore)
Louis M. Dennis, 1903 (Cornell University)
Howard W. Doughty, 1904
(Johns Hopkins University; later,
Amherst College)
George S. Forbes, 1906 (Harvard University)
Joseph C. W. Frazer, 1916–1918
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Harry C. Jones, 1903–1916
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Physics

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(National Bureau of Standards)
Henry Crew, 1902–1904
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Paul S. Epstein, 1937–1939
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1932, 1938, 1939, 1942, 1946, 1949
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1936–1938, 1947 (Bandung, Java)
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(National Academy of Sciences)
Henry S. Williams, 1902 (Yale University)
Howell Williams, 1943–1944
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Samuel W. Williston, 1903
(University of Chicago)
Robert W. Wilson,
1933–1934, 1936–1937, 1940, 1942, 1949
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Wendell P. Woodring, 1925, 1928, 1932
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Archaeology and Anthropology

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Waldemar Jochelson, 1923–1924, 1926, 1929–1930, 1934–1935 (American Museum of Natural History)
Allan C. Johnson, 1910–1911 (Princeton University)
W. Max Müller, 1904–1907, 1910–1911 (University of Pennsylvania)
Raphael Pumpelly, 1903–1906 (Newport, Rhode Island)
Hubert Schmidt, 1903–1904 (Museum für Völkerkunde, Berlin)
George W. Scott, 1904–1905, 1911–1914 (Law Librarian of Congress and Supreme Court)
Thomas D. Seymour, 1903 (American School of Classical Studies, Athens)
Esther Boise Van Deman, *Research Associate* 1906–1925 (American School of Classical Studies, Rome; later, University of Michigan)
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Andrew F. West, 1905–1911 (Princeton University)
James R. Wheeler, 1905–1912 (American School of Classical Studies, Athens; later, Columbia University)

Bibliography

Cyrus Adler, 1902 (Smithsonian Institution)
J. McKeen Cattell, 1902–1904 (Columbia University; later, editor, *American Men of Science*)
Wilberforce Eames, 1906–1908 (librarian, Bibliographical Society of America)
Fielding H. Harrison, *Research Associate* 1903–1927 (Army Medical Museum; later, Surgeon General's Office)
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Literature, Linguistics, and Philology

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John Pawley Bate, 1910–1917 (Inns of Court, London)
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Albert G. de Lapradelle, 1910–1916 (University of Paris)
Charles G. Fenwick, 1910–1916 (Carnegie Endowment for International Peace; later, Bryn Mawr College)
Ewald Flügel, 1904–1908 (Stanford University)
George D. Gregory, 1916 (Carnegie Endowment for International Peace)
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Arthur G. Kennedy, 1916–1918, 1920, 1923 (Stanford University)
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John D. Maguire, 1909–1913 (Catholic University of America)
Ernest Nys, 1917 (University of Brussels)
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James Brown Scott, *Research Associate* 1910–1917 (Department of State; later, Carnegie Endowment for International Peace)
E. W. Scripture, 1903–1906 (Yale University)
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Herbert Francis Wright, 1917 (Catholic University of America; later, Georgetown University)

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Reports of Departments and Special Studies

Mount Wilson and Palomar Observatories

Geophysical Laboratory

Department of Terrestrial Magnetism

Committee on Image Tubes for Telescopes

Department of Plant Biology

Department of Embryology

Department of Genetics

Mount Wilson and Palomar Observatories

Operated by Carnegie Institution of Washington and California Institute of Technology

Pasadena, California

Contents

INTRODUCTION

In 1904 George E. Hale, acting under the auspices of the National Academy of Sciences, invited the scientific academies and the astronomical and physical societies of a number of countries to send representatives to a meeting to be held in connection with the International Congress of Science at the St. Louis Exhibition for the purpose of establishing "co-operation among individuals and institutions engaged in Solar Research." This meeting resulted in the formation of the International Union for Co-operation in Solar Research, which held later meetings at Oxford (1905), Meudon (1907), Mount Wilson (1910), and Bonn (1913). The Mount Wilson meeting was attended by about 80 members of the Union and invited guests.

After World War I, the Union was reorganized on a broader basis to include all branches of astronomy and its name was changed to the International Astronomical Union. Assemblies of the Union were held at Rome (1922), Cambridge, England (1925), Leiden (1928), Cambridge, Massachusetts (1932), Paris (1935), and Stockholm (1938). After a ten-year intermission caused by the second World War, meetings occurred at Zurich (1948), Rome (1952), Dublin (1955), and Moscow (1958). The next General Assembly in 1961 was planned for the United States of America, and it was hoped that it might be held in Pasadena. However, a survey of the hotel situation indicated that to accommodate locally the more than 1000 members and guests who have attended these meetings in recent years would be impossible. The 1961 General Assembly of the International Astronomical Union was therefore held in Berkeley, between August 15 and 24, the University of California acting as host institution.

Several international symposia took place just before or after the Berkeley meeting. One of these, on the subject of space age astronomy, was sponsored by the Douglas Aircraft Company and was held at the California Institute of Technology on August 7, 8, and 9. It was attended by about 100 engineers and astronomers.

Because of the interest in the large telescopes at Mount Wilson and Palomar Mountain, arrangements were made by the Observatories to provide transportation from Los Angeles and entertainment on the mountains for the foreign delegates to the Assembly of the Union. Trips to Mount Wilson were scheduled on the afternoons of August 11 and 25, and to Palomar on August 12 and 26. About 275 delegates took advantage of this opportunity to visit the facilities on the mountains.

Nearly all members of the staff of the Observatories attended the Assembly at Berkeley and participated in the sessions of the various commissions of which they were members.

Throughout the history of the Observatories the major emphasis has been placed on observations of the sun, stars, nebulae, and galaxies. From time to time, however, when the Observatories' equipment was suitable, attention has been given to observations of planets and satellites. For example, satellites X, XI, and XII of Jupiter and the very unusual asteroids Icarus and Geographos were discovered at the Observatories. High-dispersion spectroscopic studies of Venus and Mars by Adams and Dunham provide the basis for the current knowledge of the composition of their atmospheres. Infrared observations by Nicholson and Pettit of the lunar surface during an eclipse led to the concept of a surface covered with dust. Recently these infrared lunar observations were refined by Dr. Shorthill and Mr. Saari of the Boeing Aircraft Company, using the 60-inch on Mount Wilson. In 1958 and 1960 the 200-inch was used by Dr. Sinton of the Lowell

Observatory to map the areas on Mars that show the absorption bands near 3.4 μ which are attributed to organic molecules.

The development in the last few years of rockets capable of going to the neighborhoods of the moon and the inner planets has focused attention on lunar and planetary problems. Because of the much lower effort and cost required for ground-based observations compared with observations made from rockets, it has become important to push these solar system observations to the limits made possible with the new photometric and infrared techniques developed in recent years.

In the past year G. Münch, with the assistance of Mr. Robert Younkin of the Jet Propulsion Laboratory, has used the Cassegrain spectrum scanner to investigate the monochromatic albedo and the total intensity of the absorption bands in the spectra of the major planets; Münch has also obtained high-dispersion spectra of Jupiter, Saturn, and Neptune, which have been studied in collaboration with Dr. Hyron Spinrad of the same Laboratory. Lines of the hydrogen molecule at λ6367.80 and λ6435.03 were found in the spectrum of Saturn, providing the first definite evidence for the presence of hydrogen in its atmosphere. Dr. Spinrad, analyzing the high-dispersion spectra of Venus available in the files of the Observatories, has found evidence for large changes in the apparent temperature of the atmosphere. Dr. Bruce Murray of the Lunar Research Laboratory at the California Institute of Technology has continued the studies of the photoelectric colorimetry of the moon with the 60-inch telescope. He has also developed and tested on Mount Wilson a special 20-inch infrared telescope which will be used for lunar studies at an altitude of 13,000 feet on White Mountain.

OBSERVING CONDITIONS

After three years in which the rainfall on Mount Wilson averaged less than 45 per cent of normal, the precipitation for 1961–1962 jumped to 46.14 inches, or within an inch of the total for the preceding three years. This increased rainfall came just in time to avoid a serious water shortage on the mountains.

Solar observations were made on 311 days; the 200-inch was in use on 287 nights, the 100-inch on 292 nights, and the 60-inch on 265 nights.

SOLAR OBSERVATIONS

Solar observations were made by Cragg, Hickox, and Utter. The numbers of photographs of the various kinds taken between July 1, 1961, and June 30, 1962, were as follows:

Direct photographs	302
Hα spectroheliograms, 18-foot focus	270
K2 spectroheliograms, 18-foot focus	258
K2 prominences, 18-foot focus	84
Number of days on which magnetograms were obtained	223

Effective September 1, 1961, a basic change was made in the method of reduction of the sunspot magnetic data. In the past, sunspot groups were numbered and a list of these groups along with average magnetic classifications and dates of central meridian passage was published, or otherwise made available, every year or so. More recently, investigators interested in this information have wanted it available rapidly, and they have frequently been interested in the appearance of the sun on some particular day. To meet these needs, and also to save time in the reduction process, a list is now prepared each month giving daily positions and magnetic classifications of spot groups for which there are magnetic measures. Copies of this list are sent each month to interested investigators. As the routine observing program, except for the magnetograms, has a low priority, the

sunspot information is not as complete as in previous years. Sunspot groups are no longer numbered, and no attempt is made to keep track of returns. The K2 prominence patrol was also ended in September.

Because spot magnetic polarities are not observed as often as in the past, and because the method of reduction has been altered, the tables of sunspot groups and classification usually published in the Annual Report will be discontinued. Magnetic classifications of spot groups were made on 161 days from July 1, 1961, to June 30, 1962.

Solar Magnetic Fields

Howard has completed a preliminary study of solar magnetograph observations made with very small apertures. Magnetic traces with an aperture about 2 seconds of arc on a side show root-mean-square fluctuations of 8.2 ± 4.4 gauss. The autocorrelation function derived from these observations shows maxima near 16,000 km and 40,000 km and in general resembles the autocorrelation function that Rogerson derived from intensity fluctuations on calcium spectroheliograms. Similar observations made recording line-of-sight velocities yield root-mean-square fluctuations of 0.39 ± 0.14 km/sec. Observations made recording velocities with an aperture held fixed show autocorrelation curves that are damped cosine curves, indicating the presence of oscillations in the solar atmosphere. The period observed is 296 seconds. The spectrum line used for all these observations was λ5250.218, Fe I. Instrumental improvements since these observations were completed in the summer of 1959 enable us now to make much better observations of this type. Further observations are planned for the near future.

The daily solar magnetograms, started in 1957, constitute a unique series of observations giving valuable information about daily configurations of the solar magnetic fields. Howard has begun an extensive study of these records, which will include classification of magnetic regions and their correlation with optical and radio phenomena. The investigation starts with the magnetograms from August 1959, when the new slant-line registration was begun. One interesting result that has appeared at this stage of the investigation concerns the UM regions first discovered by H. W. and H. D. Babcock. A large number of UM regions have been identified; invariably, at the position of the UM region the calcium (K2) spectroheliogram for that day shows mottlings somewhat brighter than the ordinary background. Thus it may be possible to detect UM regions over a period of fifty years or more using the extensive solar plate collection.

Forbidden Nitrogen Lines in the Solar Spectrum

Starting from Vitense's model of the solar atmosphere and from a recent determination of the abundance of nitrogen by Neven, the intensities of the [N I] λ10397 and λ10407 lines have been computed by Houziaux. From a comparison of these results with the intensity of the weak feature observed at λ10397 on several spectrograms recently obtained by Migeotte at the Jungfraujoch it can be concluded that the forbidden doublet $^2D_{5/2}-{}^2P_{1/2,\,3/2}$ is present in the infrared solar spectrum. The $^2D_{3/2}-{}^2P_{1/2,\,3/2}$ doublet is hidden by a strong line of unknown origin.

PLANETS

An observing program has been started by G. Münch to serve as a basis for a reexamination of problems related to the structure and composition of planetary atmospheres. One aspect of the program is concerned with the determination of the energy distributions in their spectra by photoelectric scanning. The mono-

chromatic albedos of the planets and the total intensity of their absorption bands will thus be derived. Observations of the integrated light of the major planets have been obtained with the Cassegrain scanner on the 60-inch telescope, from λ3400 out to the long-wavelength sensitivity limit of photomultipliers with trialkali cathodes. These scans have in part been studied by Mr. Robert Younkin of the Jet Propulsion Laboratory. Repeated tracings of Jupiter obtained during four different periods have provided consistent results proving that there is not a steep fall in the energy distribution shortward of λ3900, as has been reported in the past. During this preliminary work, it has been found that the amount of time involved in an exhaustive study of the tracings is so great that such study is impractical without the aid of automatic data reduction equipment. An arrangement in which the output of the scanner is fed directly into a digital voltmeter with magnetic tape recording has therefore been tested. This will be reduced with an IBM 7090 computer. Further observations of the variations in color and spectra of planetary surfaces will be carried out by means of such auxiliary equipment.

Münch is utilizing the greatly increased resolving power and speed of the coudé spectrographs to take spectra of the planets under the highest dispersion possible with the purpose of detecting new spectral features and studying the structure of known ones over the various parts of the planetary disks. Plates of Jupiter, Saturn, and Uranus in the blue and yellow-red regions of the spectrum have been obtained. Part of this material is being studied in collaboration with Dr. Hyron Spinrad of the Jet Propulsion Laboratory. In these plates, Spinrad verified his discovery of the anomalous inclination of the NH_3 lines in the spectrum of Jupiter's equator. In Saturn it was found that the lines of CH_4 band at λ6190 have an inclination greater than half that of the Fraunhofer lines of the scattered solar spectrum, by about 10 per cent—an amount twice as large as the probable error of measurement. In the same plates of Saturn two sharp lines of λ6367.80 and λ6435.03 which, with certainty, must be identified with the S(1) and S(0) lines, respectively, of the 4–0 quadruple rotation-vibration band of H_2 were discovered. The possibility of observing the quadruple spectrum of H_2 was suggested by Herzberg in 1938, but this is the first observation of the S(1) and S(0) lines of the 4–0 band in any astronomical object.

COMETS

The bright comet Seki-Lines (1962c) was observed by Greenstein. It was remarkably dust-free after perihelion, the Na I lines were very weak, and the visual region of the spectrum consisted largely of NH_2 and C_2. The distortion of the CN (0, 0) band by resonance fluorescence was quite different from that of Comet Mrkos (1957d). Spectra of 1962c will be measured by Greenstein and Arpigny.

STELLAR SPECTROSCOPY AND PHOTOMETRY

During the report year, 900 spectrograms were taken with the 200-inch telescope, 970 with the 100-inch, and 550 with the 60-inch.

Chemical Composition of Stellar Atmospheres

The program for the study of the abundances of the elements in astronomical objects continued under the direction of Greenstein with the support of the Air Force Office of Scientific Research of USAF.

Spectrophotometric analyses of the high-galactic-latitude supergiants HD 161796 (F3 Ib) and 89 Herculis (F2 Ia) have been carried out by Searle, Sargent, and Jugaku. Comparisons were made with

the standard low-latitude supergiants φ Cassiopeiae (F0 Ia) and α Persei (F5 Ib). All the elements studied are found to have the same relative abundances in all these stars. Spectroscopic absolute magnitudes were derived for the high-latitude supergiants using φ Cas and α Per as calibration stars. It is concluded that both 89 Her and HD 161796 could have reached their present heights above the galactic plane in times comparable to their estimated times of evolution from the main sequence if they were expelled from the plane at the time of their formation with velocities of the order of 100 km/sec. These results—which are in disagreement with those of an earlier study of these same stars by Abt—are consistent with the view that the high-galactic-latitude supergiants are evolved runaway stars.

Additional measurements since last year on 3 Centauri A were made by Jugaku and Sargent on a Radcliffe Observatory coudé plate of the far ultraviolet for line identifications. Most of the 40 lines between λ3500 and λ3100 can be identified with Fe II, Mn II, and Ni II, although a few fairly strong lines remain unidentified. The Be II doublet at λ3130 is absent. A plate of the visual region obtained in April 1962 shows that the longward shift of λ6678 of He I, which was interpreted as an isotope shift, is still present.

The abundance analysis of κ Cancri, B8p, an Mn star, by Jugaku and Sargent is progressing. Equivalent widths of 250 lines in the photographic region have been measured. The ratio P III/P II gives a value of $\theta_{\text{ion}} = 0.39$, which is typical of a normal star of spectral type about B7. The $B - V$ color also agrees with such a temperature. A study of the hydrogen lines gives $\log P_e = 1.92$. Using these values of θ and $\log P_e$, the preliminary abundance results are P/Si $\approx$ 1 (as in 3 Cen A—this means that P is overabundant by a factor of 100). The identification of a line at λ3984 with Hg II by Bidelman leads to an overabundance of about 30,000 for Hg. Helium is deficient by a factor of about 10 (factors of 6 were found for 3 Cen A and α Sculptoris). Be is overabundant by a factor of 100 relative to the sun.

The study of the infrared O I lines in the spectra of 20 Ap stars has been completed by Searle and Sargent. Oxygen is found to be deficient with respect to hydrogen by factors ranging from 8 to more than 100 in all Ap stars of the Si-Eu-Cr, Eu-Cr, Eu-Cr-Sr, and Sr classes, whereas in the Mn stars the oxygen abundance is normal. Assuming that they originated with normal composition, the oxygen-deficient Ap stars demand that O must have been transmuted into one or more of the cosmically abundant elements by an as yet unspecified process.

The infrared N I lines fall at the limit of plate sensitivity and for this reason have been studied in only four bright Ap stars. Two of these, α^2 Canum Venaticorum and β Coronae Borealis, are deficient in O; the remaining two, φ Herculis and μ Leporis, are Mn stars with normal oxygen abundance. In none of these stars are the infrared N I lines detectable, although they are clearly present in the spectra of standard stars from B5 to F5. It appears that nitrogen is deficient in all four Ap stars by factors estimated to be 10 or more.

Infrared spectrograms, at 20 A/mm, have been obtained by Searle and Sargent of four bright metallic-line stars and four standard stars to study the behavior of the O I lines. The metallic-line stars selected fall in the two-color and color-magnitude diagram among the extreme oxygen-deficient Ap stars, but unlike the Ap stars their oxygen abundance is normal.

Spectrograms of 30 Ap stars and 6 standard stars with types between B5 and A4 have been obtained at 10 A/mm by Searle and Sargent. The pressure- and temperature-insensitive ratios of Mg II (λ4481)/Si II (λ4128, λ4130) and C II (λ4267)/He I (λ4471) are being studied

and Balmer line profiles obtained.

For the Ap stars of earliest type, the λ4200, Si and Mn classes, there is an excellent correlation between the central depth of Hγ and the $U - B$ color, identical with the correlation obtained for standard stars. There is no systematic difference between the Balmer line profiles of the Ap stars and the standard stars, and the location of an Ap star in the two-color diagram is a good indicator of the atmospheric temperature of the star.

Among the hotter Ap stars there is no evidence for Mg abundance anomalies, and Si is overabundant (by about × 10) only in stars that show the high-excitation Si II lines at λ4200. Certain sharp-line late B-type stars (e.g., 21 Aquilae and HD 207840) which have been called Si class Ap stars have Si lines of normal strength for their colors, and normal strength of He, C, O, and Mg. It is probable that they are "peculiar" only in that they have unusually sharp lines.

The C II (λ4267) strength is normal for the color in Ap stars of the Mn class and in the so-called Si stars which do not show λ4200. In these stars, the lines of He I are normal or only slightly weaker than in normal stars of the same color.

In the stars that have definite Si overabundance (the λ4200 stars), the C II line is weak (by a factor of about 5 in the equivalent width) and the He I lines are very weak (by a factor of about 10 in the equivalent width). It seems hard to escape the conclusion that the λ4200 stars are very deficient in helium.

Spectrograms of 27 Ap stars and 4 standards at 10 A/mm in the photographic ultraviolet have been obtained by Searle, Sargent, and Jugaku to study the behavior of the Be II doublet at λ3130. In the cooler Ap stars, the Be II lines, if present, are seriously blended, but in the hotter Ap stars they are free from serious blending. No lines of Be II are to be seen in nine λ4200 stars observed. Of 10 Mn stars, 6 show no trace of Be II, but the remaining 4 have very strong lines. In the Be-strong Mn stars (112 Herculis, κ Cancri, μ Leporis, and υ Herculis), Be is estimated to be overabundant by a factor of about 100. All the stars that show strong lines of Be II also have lines of Ga II in their spectra. However, π^1 Bootis, in whose spectrum Ga II lines are present, shows no trace of Be II.

Scans of the continuous spectrum of about 20 Ap stars have been made by Jugaku and Sargent with the Cassegrain scanner. They will be examined in conjunction with hydrogen-line profiles obtained from coudé plates of the same stars to see whether there is definite evidence that the atmospheres of magnetic stars differ from those of normal stars. Preliminary results show that stars like α Andromedae (B8p Mn), which have anomalously blue $B - V$ and $U - B$ colors, have complete continuous energy distributions identical to those of main-sequence stars as hot as B5.

Greenstein, Parker, Wallerstein (University of California at Berkeley), Helfer (University of Rochester), and Aller (University of Michigan) have collaborated in an extensive analysis of three red giants with extremely weak lines: HD 122563, 165195, and 221170. In last year's report, HD 165195 and 221170 were mentioned as weak-lined G dwarfs. Further analysis and the earlier incompleted work of Greenstein and Aller on HD 122563 have shown that these stars are, in fact, giants with colors and spectra like the stars in globular clusters. In many ways, these stars are extraordinary; their colors are quite red ($B - V \approx +1.0$), yet at first glance they could be mistaken for F subdwarfs. The results are temperatures near 4100°K, log $P_e = -2.5$, metal/hydrogen ratios 500 times lower than in the sun (like the most extreme subdwarf). One problem is in the opacity, which seems to be largely Rayleigh scattering, although the expected colorimetric effects are not found. In addition, the ratio of iron-group metals to heavy elements like Sr, Zr, Ba, Ce, and Eu is abnormal, when compared with the sun, in that the heavy elements are deficient

by an additional factor of 50 in HD 122563. This is the first known example of large changes within the abundances of the metals. It indicates that the stars condensed at an early stage in the life of our Galaxy, perhaps 10^7 to 10^9 years (at the very latest), and probably 10^7 to 10^8 years after the beginning of element synthesis. Another unusual effect is that Eu behaves like the other heavy elements; although Eu in the sun and earth was synthesized by the *r* process of neutron capture, in HD 122563 it was made by the *s* process. There are other elemental deficiencies, e.g., V and Mn, of a previously recorded type, and also evidence that the heavier elements were synthesized in a very metal-poor environment.

Gunn and Kraft have completed a study based mainly on 200-inch coudé spectrograms of the hydrogen-to-metal ratio in F-type stars of NGC 752, a galactic cluster of age 1×10^9 years. It has been suggested from earlier studies of small-scale spectrograms that NGC 752 may have a lowered metal abundance relative to the sun. If this were true, it would mean that as little as 10^9 years ago different regions of the Galaxy were forming stars of different metal content at the same time, though the galactic orbit of NGC 752 is nearly the same as that of the sun.

In the present analysis, careful attention was paid to deriving accurate ionization temperatures. With the aid of Hγ profiles based on model atmospheres (Searle and Oke, 1962) and known abundances (Parker, Greenstein, Helfer, and Wallerstein, 1961), the scale of T_{ion} for Hyades F-type stars was first established from curves of growth. From the models and Hγ line strengths, ionization temperatures for NGC 752 stars were estimated relative to Hyades stars. This method of determining T_{ion} replaces the customary, and somewhat unsatisfactory, procedure of estimating T_{ion} from T_{exc}. Using these temperatures, it is concluded from a study of curves of growth that the metal abundance of stars in NGC 752 does not differ from that of the sun by more than a factor of 2.

Gunn is carrying forward a program of studying "strong-line" versus "weak-line" field F stars from spectrograms of 20 A/mm dispersion taken with the 60-inch. It will be determined whether the weak-line group can be explained only in terms of lowered metal abundance (relative to hydrogen) or whether differences in mean turbulence and mean degree of ionization can explain the existence of the group.

Line Blanketing

Sandage and Smith completed an observational study of the differential blanketing effect of weakening the Fraunhofer lines in stellar spectra. A four-color, broad-band photometric system was devised using an RCA 7263 photomultiplier cell with an S20 trialkali photocathode. The system is close to the standard *U*, *B*, *V* but adds a fourth color, *R*, at an effective wavelength of 6800 A. Observations were made with the Palomar 20-inch telescope of 64 standard stars whose *U*, *B*, *V* values are well known and of 32 subdwarfs of intermediate to large ultraviolet excess. Three results came from the study. (1) It is possible to transform the natural photometric system of the S20 photocathode to the *U*, *B*, *V* system (usually observed with an S4 cathode), with a systematic accuracy of $0^m.02$ in $B - V$ and $0^m.05$ in $U - B$. The data show a nonlinearity in the $(u - b)$ natural $= f(U - B)$ transformation curve of amplitude $0^m.05$ which is undoubtedly due to the different ultraviolet response of the 1P21 and RCA 7263 multiplier. Therefore, precise transformations of S20 data to the $U - B$ system must be done with a nonlinear equation. (2) The effect of differential line blanketing on the positions of stars in the $U - B$ versus $V - R$ diagram is clearly seen. The subdwarfs, as expected, stand high by $\delta(U - B) = 0^m.2$. (3) An extension of the blanketing theory discussed in *Year Books 59* and *60* was made to include the *R*

point, and it was shown that the theory can with great accuracy correct the subdwarf positions in the $U - B$ versus $V - R$ and in the $B - V$ versus $V - R$ diagrams to the position of stars of high metal abundance. Therefore, most and perhaps all of the previously observed peculiarity in the energy distribution of subdwarfs over the spectral range λ3300 to λ6809 can apparently be explained as due to the effects of weak Fraunhofer lines on broad-band photometric measurements. Unpublished data of Sears and Whitford at the Lick Observatory suggest that this is also true all the way to the infrared point at λ = 10,000 A on their six-color system.

Spectroscopic scans are being obtained by Oke for a selection of very metal-deficient stars. Because of the weakness of the lines, the absolute energy distribution in the spectrum can be accurately obtained over a large wavelength interval. This facilitates comparisons with model atmosphere fluxes. In addition, model atmospheres for these stars can be computed with higher accuracy than for corresponding metal-normal stars. The hydrogen-line profiles will also be studied. Scans of the extremely metal-deficient red giant HD 122563 have been obtained between λ3400 and λ8000. Assuming that the continuous opacity is due to the negative hydrogen ion, comparison with model atmospheres gives an effective temperature of about 4200°K, in good agreement with the value determined by Greenstein, Wallerstein, and Parker.

Color-Magnitude and Chemical-Composition Relationships

Eggen and Sandage reexamined the problem of the position of the main sequence as a function of chemical composition in the M_v, $B - V$ and the M_{bol}, log T_e diagrams. A sample of stars was chosen (1) which were known to be dwarfs from spectroscopic luminosity criteria, (2) which had trigonometric parallaxes larger than 0″034, and (3) for which photometry on the U, B, V or U_c, B, V was available. From this extensive material, Eggen and Sandage confirmed their previous result (1959) that the displacement of a star below the Hyades main sequence in the M_v, $B - V$ diagram is directly proportional to the ultraviolet excess. Assuming that the ultraviolet excess is due to Fraunhofer-line weakening caused by low metal abundance, and applying the blanketing theory reported in previous years, it was shown that differential blanketing corrections move all stars, independently of the size of their ultraviolet excess, onto the Hyades main sequence with good accuracy. In particular, 16 extreme subdwarfs with well determined absolute magnitudes and with excess values averaging $\delta(U - B) = 0^m.21$ were observed to be $1^m.05 \pm 0^m.04$ fainter than the Hyades before blanketing corrections were applied but are moved onto the Hyades main sequence to within $+0^m.03 \pm 0^m.05$ after the corrections were made. These results provide the observational justification for the main-sequence fitting procedure to find distances of star clusters where it has always been assumed that the main-sequence positions are identical in the M_v, $B - V$ diagram for clusters of different chemical composition. These reported results show that the assumption is correct, but only if differential blanketing corrections are applied before the modulus fit is made.

It was further shown that the large scatter in the main sequence, between $B - V = 0^m.4$ and $B - V = 0^m.8$ of trigonometric parallax stars, is due not only to errors in the parallaxes but primarily to the line-blanketing effect on the colors. The distribution of ultraviolet excess shows that large variations in chemical composition exist among the parallax stars closer than 29 parsecs ($\pi \geq 0''.035$). The main-sequence scatter is markedly reduced when blanketing corrections are applied to the observed $B - V$ colors.

The fact that subdwarfs are moved onto the Hyades line after applying blanketing corrections shows that a

separate subdwarf sequence does not exist in the M_{bol}, log T_e diagram, despite the low metal abundance of these stars. This remarkable result permits the determination of the helium abundance in the interior of subdwarfs from the theory of the stellar interior. Decreasing the metal abundance will, in general, move the star below the main sequence. A corresponding decrease in the helium abundance works in the opposite direction. The effects can be predicted qualitatively by homology arguments, but computed stellar models are needed for accurate abundance determinations. Sandage used the models of DeMarque to show that the hydrogen (X), helium (Y), and metal (Z) abundances by weight of the extreme subdwarfs with well determined absolute magnitudes are $X \approx 0.95$, $Y \approx 0.05$, $Z \approx 0.001$, compared with adopted solar values of $X = 0.65$, $Y = 0.31$, $Z = 0.04$. Subdwarfs are the oldest stars we know. Therefore, this result suggests that the primeval abundance of both helium and the heavy elements was very low, a result in agreement with theories of element enrichment of the interstellar medium with time. The results are not final. Interior models with more closely spaced abundance differences are needed. Ikco Iben of the California Institute Physics Department spent two months at Los Alamos computing better interior opacity values from an IBM 7090 computing program developed by A. N. Cox and Robert Brownlee. Iben's resulting models will be used when they are completed for a second solution to the problem.

From a photometric study of three separate samples of main-sequence stars, Eggen has found that, judged by the distribution of ultraviolet excesses with respect to the Hyades stars, two-thirds of the stars in the solar neighborhood have a higher ratio of metals to hydrogen than the sun. The distribution of ultraviolet excesses suggests that, if enrichment of the interstellar medium has been uniform with time, the rate of star formation between 5×10^9 (formation of the sun) and 5×10^8 (formation of the Hyades) years ago was nearly uniform.

Color–Spectral-Type Relationships

Two years ago a report was published by Wilson in which it was indicated that, among main-sequence field stars later than type G5, a considerable spread of color for a given type, or of type for a given color, was present. The data used for this purpose were the old Mount Wilson spectral types and the photoelectric colors measured by Eggen. Subsequent spectrograms of some of these stars revealed that the Mount Wilson spectral types were unreliable. As a result, new spectrograms of 10 A/mm dispersion have been obtained by Wilson for more than 100 of these stars, and new types, based on the Yerkes system, have been derived. When the revised types are plotted against Eggen's colors, a considerable spread, amounting to 0.2 mag at some types, is again found, although many stars no longer occupy the same locations in this diagram as in the former one.

In addition, many members of the Hyades cluster were also observed spectroscopically (although at smaller dispersion), and a similar diagram was constructed for them. When these two diagrams are compared, the correlation between spectral type and color appears to be tighter for the Hyades members than for the field stars. In the light of current knowledge, the simplest explanation of this result is to suppose that, as regards chemical composition, the Hyades stars represent a more uniform group than the field stars. This conclusion is not especially surprising.

In recent Year Books Wilson has reported the discovery of a definite relationship between the width of the reversals of the H and K lines and the absolute magnitude of the star. However, the total emission intensity of the H and K reversals seems to have no obvious correlation with other features of the spectrum. This raises the question: why

is it that among the late main-sequence field stars there are stars which appear spectroscopically identical in every respect except that one has strong central H and K reversals and the other little or none? In the previously published work mentioned above, it appeared that there was a strong tendency for the redder stars of a given type to have stronger reversals than their bluer counterparts. With the revised types, however, little if any of this tendency remains, and thus what appeared to be a promising clue has proved to be illusory.

As an outgrowth of the present research more definite light is being shed on this problem. That H and K reversals are unusually frequent in the Hyades stars has long been known and is fully confirmed by the present work; indeed, if the discussion is restricted to types G5–K0, inclusive, the fraction of Hyades members in this range which has strong reversals seems to approach 100 per cent. The same thing is true of main-sequence members of the Praesepe cluster. Even in the Coma cluster, which is relatively poor in known main-sequence members, the frequency of strong reversals appears to be greatly in excess of that for the local main-sequence objects of the same range of spectral types, where the frequency is less than 20 per cent.

Thus, on the available evidence, stars formed in clusters have a much higher probability of possessing strong H and K reversals than the noncluster field stars of the same spectral types. This property may then, perhaps, be thought of as a genetic factor that can supply information of some kind as yet unspecified about the circumstances under which the stars were formed.

A natural extension of the investigation along the line of genetic thinking is to look at the reversals in double and multiple main-sequence systems. This has been done for a number of such objects, and the results very strongly support the genetic viewpoint. That is, there is a very decided tendency for the members of double and multiple systems to have H and K reversals of similar strengths when due allowance is made for differences in spectral type. The few exceptions to this rule seem to be restricted to members of systems that are themselves short-period spectroscopic binaries.

Clearly, Wilson's work summarized above is still in a preliminary stage, and definite conclusions should be avoided at present. Nevertheless, enough has been accomplished to justify pursuing it further in attempting to understand its significance and, perhaps, eventually in making use of it as a tool for the furtherance of other aims.

Photometry of Stellar Clusters and Associations

Sandage and Smith completed the study of the color-magnitude diagram of NGC 6712, a globular cluster of relatively high metal abundance situated in the Scutum Cloud. A photoelectric sequence was determined to $V = 17^{m}.5$, $B = 18^{m}.5$ with the 200-inch, and short-exposure plates were measured for the color-magnitude diagram. It suffices to remark that the earlier conclusion reached from the study of NGC 6536, that the absolute magnitudes of the brightest giant stars in globular clusters are a function of their chemical composition, is confirmed.

Plates of the globular cluster NGC 6712 taken by Sandage were photometered for RR Lyrae stars and reduced by Norton and Lynden-Bell.

Sandage continued the photoelectric measurement of faint stars in M 15 and M 92. Complete color-magnitude diagrams for these clusters were prepared. Katem measured a special series of plates from $V = 17^{m}.5$ to $V = 22^{m}.0$ in both clusters to obtain the main-sequence positions using the photoelectric stars as standards. The results disagree by about $0^{m}.05$ in the color of the main-sequence termination point with the definitive photometry of M 13 reported several years ago, and a special program of photo-

electric intercomparison of the five clusters M 3, M 5, M 13, M 15, and M 92 was begun. This study must be completed before the M 15 and M 92 results will be published.

A photometric investigation of the clusters and surrounding associations of h and χ Persei using combined photoelectric and photographic techniques has been made on the U, B, V system to $V = 17.0$ by Wildey. A nonunique epoch of star formation is suggested both by an apparent fine structure in the bright end of the C-M diagram and by the presence of main-sequence stars to the photometric limit even though apparently contracting stars are found at brighter magnitudes. Nuclear and gravitational time scales are in agreement. The data, when compared with the theoretical evolutionary tracks of Hayashi and Cameron, suggest that helium burning takes place on the blue side of the Hertzsprung gap. The differences in apparent evolutionary tracks between the Galaxy, the Large Magellanic Cloud, and the Small Magellanic Cloud are reconfirmed. The two-color diagram of the bluest stars is of a gray-body character. Sp versus $(B - V)$ is monotonic for all stars.

The publication of the analysis of the color-magnitude diagrams of M 5, which indicated ages of 20×10^9 years, emphasized the serious discrepancy between these ages and those yielded by the measurements of cosmological expansion. In connection with another investigation, Arp has reexamined the assumptions in regard to space reddening made in the analysis of the color-magnitude diagrams of globular clusters. He found the following results: (1) The cosecant reddening law applies to high-latitude globular clusters despite the fact that photometric analysis of stars within 500 parsecs shows on the average 0.06 mag less reddening than that given by the law. The implication that reddening in the Galaxy is not concentrated entirely within a thin sheet needs to be examined further. (2) If the cosecant reddening law is used for all the globular clusters so far analyzed, the derived ages drop to between 9 and 14×10^9 years. The mean absolute magnitude of the RR Lyrae stars should be adopted as $M_V = +0.3$, $M_B = +0.5$ mag.

Investigation of clusters of intermediate age (10^9 years) and low metal content (½ to ⅓ of normal) has been completed by Arp. Work continues in the clusters near the Galactic nucleus. A very clear giant branch in the color-magnitude diagram of NGC 6838 has been obtained. The brighter sequences appear somewhat similar to those in 47 Tucanae and NGC 6356. The photometric fitting of the main sequence and derivation of accurate reddening are now in progress.

Eggen has isolated the "Pleiades Group," which, together with the Hyades and Sirius Groups, accounts for nearly 25 per cent of the A-type stars in the solar neighborhood. Also, two groups of high-velocity stars, in addition to the Groombridge 1830 Group, have been found. One of these, Kapteyn's Star Group, contains at least two RR Lyrae variables, SU Draconis ($0^d.66$) and ST Leonis ($0^d.48$). The derived median, visual absolute magnitude is $+0^m.8$ for both stars. Another possible member of this group is ST Comae Berenices ($0^m.60$). The subdwarfs HD 106038, CC835, and CC486, as well as the horizontal branch A-type star HD 139961, are also members. The mean ultraviolet excess of the group is $+0^m.21$, and the variables all have $\Delta S = 6$. No RR Lyrae variables have been identified as belonging to the third high-velocity group, which contains the subdwarfs HD 74000, Ross 626, Ross 451, Ross 453, HD 108177, and −35°-14849.

Photometry of Double Stars

Eggen began a program of U, B, V photometry of the components of visual double stars with the 200-, 100-, and 60-inch reflectors. The components of about 100 pairs have so far been observed, including the following of special interest:

(1) Red dwarf–white dwarf pairs. Observations of the components of six wide, proper-motion pairs discovered by Giclas, Slaughter, and Burnham at the Lowell Observatory, G39-27/28, G14-57/58, G87-28/29, G102-39/40, G111-71/72, and G61-16/17, have confirmed the discovery suspicions that one of the components in each pair is a white dwarf. An additional pair, G24-9/10, has also been found to contain a late-type white dwarf. (2) Intrinsic variables. The results for the main-sequence companions of the β Cephei variables, β Cephei and σ Scorpii, indicate that the luminosities of these variables may be about a magnitude fainter than usually supposed. Photometry of the solar-type, main-sequence companions of the Mira variables, R Cassiopeiae and RU Cygni, indicates a luminosity near -1 for both variables. A weak-lined G-type subdwarf, 10 seconds from the RR Lyrae variable AP Serpentis ($P = 0^d.25$), shows an ultraviolet excess of $+0^m.12$ and gives an absolute median luminosity near $+2^m$ for the variables if the stars form a physical system. (3) A wide range of eclipsing stars with visual companions is included in the program to help in standardizing mass-luminosity and radius-luminosity relations. Also, observations of the physical companions to several bright W Ursae Majoris systems indicate that the ultraviolet excesses are the same for the variable and the nonvariable components. The system of VW Cephei shares a large space motion with HD 199476, although the stars are separated in the sky by about 1 degree. W Ursae Majoris, BD $+55°1351$, has a faint companion ($V_E = 12^m.35$, $B - V = +1^m.70$), a degree away, with which it forms ADS 7497. Although the two components are not physically connected they share a common proper motion. The radial velocity of the BD star is not available. From the companions of these two variables, as well as that of AM Leonis (ADS 8024), the variables are found to lie $0^m.75$ above the main sequence. A faint star ($V_E = 13^m.84$; $B - V = +0^m.65$; $U - B = +0^m.11$) about 10 seconds from UY Ursae Majoris may be physically connected to that variable. (4) Structure in the mass-luminosity relationship has been found for stars with different metal contents as judged by their ultraviolet colors compared with Hyades stars. The colors of all binaries for which orbital elements are available are being obtained by Eggen with the 20-inch and 60-inch reflectors for further study of this structure.

Photometry of Variable Stars

Eggen has undertaken three-color photometry of all northern cepheids not already observed photoelectrically. One preliminary result from this program is that Baade's faint cepheids in Cygnus are reddened by about $1^m.0$. Also, all known contact binaries are being observed for color. Two variables classified in the literature as contact binaries have been found to violate the period-color relation previously established by Eggen. Extensive observations of one of these, SZ Lyncis, shows it to be a short-period, RR Lyrae variable (period near $0^d.13$) and not a contact binary. A previous conclusion that T Tauri variables and contact binaries do not coexist in space is apparently violated by the recent discovery by Götz of V449 Orionis (W Ursae Majoris, $P = 0^d.44$) and V441 Orionis (T Tauri Variable) which are separated by less than 30 minutes. Observations on five nights in February with the 100-inch indicate that V449 Ori is not a contact binary. The star showed very little variation in visual magnitude, $V_E = 15^m.10$ to $15^m.28$, or $(B - V)$ color, $+1^m.05$ to $+1^m.26$, but the ultraviolet color showed erratic variations of more than a magnitude. V441 Ori ($V_E = 14^m.72$, $B - V = 1^m.74$, $U - B = +1^m.11$) showed no variation. Other variables observed include Nova Orionis (1667 and 1894) and X Leonis. The nova showed no variation in the blue and visual ($V_E = 14^m.14$, $B - V = +0^m.48$) on five nights, but there are erratic variations in the ultraviolet. A bright maximum of

X Leo was observed from February 2 to 5, 1962.

Photometry of the Giclas Proper Motion Catalogue

A routine program of photoelectric observation of stars of high proper motion taken from the Giclas Lowell Observatory Proper Motion Catalogue was started in September by Kowal under the supervision of Sandage. The pilot program for the discovery of new subdwarfs, reported last year, was so successful that this regular discovery program was begun. Good progress was made during the report year with more than 700 stars observed and reduced in the three colors U, B, V. Both the 60-inch and 20-inch reflectors were used to make the observations. Over 100 new subdwarfs have been found. At least 30 of them have the extreme line-weakened characteristics of the globular-cluster main-sequence stars. Sandage continued his routine observational program of determining radial velocities of the stars in Kowal's lists that have ultraviolet excess values greater than $\delta(U - B) = 0^{m}.14$. This is a standby program with the 200-inch coudé, which is used only when sky conditions prevent more critical photoelectric work at the prime focus. But poor seeing conditions and partly cloudy weather were prevalent enough during the winter season so that spectra for 35 program stars were obtained in this way with the 18-inch camera giving a dispersion of 18 A/mm. Again, the results show that stars with high ultraviolet excess values invariably have large space velocities relative to the sun.

The photometric discovery program will continue for another year, by which time it is hoped that more than 2000 stars of the Giclas Catalogue will have been observed.

Subdwarfs

Greenstein has completed a velocity program on some 150 F–K subdwarfs and halo B and A stars. The number of spectroscopic binaries found is very low. Nevertheless, spectroscopic examination of the high-velocity stars revealed many types of peculiarities. After several attempts, an apparently reliable scheme of visual classification at 18 A/mm was developed with the following results: extreme weak-line subdwarfs of F–K types, 30 per cent; moderately weak-line subdwarfs, 18 per cent; slightly weak-line, 7 per cent; subgiants or giants, 10 per cent; horizontal-branch A–G stars, 4 per cent; ionized lines enhanced, 15 per cent; CH enhanced relatively to metals, 16 per cent. Some of the so-called subdwarfs are clearly above the main sequence spectroscopically, and the 10 per cent figure for giants or subgiants means that many very high space motions are included. The radial velocities have internal probable errors from 0.5 to 1.3 km/sec.

Jones has discussed a collection of 200 plates of late-type subdwarfs taken by Greenstein and Sandage with the coudé spectrograph of the Hale telescope at 18 A/mm. An attempt has been made to set up a three-dimensional classification scheme which estimates the spectral type and luminosity of the star independent of any weakening of the spectral lines. The spectral type is based on four ratios of line pairs of differing excitation potential and the strength of the hydrogen lines; the luminosity is estimated from three ratios of ionized to neutral lines in a manner closely following the Mount Wilson Catalogue. The strength of six of the 1.5-volt lines of Fe has also been estimated, and the values have been combined to form an index of the line strength. The principal conclusions are as follows: spectral types on this system are well correlated with the $B - V$ colors corrected for blanketing as far as the main sequence is concerned, but stars above the main sequence appear to obey another relation. Stars whose lines are among the strongest for their assigned spectral type have types on this system that correlate very well with the MK and Mount Wilson systems. This correlation was used to convert the new types from an arbitrary numerical scale to the well

known A–F–G–K scale. Line weakening occurs more frequently for stars earlier than G5, where it may amount to 0.7 logarithmically. Later than G8, it rarely exceeds 0.1. Owing to the small amount of material, only one curve was derived to reduce the luminosity characteristic to absolute magnitude based on trigonometric parallaxes, but the absolute magnitudes so derived agree very well with the Mount Wilson Catalogue. The Hertzsprung-Russell diagram shows a marked main sequence with a large number of weak-line stars which appear to define a "turnoff" at about G2. There are also several subgiants, some with markedly weak lines.

White Dwarfs

The discovery or spectroscopic confirmation of white dwarfs was continued by Greenstein, who finds Feige 22, 24, Giclas 21-16, 24-10, 28-13, 29-38, 61-17, 67-23, 93-53, LDS 235B (helium-rich), −37°10500, Wolf 457 (essentially continuous) to be of this type. A program of observation of white-dwarf members of double stars has been started by Greenstein in collaboration with the photometric work of Eggen. One λ4670 white dwarf, i.e., molecular carbon-rich, is a member of a wide binary.

Faint Blue Stars

A project was started by Zwicky with the aid of a grant from the National Science Foundation to obtain spectra of special types of blue stars for the purpose of determining their radial velocities and spectroscopic characteristics. The data thus obtained will be used to investigate the statistical distribution and proper motions of these objects.

The spectrographic observations were made by Berger with the 4-inch camera on the 60-inch Cassegrain spectrograph. Most of the stars observed are in the list of subluminous hot stars discovered by Feige. A few additional stars were supplied by Haro and by the list of blue stars published by Cowley. One to four spectra of 17 stars have been obtained in the magnitude range between 7.5 and 11.3. Preliminary measures of part of the plates do not indicate a large velocity for these blue stars, $|V| < 50$ km/sec.

A search for very faint blue stars ($15 < m < 19$) near the north galactic pole has been started by Berger with the 48-inch schmidt using three-color photography. These observations will be combined with the observations of 8500 faint blue stars near the south galactic pole made by Haro and Luyten for an investigation of the statistical distribution of the halo population. These plates will also be compared with the National Geographic Society–Palomar Observatory Sky Survey plates taken in the early 1950's for the detection of faint blue stars with large proper motions.

Balmer Lines in Early-Type Stars

Spectra of 57 B and Be stars have been obtained by Houziaux in the region λ3900 to λ3550 in order to study the confluence of the Balmer lines. It has been found that this emission-free region provides a good observational criterion for the determination of the gravity of early-type emission-line stars. The ratios of the intensities between the Balmer lines, corrected for atmospheric absorption, to the intensity at λ3862 are plotted versus the principal quantum number. Stars of the same spectral type and of different luminosities are clearly separated. These observational data are now compared with the results of flux computation for high lines at 41 wavelengths performed for 40 model atmospheres in the temperature range 9510°K to 29,000°K and for $\log g = 1(1)5$.

Changes have been observed by Houziaux in the infrared spectrum of Pleione. The strong O I absorptions at λ7771 and λ8446 have disappeared. From a spectrophotometric study of the infrared region, it has been shown that the O/H ratio during the shell episode was 0.6×10^{-4}, a value similar to the one observed in other B-type stars.

RR Lyrae Variables

The photoelectric spectrum scanner has been used by Oke in a continued program to measure absolute energy distributions in the spectra of RR Lyrae variable stars. The stars being studied are RR Lyrae, SU Draconis, X Arietis, SW Andromedae, and VZ Cancri. Photographic spectra with a dispersion of 9 or 18 A/mm are also being obtained. The absolute energy distributions are compared with fluxes computed from model atmospheres to obtain values of the effective temperature at each phase. These profiles can also be used, along with the photoelectric scans, to determine space reddening with high accuracy. After correction for reddening, RR Lyr, SU Dra, and X Ari all appear to have the same temperature range, 6000°K to 7500°K. The temperature curves, as a function of phase, for these three stars are similar but not identical. The radial-velocity curves for SU Dra and RR Lyr demonstrate conclusively that differential radial motions exist throughout the atmospheres at all phases. Since the continuous opacity changes with phase, different mass layers are observed with different velocities at various phases. Consequently, the observed radial-velocity curve does not represent the motion of the star's photosphere, and integration of the velocity curves does not give the radius-displacement curve. Therefore, the Wesselink-Baade method cannot give correct radii for RR Lyrae stars. It is found, however, that a modification of the method can be used successfully to determine the absolute radius. For SU Dra the mean radius is $5.2R_{\odot}$. Using the temperatures obtained from the scans, this leads to a mean absolute visual magnitude of $+0.8 \pm 0.4$. The error can be reduced if only differences of absolute magnitudes are required.

The scanner is being used by Oke to measure absolute energy distributions of suspected horizontal branch stars. These measures will be used in conjunction with temperatures obtained by fitting $H\gamma$ to theoretical profiles to obtain the reddening. The value of the effective gravity determined from the scans indicates whether or not a star can be a horizontal branch object. A comparison will be made of effective gravities of RR Lyrae stars and nonvariable horizontal branch stars to study the effects of the dynamics of pulsation on the atmosphere. One star, HD 161817, is confirmed to be a horizontal branch star similar to RR Lyrae at maximum light.

Supernovae

The search for supernovae has continued under the direction of Zwicky and with the support of the National Science Foundation. Between July 1, 1961, and May 31, 1962, a total of 15 supernovae was discovered at Palomar, all of them on plates taken with the 48-inch schmidt telescope. Of this number, 4 were discovered by Humason, 2 by Kearns, 6 by Zwicky, and 1 each by H. S. Gates, Rudnicki, and Berger. Of these, 2 were in the Coma cluster, as identified through determination of their symbolic velocities of recession.

The supernova in NGC 4303 developed into a type II spectrum after a peculiar early behavior. Greenstein found, near maximum, large negative displacements of the emission lines which were accompanied by very greatly displaced absorption edges. Within a month, the emission lines became sharper and returned to zero velocity. Apparently both absorption and emission were formed at the leading edge of an opaque expanding shell. It is unclear whether the apparent deceleration is real or caused by the appearance of the far (receding) side of the star, but there is no doubt that the velocity spread was greatly reduced.

Observations by Greenstein showed that the supernova in NGC 1058 had many extremely sharp lines in November 1961, some accompanied by absorption edges. The spectrum resembled that of a type II object with low velocity dispersion. H, He I, and C III were present.

During a secondary light maximum in December a spectrum was obtained at 18 A/mm providing excellent line profiles of the shallow, broad lines. In January 1962 the spectrum was like that 75 days earlier except that the lines had become sharper again. This object was extremely complex in light and spectral variations, and it might be taken to be a distinct subclass of type II.

Additional spectra of several of the brighter supernovae were obtained by Zwicky. From a study of the spectra and the light curves, Zwicky believes that it may be necessary to postulate several new types of supernovae in addition to types I and II. The supernova in NGC 1058 may be a representative of a type intermediate between ordinary novae and supernovae.

U Geminorum Stars (Dwarf Novae)

An extensive study of several U Gem variables at minimum light was continued by Kraft with the prime-focus spectrograph of the 200-inch. A spectroscopic binary orbit for the emission-line component of Z Camelopardalis was obtained with $P = 6.5$ hours; SU Ursa Majoris was found to vary in radial velocity, but a period has not yet been determined. Forty-four per cent of the U Gem stars that can be reached from Palomar have been studied so far; all have proved to be binaries with $P < 9$ hours. A study of the motions leads to $<M_V> \sim +9.5$ at minimum.

An hypothesis was advanced suggesting U Gem stars are descendants of W Ursa Majoris binaries. The two kinds of variables have comparable space distributions and velocities. Plausible arguments on mass transfer between the components of a typical W UMa system show that it might well become a U Gem star after 10^7 to 10^8 years.

Old Novae

A search for binary stars of short period among old novae has been started by Kraft using the 200-inch prime-focus spectrograph (180 A/mm). Nova Persei (1901) has a definitely composite spectrum (sdBe + K), and Nova Lacertae (1910) shows some peculiar, as yet not understood, variations in the velocity of He II (λ4686) in emission.

Nova Sagittae (1913, 1946) proves to be a spectroscopic binary with the shortest known period: 81½ minutes. This could be detected only by trailing the star over a long slit without repetition. An "*S* wave" was found in the hydrogen emission lines superimposed on the absorption lines of a white dwarf already found by Greenstein. Meanwhile, Krzeminski at Lick discovered that Nova Sge is an eclipsing binary, as well.

Greenstein and Kraft, together with Jon Mathews of the California Institute Physics Department, collaborated in pointing out the possible importance of Nova Sge as a test for the part of Einstein's general theory that predicts the existence of gravitational waves. However, since the mass ratio is unknown in Nova Sge, it cannot be determined whether the star emits a significant amount of gravitational energy. If $\mathfrak{M}_1/\mathfrak{M}_2 \sim 5$, for example, the emission of gravitational energy is found to be 30 times that of the luminosity, and the system would collapse in only 20 million years. The eclipse period would be out of phase by a minute in 15 years—an easily detectable quantity.

Another object that may be similar to Nova Sge is the suspected U Gem variable EX Hydrae, for which both Krzeminski and Kraft find $P \approx 99$ minutes. These two, together with Herbig's similar object VV Puppis, for which $P = 100$ minutes, might all be emitters of gravitational radiation.

Shell Stars

Further plates of 89 Herculis have been obtained by Sargent to continue work on the circumstellar envelope. During 1961, the shortward displaced absorption components at the Balmer lines weakened considerably in an interval of less than 60

days. The other peculiar features—the emission in the redward wing of Hα, the emission at the intercombination lines of the neutral metals, and the shortward displaced absorption lines at H and K and the D lines—did not change. A preliminary curve of growth for the circumstellar H lines shows that the turbulent velocity is very large—greater than 20 km/sec.

Mass Loss from Stars with Extended Atmospheres

In connection with his continuing study of mass loss from late-type giants, Deutsch has observed the differential motions occurring in the atmospheres of some M-type supergiants. At 4.5 A/mm, spectra of μ Cephei (M2 Ia) reveal the contributions of at least five atmospheric layers with distinguishable radial velocities in the range -29 to $+29$ km/sec. The radial velocities of individual lines correlate with equivalent width and excitation potential. Asynchronous velocity variations can be clearly seen in three of the atmospheric layers.

For the further elucidation of the mass-loss process, Deutsch has under continuing observation a number of late-type giants which are spectroscopic binaries. Several of these exhibit circumstellar lines at the D line as well as at H and K.

A reconsideration of the pronounced line weakening found in early-type Mira variables has led Deutsch to the conclusion that these stars, like others belonging to the halo population, probably have metal deficiencies of the order of 10^{-2} as compared with the sun or other normal stars. The chief source of the opacity in these very cool atmospheres remains unidentified, however, and until it is known the degree of metal deficiency will remain uncertain.

Segregation of Elements in Magnetic Stars

The problem of the anomalous abundance of elements in the magnetic stars of type A has been investigated by H. W. Babcock. These stars generally show an abnormally high abundance of several elements such as Cr, Sr, Mn, Si, Eu, and other rare earths; further, in the outstanding spectrum variables, Eu and Cr are observed to undergo large variations in antiphase. Whatever the basic mechanism of this variation, the process by which the particular elements are segregated and the manner in which this segregation is maintained in the face of gaseous diffusion are matters of considerable interest.

In 1947 it was pointed out that many of the anomalous elements in the magnetic stars belong to the iron group or the rare-earth group, and that their atoms generally have large magnetic susceptibility owing to the occurrence of partly filled internal electron shells. Each such atom has a magnetic moment, μ, the effective value of which, measured in Bohr magnetons, is expressed by the product gM, in which g is the Landé factor and M is the magnetic quantum number. In a magnetic field H, having a gradient ∇H, the atom will experience a force $gM\nabla H$ in the direction of stronger or weaker field, depending upon whether its alignment is parallel or antiparallel to the field. The idea that, as a result of this force, a selective paramagnetic migration of atoms might occur in a stellar atmosphere was set aside because in thermal equilibrium the magnetic sublevels will be very nearly equally populated. Therefore the net magnetic moment would be vanishingly small.

The phenomenon of "optical pumping," recently investigated by microwave physicists, now offers a nonthermal process by which atoms in a magnetic field can be polarized; i.e., they can be given a preferential orientation. It has been found that irradiation of atoms by polarized light, in a magnetic field, can drastically alter the relative population of the magnetic sublevels. This results in a net paramagnetic susceptibility and in a migration toward stronger or weaker regions of the field provided that the magnetic gradient is

sufficient. It is also known, as a result of recent laboratory investigations, that polarized atoms have a rather remarkable resistance to disorientation by collisions with other atoms. This disorientation resistance, according to Princeton investigators, is particularly marked for spherically symmetric atoms—those in S states—as compared with those in states having orbital anisotropy.

The possible application of the foregoing facts relating to optical pumping and disorientation resistance has been considered with respect to the abundance anomalies of the magnetic stars. For the elements of the periodic table, the magnetic moment of the ground state of the neutral atom, as well as for the first two stages of ionization, has been computed. Elements whose ground states are not S states have been rejected by reason of insufficient disorientation resistance. Then elements of very low astrophysical abundance have been deleted, and, finally, in the accompanying tabulation, all remaining atoms having a magnetic moment greater than 2 Bohr magnetons have been listed.

Atomic Number	Element	$\pm gM$
7	N I	3, 1
8	O II	3, 1
15	P I	3, 1
16	S II	3, 1
24	Cr I	6, 4, 2
	Cr II	5, 3, 1
25	Mn I	5, 3, 1
	Mn II	6, 4, 2
	Mn III	5, 3, 1
42	Mo I	6, 4, 2
	Mo II	5, 3, 1
63	Eu I	7, 5, 3, 1
	Eu II	8, 6, 4, 2

It is seen that a few atoms best fitting the conditions for paramagnetic migration in a magnetic-field gradient are Cr, Mn, Mo, and Eu. With the exception of Mo (which has no prominent lines in the commonly observed spectral region), these elements are known to show striking abundance anomalies in the magnetic stars. Indeed, Cr and Eu are the most outstanding peculiar elements in the magnetic spectrum variables. This result lends support to the initial suggestion that migration or segregation of paramagnetic elements actually occurs in magnetic stars, even though this idea seems a priori quite unlikely because of the requirement of a large magnetic gradient if the paramagnetic force is to overcome backward diffusion. If the selective migration occurs horizontally, an increase in concentration of one order of magnitude over a distance of 10^{11} cm is a minimum need. The diffusion equation, relating the concentration gradient to the selective force on a particular kind of atom, then shows that a magnetic gradient of at least 10^{-3} gauss/cm is required. This is about 10^{3} times the gradient over a large sunspot. If paramagnetic concentration of elements actually occurs—and no other theory has been offered to maintain the segregation of particular elements—this will have a decided bearing on possible models of magnetic stars.

Radial Velocities of Magnetic Stars

As a by-product of the study of stellar magnetic fields, accurate radial velocities have been derived by H. W. Babcock for several A-type stars, most having very sharp lines. In nearly all cases the spectrograms, made with the 200-inch telescope, have a dispersion of 4.5 A/mm. Velocities depend on upward of 20 lines measured on each plate by Miss Burd. For some 36 stars brighter than magnitude 6.5, the new radial velocities substantially augment the data of the Yale Bright Star Catalogue. Of these 36 stars, 25 are found to have variable velocity. For most of them the range is a few kilometers per second. For two of these stars, HD 15144 (HR 710) and HD 187474 (HR 7552), which are evidently spectrosopic binaries with periods of 2.9978 days and 700 days, respectively, Miss Burd has computed orbital elements.

Stellar Polarization

The first form in which stellar polarization was detected was the circular and elliptical type due to the longitudinal Zeeman effect on line profiles. It is this that permits the line-of-sight component of the star's magnetic field to be measured. But, if the direction of the stellar field is essentially perpendicular to the line of sight, the transverse Zeeman effect can be expected. If the absorption lines are numerous and strong, there can occur a resultant plane polarization of the light which has been called polarization by magnetic intensification. Owing to an imbalance in equivalent width between the "perpendicular" and the "parallel" components of the saturated, Zeeman-broadened profiles, an excess of polarization in a plane parallel to the magnetic field will result. The integrated effect in a broad region of the spectrum of a star having a field of a few kilogauss may be of the order of 1 per cent. Plane polarization due to magnetic intensification should be observable not only in sharp-line stars but also in those with lines broadened by axial rotation. This kind of polarization should be distinguishable from the well known interstellar polarization, due to dust grains in space, because that is constant, whereas intrinsic stellar polarization may be expected to show variations in intensity and in position angle as the star rotates or as its magnetic field changes. Observation and analysis of intrinsic plane polarization should give valuable supplementary data on which to base models of magnetic stars.

A sensitive polarimeter for the investigation of such effects has been under development during the year by H. W. Babcock, after initial tests with a simple rotating analyzer in front of a photometer indicated promise for this approach. The instrument is designed to work at the prime focus of the 200-inch telescope, which is ideal for the purpose because there is only one reflection (nearly normal) and because the large light-gathering power provides an excellent signal-to-noise ratio.

The new polarimeter is of an unconventional design, developed with the intent of overcoming the effects of stellar scintillation and of the nonuniformity of the cathode of multiplier phototubes. These effects have placed a serious limitation on precision of measurement with existing stellar polarimeters. Scintillation, closely related to astronomical seeing, is an intensity fluctuation in the low-frequency range (<500 cps). To overcome it, the polarization vector of the light can be resolved into two orthogonal components that are chopped at a frequency considerably greater than the scintillation frequency and admitted alternately to a single, stationary phototube. The instrument employs a slowly rotating electrooptic crystal (ADP plate), excited by a 3500-volt square wave at 2000 cps. The crystal becomes birefringent, with a phase shift alternating between $+90°$ and $-90°$ for blue light. This is followed by a fixed circular analyzer. The plane-polarized components of star light parallel to the two axes of the crystal are alternately transmitted as these axes alternate between optically "fast" and "slow" at the applied frequency. The output of the photomultiplier is amplified, demodulated, and filtered by an amplifier of the so-called "lock-in" type, which has already proved indispensable in other astronomical instruments required to measure a weak signal in the presence of noise.

The ADP crystal is rotated at a rate of about 1 turn in 5 minutes. As a result, the demodulated signal produces a sine wave on a strip-chart recorder. The amplitude and phase of the sine wave are readily related to the percentage polarization and position of the electric vector in the light of the source. Calibration is accomplished by inserting a depolarizer followed by a tilted glass plate designed to introduce either 1 per cent or 4 per cent polarization.

Tests of the polarimeter during various stages of development have shown that

the 2-kc/sec modulation frequency and the very narrow band width of the lock-in amplifier result in a satisfactory signal-to-noise ratio, and that a precision of a few hundredths of 1 per cent can be obtained in measures of the polarization of stars brighter than about seventh magnitude. Observations of fainter sources are generally limited by shot noise (randomness of arrival of the incoming photons) rather than by scintillation. It is satisfying to find that no detectable plane polarization is introduced by reflection from the 200-inch mirror. An upper limit at present is 0.1 per cent.

Observations of some 80 stars have now been obtained. Practically all show at least a small degree of polarization, and it is evident that considerable care will have to be exercised to maintain standard instrumental conditions and consistent calibration in the determination of small variations due to intrinsic stellar causes. Preliminary results for the percentage polarization of a few stars are: WY Geminorum 1.75, 9 Geminorum 3.26, R Leonis 2.47, XX Ophiuchi 5.15, ϵ Ursae Majoris 0.06, α^2 Canum Venaticorum 0.04–0.11, AD Leonis 0.54–4.16, HD 153882 0.13–0.34, and HD 154445 4.0.

GASEOUS NEBULAE AND INTERSTELLAR GAS

A program has been initiated by O'Dell to test the feasibility of using the doublet ratios of [Ar IV], λλ4711, 4740, and [S II], λλ6717, 6731, in a manner analogous to [O II], λλ3726, 3729, for the determination of the electron density and temperatures in gaseous nebulae. The former ratios should be excellent criteria for nebulae of densities above 10^4 electrons/cm^3, where (O II) becomes insensitive. It is hoped that forthcoming theoretical calculations will aid in placing the calibration on a reliable basis. Measures are being made on a number of planetary nebulae covering a large range in densities with both photographic and photoelectric spectrographs on the 60-inch and 100-inch telescopes.

Although the spectra of the planetary nebulae have been the subject of numerous photographic studies, substantial errors exist in the relative intensities of the emission lines, due to the inherently small photometric range of the photographic plate. With this difficulty in mind, O'Dell has begun a systematic survey of the bright planetary nebulae with the photoelectric scanning spectrograph. Particular attention is being paid to emission lines that should be indicative of the conditions in these nebulae. Since the effect of interstellar extinction can become large for even moderately reddened nebulae, the interstellar extinction is being determined from observations of Paschen and Balmer series lines of hydrogen arising from the same upper level. Although the detailed recombination theory of Burgess predicts a small variation in the ratio of these Paschen and Balmer lines with electron temperatures, the interstellar extinction correction should be much more accurate than the result by any other current technique. As part of this program, the relative energy distribution of the continuum from the central stars is also being determined.

The prints of the National Geographic Society–Palomar Observatory Sky Survey plates were used by Struve to prepare a list of 74 interesting nebulae located in and near obscuring clouds in the Milky Way. Struve finds that the great nebula near Antares extends to an angular distance of several degrees from the star. The reddish glow appears to illuminate the southern edge of the long, opaque lane which extends from 22 Scorpii toward the east.

Several faint red and blue nebulosities were found in the vicinity of ρ Ophiuchi and CoD $-24°12684$. Several illuminating stars are reduced in brightness by 3 mag, but no luminous nebulosity could be assigned to a star whose light is dimmed by more than 3 mag.

The nature of the emission nebulosity near σ Scorpii confirms the previous result that this object does not coincide with the reflection nebula belonging to the same star.

There is a pronounced tendency for luminous nebulosities (probably of the reflection type) to be more frequent in the Taurus complex of dense clouds than in the Ophiuchus-Scorpius complex. The quantity discussed is the number of nebulosities per square degree of dark cloud. Possibly this is connected with the fact that the average absorption of the clouds is smaller in Taurus than in the Ophiuchus-Scorpius complex.

Parker has continued his study of S22, S147, NGC 6888, IC 443, and the Cygnus Loop, which are possible supernovae remnants. The observing program, which has been completed, includes spectrograms and spectral scans of 16 separate filaments in these objects. On the program of reduction, relative intensities of all lines, corrected under various assumptions for reddening, have been obtained. For the condition of collisional excitation and ionization, relative intensities have been computed for the emission lines involved for several values of the electron pressure and electron temperature. Comparison of the computed and observed ratios will enable statements to be made about the temperature, density, and abundances in the filaments. Monochromatic net fluxes are also being measured for the objects, both to investigate the mass of the visible filaments and to investigate the amount of free-free radio emission that could be expected. The dynamics of the five objects has also been examined.

G. Münch and Wilson have prepared a reply to the criticism raised by K. Wurm against the model of the Orion nebula proposed earlier by them. The lack of agreement between the radial velocities of the He I nebular absorption lines and the emission lines at the same position, the essence of Wurm's criticism, is explained in terms of the density fluctuations existing in the nebula. Wilson and Münch have therefore reviewed the variety of observational data related to such density fluctuations and, within this framework, have discussed the radial-velocity data provided by their early observations. On the whole, they find that the model does not need as drastic a revision as Wurm proposes.

G. Münch and Dr. A. Unsöld observed that the star α Ophiuchi, at a distance of 25 parsecs, shows a K-line component undoubtedly of interstellar origin. The observations of other near-by stars in the same area of the sky lead them to infer that the interstellar cloud in front of α Oph has linear dimensions no larger than 1 parsec. The study of this near-by complex of interstellar matter, which may extend right to the sun, is being continued by observing additional near-by stars.

GALAXIES

Structure and Internal Motions of the Galaxy

A determination of the solar motion and the parameter A of differential Galactic rotation from cepheid variables is being finished by Schmidt and Kraft. The total solar motion is found to be 16 or 17 km/sec, rather less than the value 21 km/sec adopted by Blaauw and Morgan. The value of A is found to be 14 to 15 km/sec kpc. No reliable determination of the curvature of the angular velocity curve can be made. A small negative K term is found, but the observations are equally well represented by a constant error of about -3 km/sec in the radial velocities.

The (U, V, W)-velocity vectors for 221 well observed dwarf stars have been used by Eggen, Lynden-Bell, and Sandage to compute the eccentricities and angular momenta of the galactic orbits in a model galaxy. It is shown that the eccentricity and the observed ultraviolet

excess are strongly correlated. The stars with the largest excess (i.e., the lowest metal abundance) are invariably moving in highly elliptical orbits, whereas stars with little or no excess move in nearly circular orbits. Correlations also exist between the ultraviolet excess and the W-velocity. Also, the excess and the angular momentum are correlated; stars with large ultraviolet excesses have small angular momenta. These correlations have been discussed in terms of the dynamics of a collapsing galaxy. The data require that the oldest stars were formed out of gas falling toward the galactic center in the radial direction and collapsing from the halo onto the plane. The collapse was very rapid, and only a small number $\times 10^8$ years was required for the gas to attain orbits in equilibrium (i.e., gravitational attraction balanced by centrifugal acceleration). The scale of the collapse was tentatively estimated to be at least 10 in the radial direction and 25 in the Z direction. The initial contraction must have begun near the time of formation of the first stars, some 10^{10} years ago.

In connection with the study of the collapsing galaxy, mentioned above, Eggen has prepared for publication a catalogue of some 700 stars whose space motions with respect to the sun are almost certainly greater than 100 km/sec. In addition to astrometric and photometric data, the catalogue contains the values of the "modular velocities" from which new space motion vectors can be computed for any future changes in the available radial velocity, proper motion, or luminosity estimates of these objects.

The globular cluster system in our own Galaxy has been reanalyzed by Arp. The globular clusters in the Galaxy are shown to be 90 per cent discovered. The best available moduli with the new RR Lyrae zero point ($M_V = +0.3$, $M_B = +0.5$ mag) give a distance to the center of the Galaxy of $R_1 = 9.9$ kpc $\pm$ 0.5 kpc (minimum error for an error of 0.1 mag in RR Lyrae absolute magnitude).

The analysis of the stars in Baade's field near the Galactic center has been completed by Arp. There is a definite giant branch which clearly emerges in the color-magnitude diagram and represents the population in the nucleus or nuclear bulge. This giant branch is not made up of globular clusterlike giants. More detailed consideration of this diagram will be made shortly.

The material gathered by Guido and Luis Münch for the determination of motions and distances of faint OB stars in directions near that of the Galactic center has been studied and reduced. All together, they determined U, B, V colors and spectral types for 35 stars and radial velocities for the 24 objects of higher luminosity from 90 plates obtained in a variety of dispersions with the X spectrograph and the coudé spectrographs. This material is now being prepared for publication and will be discussed with the earlier results obtained by them, as well as with the data for the interstellar lines in these objects obtained during the past few years.

Rotation and Internal Motions of Galaxies

The rather extensive observations of the internal motions in the elliptical galaxy NGC 3115 obtained by Minkowski and Oort in 1958 and extended by Minkowski during the following observing season were discussed by Oort. The principal aims were to find the distribution of mass and to obtain data on the distribution of random motions in the galaxy. It appears that, whereas up to a distance of about 60 seconds from the center along the major axis the distribution of mass seems to be roughly the same as that of the light, the mass density in the outermost parts observed decreases more slowly than the light density. The ratio of mass density to light density, M/L, expressed in terms of the mass and light of the sun as units, is about 15 in the inner parts, and rises to values of 100 in the outer shells. For the entire galaxy, M/L was found to be about 60. Such a high value has never yet been found from observations of rotation,

presumably because they have never reached such large distances. The result is important because of its bearing on the problem of stability of groups and clusters of galaxies.

The preliminary reduction of the Hα plates taken by Brandt with the Mount Wilson B spectrograph for the purpose of redetermining the rotation curve of M 33 is complete. The rotation curve determined optically extends to 30 minutes from the center of M 33, but it can be extended 60 minutes from the center with the aid of the radio observations. The shape of the rotation curve is very similar to that of M 31; hence, the relative mass and density distributions should be similar. For an assumed distance of 630 kpc, the approximate mass becomes $2.3 \times 10^{10} M_{\odot}$.

Emission Nebulae in Galaxies

Work done by Baade on the precise position of all emission nebulae in M 31 is being prepared for publication by Arp. An effort will be made to give illustrative data on the connection between these emission nebulae and the spiral structure in M 31.

Another attempt was made by Schmidt to determine the helium abundance of the interstellar gas in H II regions at various positions in the Andromeda galaxy. The observations were planned to furnish absolute values of the helium-to-hydrogen abundance ratio and the diameters of both the hydrogen emission region and the helium emission region. The first objective was attained by plates taken in the Orion nebula, where Mathis has obtained an absolute calibration. Exposures on the H II regions in Andromeda, of which one was a multinight exposure, were carefully guided with the spectrograph slit over the H II region and a near-by star. The star spectrum allows evaluation of effects of seeing, guiding, and scattering in the photographic plate, and the diameters of the emission regions are corrected for these effects. Once these diameters are determined for both hydrogen and helium emission, the abundance ratio can be determined independent of a possible variation with abundance ratio of the far ultraviolet continua of the exciting stars. The observational problem is a marginal one for the 200-inch prime focus spectrograph, and the photographic photometry on the plates is difficult. Results thus far give a helium-to-hydrogen number ratio of 0.08 at 89 minutes from the center of M 31, 0.14 at 70 minutes, and 0.17 at 25 minutes. Each determination is uncertain by a factor between 1½ and 2, so that the variation as well as the difference from Orion nebulae (0.13) is hardly significant. The implication of an essentially constant helium abundance would be far reaching, as was briefly indicated in the Annual Report for last year.

The observation of the emission lines of [O II] in the nuclear region of M 31 was continued by G. Münch. An area approximately rectangular with dimensions 100 $\times$ 300 seconds centered at the nucleus has now been covered with slits at various position angles. The emission lines at greater distances from the nucleus become so faint that it is impractical to attempt to follow them with existing equipment. The material so far collected, therefore, will be discussed shortly and published.

Münch's lack of success in detecting emission lines in the patches which were observed by Baade in the disk of NGC 4594, and which Lindblad considered to be H II regions, was reported last year. Confirming the tentative explanation then given, it has been found that an Hα exposure through an interference filter, with the f:3.67 focal ratio of the 200-inch, does not show such features with stronger contrast than broad-band photographs do. The patches undoubtedly, then, do not have an emission spectrum, and their true nature raises an important problem. Further work on these objects is being planned with a photoelectric scanner.

A systematic search for planetary

nebulae in other galaxies in the local system is being executed by O'Dell, by means of photographic plate plus filter combinations that give sensitivities around the Hα, N1 + N2, and λ5400 regions, since the nebulae should appear in the strong emission regions and not in the relatively line-free region at λ5400. Thus far, four suspected nebulae have been found in the Leo I system.

A program of direct photography of nearly resolved, Irr, Sc, and late Sb galaxies whose redshifts are less than 2000 km/sec was begun by Sandage using a 4 by 4 inch Hα filter of 80 A half-width. The purpose is to isolate the H II regions and to measure their apparent diameter as distance indicators. Absolute calibration of the linear diameter will be made using galaxies in the local group, such as the Large and Small Magellanic Clouds, M 33, IC 1613, and NGC 6822, whose distances are known from the cepheid variables. Preliminary calibration during the report year gave the linear diameter of the largest H II region as 245 parsecs and the mean diameter of the first five largest as 175 parsecs. Plates were obtained of NGC 2403, M 101, NGC 925, NGC 2903, and M 51 in a first trial of the problem, and it will be feasible to measure diameters in galaxies with redshifts as large as 2000 km/sec. The ultimate aim of the problem is to improve the values of the Hubble constant (H). Preliminary results using H II sizes determined in NGC 925 and NGC 4321 show that H lies in the neighborhood of 75 to 100 km/sec 10^6 psc, in agreement with Sérsic's earlier study of the H II region problem using blue plates of galaxies taken with the 200-inch, and in agreement with the value of 75 km/sec 10^6 psc which has been generally used during the past several years.

Widened spectra of NGC 1068 and NGC 4151 have been obtained by Sargent at a dispersion of 86 A/mm. Exposures of stars whose continuous energy distribution have been obtained with the Cassegrain scanner have been made on the same plates. Line profiles have been measured for NGC 1068. In NGC 4151 the broad wings at the Balmer lines, which were reported by Seyfert, extend to ±5000 km/sec. There are no absorption lines in NGC 4151 that can be attributed to stars. Two strong absorption features which occur at λ3885 and λ3732 and which are probably to be identified with He I are displaced by about −200 km/sec relative to the emission line centers. NGC 4151 is seen almost face-on, and this indicates that material is being expelled from the nucleus along the direction of the axis of rotation.

Variable Stars in Galaxies

Sandage completed the photoelectric measurements of selected stars of Baade's variable star sequences in IC 1613 in order to put Baade's extensive photometric material of the cepheids in this galaxy on the Progson scale. The photoelectric sequence extends from $B = 12^m$ to $B = 22^m.0$, which is not yet faint enough to correct the cepheid photometry at minimum light but is faint enough to correct all Baade's data at maximum light. This has been done, with the result that the slope of the period-luminosity relation for IC 1613 is identical with that found by Arp for the Small Magellanic Cloud. The relation is

$$B_{max} = 22.61 - 2.25 \log P$$

which gives an apparent modulus of $(m - M)_B = 24^m.33$, using Kraft's zero-point calibration of the cepheids in galactic clusters.

Extensive U, B, V photometry of field stars in the direction of IC 1613 indicates a reddening due to our Galaxy of $E(B - V) = 0^m.03$, which gives $(m - M)_0 = 24^m.2$ for the true modulus of IC 1613. Sandage expects to extend the photoelectric sequence to $B = 23^m.5$ next season. All Baade's material will then be published on this photometric scale.

Extensive material on three variable-star fields in M 31, accumulated by

Baade during the past ten years, is being analyzed by Miss Swope. There are about 120 variables in a field 15 minutes of arc from the nucleus, 330 variables in a field 45 minutes from the center, and more than 50 that are in a field 96 minutes south preceding the nucleus. This last field is the only one which is suitable for precision photometry and in which there is a photoelectric sequence (Arp, *Year Book 58*). In this field there are 20 cepheids with both photographic and photovisual light curves and also a preliminary color-magnitude diagram. Miss Swope finds that the apparent distance modulus of M 31 is $B = 24^{m}.75$ or $V = 24^{m}.60$, assuming a zero point of the cepheids from Arp and Kraft's period luminosity curves. There is probably about $0^{m}.15$ general reddening due to our Galaxy, which gives a distance modulus corrected for absorption of $24^{m}.15$ in both B and V for M 31.

The slope of the period-luminosity curve is essentially the same as that found by Arp for the Small Magellanic Cloud.

Baade also obtained a series of plates of the Sculptor-type system in Ursa Minor. These plates are being assessed by Dr. S. L. Th. J. van Agt of the Leiden Observatory. In this system 91 variable stars are known. They were discovered on plate pairs, each of which covered only part of the system. The outlines of the Sculptor-type system as inferred from the positions of the variable stars are roughly elliptical and indicate dimensions on the sky of 60 by 22 minutes. About 50 per cent of the variable stars discovered in this system are concentrated on the plates that cover the center of this Sculptor-type system. The number of plates is large enough to determine the periods of the cluster-type variable stars in the central area only. Estimates of the magnitudes for 28 stars in the central area were obtained by way of inspection with an eyepiece and comparison with a sequence of close-by comparison stars. Two of the investigated stars turned out to be nonvariable. For 22 variables in the same area, periods could be derived from the brightness estimates. Among these 22, 16 belong to Bailey's type $(a + b)$. The mean period of these variable stars is $\bar{P} = 0^{d}.617$, almost the same mean period as Miss Swope found in the Sculptor-type system in Draco. For 6 variable stars, van Agt determined periods shorter than $0^{d}.45$, but, since the scatter in the estimated brightness relative to the brightness-amplitude is larger for variable stars with smaller amplitude, these periods are established with less certainty. For 4 variable stars in the group of 28 it has not yet been possible to determine a period.

One bright variable star at the northern border of the system was also estimated by way of the eyepiece method. From a limited number of plates, the period of this star was determined to be $2^{d}.697$. A rough estimate of the maximum and minimum B magnitude was max = 18.2, min = 18.7. Scale transfers for blue plates only are available for the system in Ursa Minor. From a scale transfer of Baum's sequence in M 13 a provisional magnitude sequence in the Sculptor-type system could be established. The median magnitude of 8 variable stars the periods of which are known and for which the brightness estimates were transferred into magnitudes is $20^{m}.04$. Because of the scatter in the light curves of the 8 variables, all within 6 minutes of the center of the system, this mean value of the median magnitudes is still rather uncertain.

All stars visible on a plate with an 103aO emulsion and exposed with a WG2 filter in front of the plate were measured in the iris photometer of the Leiden Observatory. In addition, the same stars were measured on a plate with 103aD emulsion and exposed with a GG11 filter. This plate was exposed immediately after the first one mentioned. No scale transfers for either of these two plates exist. The iris scale readings were used, therefore, to construct a pseudo-color-magnitude diagram for the central part

of the Sculptor-type system. All stars used in this diagram are located within 305 seconds of the center of the plates measured. These plates cover the center of the Sculptor-type system, and, since the center of the system and the plate centers are very close together, the pseudo-color-magnitude diagram represents the general trend in the central part of the system. The pseudo-color-magnitude diagram shows a distinct giant branch and a clearly developed horizontal branch. In the region where the horizontal branch meets the upgoing giant branch, the number of stars is considerably lower than in the part of the horizontal branch immediately before that region. To the blue end of the horizontal branch only one star was found. The ratio of the number of stars at the blue end and at the red end of the horizontal branch is about 1:50. The position of the gap of the short-period cluster-type variable stars is such that only the one blue star mentioned is found to the blue side of the gap.

The faint part of the giant branch sets in at about 1½ mag below the horizontal branch. At the point of bifurcation there is no clear indication of a doubling of the giant branch. The plates used for the construction of the pseudo-color-magnitude diagram were exposed under conditions of poor seeing. Much of the scatter in the branches of the diagram is due to the low quality of the two plates. The scatter outside the branches is low; therefore, we may conclude that the number of faint field stars entering the diagram is limited.

Photometry and Stellar Content and Evolution

The distribution of stars in a number of local group dwarf galaxies was studied by Hodge. These include the Fornax, Sculptor, Leo I, Leo II, Ursa Minor, Draco, NGC 147, NGC 185, and NGC 205 systems. In all, the projected density was found to obey Hubble's law in the central regions but to fall off more steeply in the outer regions. A cutoff is found which corresponds in location to the expected tidal cutoff. Photoelectric surface luminosity distributions in two colors were obtained for other dwarf galaxies, specifically the irregular systems NGC 6822, IC 1613, Sextans, and WLM.

Photoelectrically calibrated measures of the distribution of luminosity and color have been made by Hodge for 29 galaxies primarily of the S0 type. The results, not yet completely reduced, show a clear difference in physical properties between Sandage's subgroup in this class as given in *The Hubble Atlas.*

Two near-by dwarf galaxies were observed photoelectrically by Baum during the report year. The integrated light of these very faint tenuous systems must be measured by making a series of slow scans across them with a photoelectric photometer. For this purpose the prime-focus photometer at the 200-inch has been equipped with a scanning motor.

The purpose in observing these dwarfs is to obtain their color indices and absolute magnitudes so as to fit them into a color-luminosity diagram for elliptical galaxies. This diagram divides itself into two color-index groups, the large elliptical galaxies having color indices 0.2 to 0.3 mag redder than the dwarf systems. All evidence indicates that the dwarfs are truly Population II, whereas eight-color observations show that large ellipticals must be mainly old Population I. The newly observed dwarfs add two points where they are most needed in the difficult part of the color-luminosity diagram. One of these, NGC 6822, is clearly in the true dwarf class, whereas the other, NGC 185, is in the transition region between classes.

Any interpretation of the redshift-magnitude relation in terms of cosmological models depends on the way in which evolutionary effects are taken into account. Since individual stars undergo large changes in luminosity and temperature as they age, the integrated light of a galaxy will tend to change with time.

Owing to the light-travel time, distant galaxies are seen at an earlier age than near-by ones. It is therefore necessary to know something about the stellar content of galaxies of the kind used for redshift-magnitude work.

Photoelectric observations on the eight-color system were used in 1958 for computing a population model for large elliptical galaxies. At 4830 A the model could be described as 25 per cent Population II plus 75 per cent old Population I. During the current year, a first attempt was made by Baum to extend this eight-color population analysis to sample regions in spiral galaxies. Experimental results were obtained by scanning across selected regions of M 74 (NGC 628), which is a face-on Sc spiral of unusually good symmetry. As expected, the disk population underlying the inner spiral arms has a spectral energy distribution roughly similar to that of an elliptical galaxy. The outer regions of the disk, however, are evidently bluer. The arms themselves, although photographically impressive, contribute astonishingly little, perhaps 10 per cent photovisually, to the total light of M 74. The width of an individual arm is found to increase systematically with wavelength, the infrared width being about 1.5 times the ultraviolet width.

The most interesting feature of Baum's scans is a slight asymmetry in the color distribution across a spiral arm. This provides a clue to the solution of an old dilemma. Since its birth, a typical spiral galaxy like M 74 has had time for more than 50 revolutions of its disk, but its arms are coiled to the extent of less than two visible turns. If the arms rotate with the disk, the outer ends of the arms must share most of the rotation. If, on the other hand, the outer ends of the arms are tied to intergalactic magnetic fields, the disk must slip through the arms, which are zones of new-star formation, with only a small drag. The clue is this: Relative to other colors the strengths of violet and ultraviolet are found to be greatest at the center of an arm, weakest near the inner edge of an arm, and intermediate near the outer edge. The wavelength dependence is such that asymmetry in the dust lanes does not by itself appear to be an adequate explanation. Evidently the relative number of early-type stars tapers off less sharply at the outer edge than at the inner edge. Such would be the situation if the rotating disk of the galaxy slips through the arms, dragging them slightly and coiling them up.

The evolution of the integrated properties of clusters of stars has been computed by Arp. The total colors and magnitudes of groups of stars of different ages have been derived. The results indicate: (1) The E and S0 galaxies contain about 30 per cent more K7 dwarfs (or equivalent) by number than galactic or globular cluster populations. This conclusion supports evidence from M/L ratios and computations by Spinrad on the composite spectra. (2) The total increase in magnitude from a "young" E or S0 galaxy to brightness at 10^{10} years is about 4 magnitudes. The brightness-age curve also enables the evolutionary magnitude correction for large redshifted nebulae to be read off. This completely empirical method yields the same K correction as was computed by Sandage for the latest cosmological solution from the redshift data. (3) By classifying galaxies in the two nonevolving parameters of mass and angular momentum it is shown that evolution probably does not take place from one type of galaxy into another. It is suggested that only relatively slowly rotating masses contract into E0 giants, and higher rotation in the more-flattened galaxy types sets increasingly smaller mass limits. Groups and clusters of galaxies are considered, and it is shown that a high density (in a cluster of galaxies) is strictly correlated with E and S0 membership, and low average density with high membership, of spirals. This gives a physical explanation for Hubble's often-made statement that spirals tend to be field nebulae. The above

observations on cluster composition are interpreted as confirmation of the hypothesis about formation.

Recomputation by Arp of Seare's early work using modern magnitude scales yields a surface brightness of the Galaxy in the solar neighborhood of $\delta_B = 23.8$ mag/sq sec (perpendicular to plane with absorption layer allowed for). The comparable surface brightness occurs 11 kpc from the center of M 31, assuming cosecant reddening models for both galaxies. It is also shown that with such reddening models the conclusion of Kron and Mayall is incorrect and that the M 31 globular clusters are, in fact, the same intrinsic color as in our own Galaxy. The following comparison has been made:

	M 31	Milky Way
Mass, $M_\odot$	34×10^{10}	7×10^{10}
Radius (to solar-brightness isophote), kpc	11	10
Number of clusters	300–400	130
Per cent of gas	0.7	4–6

All these characteristics indicate that our own Galaxy is more like an Sc than M 31 is, and of course the Milky Way is more like an Sb than M 33 is, but it seems difficult to classify our own system more quantitatively at present.

Work is being continued by Oke on the measurement of absolute energy distributions in the spectra of the central regions of galaxies. Measurements are now complete between λ3400 and λ6000 for about 20 galaxies. Nearly all observations have been made on giant elliptical systems. The two brightest galaxies and two fainter ones in the Coma cluster have been observed. The data are being used to compute K corrections for distant galaxies and for studying the stellar content of ellipticals.

Catalogue of Galaxies and of Clusters of Galaxies

Volume I of the *Catalogue of Galaxies and of Clusters of Galaxies* by Zwicky, Herzog, and Wild was published in October 1961. In the meantime, work on volume II has progressed to the point that the data on all clusters (about 2500) have been analyzed and prepared for publication, and the work on the galaxies involved is expected to be finished in October 1962. Volume II covers the area from Milky Way to Milky Way between the declinations +15° and +35°. Volume III, which covers the south galactic cap north of declination −3°, has been started. This project was supported in part by the National Science Foundation.

The data of the catalogue have been reduced in a dozen different ways and have been used to test for interstellar and intergalactic absorption. The fact has also been confirmed that clustering among galaxies is universal and is statistically of the same nature at all distances up to redshifts corresponding to symbolic velocities of recession of the order of 100,000 km/sec. It has been further confirmed that no clusters of clusters of galaxies exist.

The relative areas of the sky that are covered by open, medium compact, and compact clusters of galaxies have been investigated by Zwicky and Rudnicki. The largest clusters are all of the same linear size, independent of type and distance.

The so-called "cluster cell," every one of which contains the equivalent of one large cluster of galaxies, on the average was found to have a diameter of 45 million parsecs, assuming a redshift constant of 100 km/sec per million parsecs.

In the catalogue of galaxies by Zwicky, Herzog, and Wild the peripheral contours used for the delineation of clusters of galaxies are the isopheths or equal-population contours along which the numbers of galaxies per square degree are equal to about twice the number of galaxies per square degree in the adjacent

fields. The clusters thus drawn, not including the near-by Virgo cluster, however, cover 13 per cent of the 3024 square degrees of the sky included in volume I of the catalogue.

In the course of the work on volume II of the catalogue it was found that the field (of 36 square degrees) centered at R.A. 11^h17^m and decl. $+35°30'$ (1950) is the richest field of galaxies and of clusters of galaxies observed thus far, containing about 150,000 galaxies and 113 clusters of galaxies as counted on a limiting 103aE plate (+ red filter) obtained with the 48-inch schmidt telescope.

Internal Motions of Clusters of Galaxies

About 60 spectra of galaxies in the cluster Cl 0123-0138 have now been photographed by Zwicky, and the following results have been derived: (1) The average symbolic velocity of recession is $\overline{V}_s = 5321$ km/sec. (2) The dispersion in velocities (radial) is $\Delta V_s = 406$ km/sec. (3) The dispersion in V_s is essentially constant from the center of the cluster to the periphery, a fact indicating that the cluster is stationary and is neither expanding nor contracting. (4) The dispersion in V_s is greater for the fainter galaxies than for the brighter ones, although the average values of V_s are the same.

The distribution of the galaxies within the cluster indicates an elliptical shape of the cluster. There is no indication of any rotation, however, from the analysis of the radial-velocity data.

Additional spectra have been obtained of member galaxies of clusters in Cancer, Hydra I, and the Coma cluster for determining velocity dispersions and mass-luminosity ratios.

Redshift-Magnitude Relations

In principle, there are several observational tests for distinguishing between an exploding universe and a steady-state universe. With methods available today, the best test is the relation between the redshifts and the distances of large clusters of galaxies. More exactly, the observable parameters are the redshifts and the apparent bolometric magnitudes of representative cluster members.

Photoelectric observations collected by Baum since 1955 have been mentioned in previous Year Books. The absolute amounts of energy received from various galaxies are measured photoelectrically in eight colors ranging from ultraviolet to infrared. Effective wavelengths and bandwidths of the eight colors are:

Color	Effective λ, A	Bandwidth, A
Ultraviolet	3730	500
Violet	4335	740
Blue	5065	430
Green	5525	470
Red	6705	850
Infrared I	7525	600
Infrared J	8520	800
Infrared K	9875	1100

When the resulting spectral-energy distributions of galaxies are compared, the displacements between them yield both their redshift and their relative bolometric magnitudes. In this way, the redshift-magnitude relation has been explored to a much greater distance than before. Final photoelectric values for the three observed clusters of largest redshift are as follows:

Cluster	Redshift $\Delta\lambda/\lambda$	Symbolic Velocity $c\Delta\lambda/\lambda$, km/sec	Probable Error in Redshift, %
0024+1654	0.29	84,000	2.5
1448+2617	0.36	108,000	4.0
1410+5224	0.44	132,000	7.1

Cluster 1410 + 5224 is the cluster found by Minkowski at the position of Cambridge radio source 3C295. An optical emission line at λ5448, presumed to be O II 3727, provides a good check on the redshift above.

When these photoelectric redshifts were reported earlier, the corresponding bolo-

metric magnitudes could not be specified with the fullest attainable certainty, and the apparent shape of the redshift-magnitude relation had to be taken as tentative. Observations by Baum during the report year have been devoted to resolving this difficulty. The uncertainty arose, not because of limited precision in the photometry, but because auxiliary data were needed for intercomparing the magnitudes in one cluster with those in another in the best possible way. During the report year, photoelectric and photographic observations have been collected for constructing the needed luminosity functions of seven key clusters of galaxies distributed in redshift from $\Delta\lambda/\lambda = 0.02$ to $\Delta\lambda/\lambda = 0.44$. The new photoelectric sequences include 57 additional galaxies, many of them relatively faint. As before, most of the observations were made with the pulse-counting photometer at the 200-inch prime focus.

RADIO SOURCES

Schmidt has engaged in a spectroscopic investigation of galaxies believed to be connected with radio sources. This program is planned in close cooperation with Dr. Thomas Matthews, who made most of the identifications. Galaxies connected with radio sources 3C33, 88, 98, 171, 198, 219, 234, 317, 433, 445, from the third Cambridge Catalogue, and Coma A were investigated. All spectra show emission lines, the number ranging from 1 to 13. The magnitudes are in the range 15 to 20; the redshifts ($\Delta\lambda/\lambda$) vary from 0.03 to 0.24. The absolute magnitudes of all galaxies are close to -20. The largest number of emission lines is seen in 3C234, the spectrum of which resembles that of a planetary nebula of high excitation. In a case like this, the spectrum constitutes a strong confirmation of the identification. For 3C88, where the spectrum shows weak λ3727 emission only, the spectrum has hardly any confirmatory value. Spectra taken of some three or four other galaxies identified with radio sources show no emission features. At least one of these is now known to be a misidentification. It may be estimated conservatively that 70 per cent of the galaxies fainter than 15 mag that are identified with bright radio sources show emission lines in the spectrum. A spectrum of the galaxy identified with the distant radio source 3C295, observed and discussed earlier by Minkowski, shows that the λ3727 emission relative to the continuum is rather weak in comparison with that observed in some other radio sources. Spectroscopic work on some stellar objects believed to be connected with radio sources is continuing.

Greenstein obtained spectra of the radio source 3C442, which is a double elliptical, with only λ3727 emission. A common feature of the radio galaxies in Herculis A, 3C278, and 3C442 is a low gradient of surface brightness. The systems seem to be ellipticals, but their brightness distribution optically is more like that of an Sc pole-on system.

Further spectra of the radio star 3C48 reveal no changes, and one in the visual region showed two more unidentifiable emission lines. He II, λ5411, was absent.

Extending the work reported last year, Dr. Thomas Matthews and Sandage identified two additional radio stars similar to 3C48. This brings the total of such identifications to three. The objects are 3C48, 3C196, and 3C286. The radio positions of all three sources were determined before optical identification was made, and the only object within the error rectangle of the radio position is stellarlike in each of the three cases. The agreement of the radio and optical positions is remarkable, being within 4 seconds of arc in all objects. Photoelectric photometry of the stars shows that each has very unusual colors. The photometry

gives $V = 16^m.20$, $B - V = 0^m.40$, $U - B = -0^m.59$ for 3C48; $V = 17^m.79$, $B - V = 0^m.57$, $U - B = -0^m.43$ for 3C196; and $V = 17^m.25$, $B - V = 0^m.26$, $U - B = -0^m.91$ for 3C286. Spectra of 3C196 and 3C286, obtained by Schmidt, also show that the stellar objects are peculiar and uniquely new.

The optical flux of 3C48 was found to vary from $V = 16^m.02$ to $V = 16^m.44$ over the 13-month observation period since its initial discovery. The time resolution of Sandage's observations is not great, and so nothing is known about variations in the order of minutes or hours, but the flux does vary from night to night. Special observations at radio frequencies by Matthews showed that the radio flux is constant to within the probable error of the determination even though the optical flux varies.

The optical U, B, V measures for the three sources were transformed to absolute flux units and compared with the radio data. For 3C48 and 3C196 the power spectrum computed from the theory of synchrotron radiation was shown to predict the U, B, and V values to within a few hundredths of a magnitude when all but one of the adjustable parameters of the theory are determined from the radio data. The remaining parameter is the critical frequency at the high-energy cutoff, and this could be adjusted for the excellent fit. This fit bridges a gap of more than 20 octaves of the power spectrum. But Matthews and Sandage were not convinced that this agreement shows that the optical radiation is necessarily due to synchrotron emission alone, and the question remains open for the future. If the optical flux were due to synchrotron emission, the energy of the relativistic electrons responsible for the radiation would be about 5 bev in a field of 1 gauss, or 50 bev in a field of 10^{-2} gauss.

Additional data at hand suggest that future identifications of radio stars can be expected, and many of the questions raised by these unique objects will undoubtedly be better understood in the near future.

One of these sources, 3C48, studied by Sandage has also been observed photoelectrically by Baum on the eight-color system used in connection with the redshift-magnitude program. This observation permits the optical energy distribution to be investigated over a broader range extending from the ultraviolet to the infrared. The eight colors were found to fit a slope of -2.25 db per 10^{14} cps. In absolute terms, the observed flux of 3C48 at 5490A (5.46×10^{14} cps) on two nights in December 1961 was found to be 1.04×10^{-29} watt/m^2 per cps.

Over the past few years, plates of the Crab Nebula have been taken by G. Münch with the object of following changes in the large-scale structure of the amorphous mass emitting synchrotron radiation. Particular attention is being given to the moving ripples, which at irregular intervals appear near the hypothetical central star. On February 2, 1962, a diffuse wisp, about 4 seconds long, at a position angle 45° and at about 2 seconds distance from the nuclear star, was detected for the first time. The other moving wisp discovered by Baade and discussed by Oort appears somewhat farther out. Poor weather and lack of observing time prevented following the development of this ripple, but its observation at a smaller distance from the central star points to the likelihood that the central star is still very active in injecting into the nebula large numbers of relativistic particles.

THEORETICAL STUDIES

Stellar Atmospheres

Line profiles of Hγ have been computed on the Kolb-Griem-Shen theory by Searle and Oke for a range of effective temperatures and surface gravities corresponding to those of F-type stars of different lumi-

nosity classes, and compared with observed profiles for (1) the cluster-type variables RR Lyrae and SU Draconis, (2) two F-type subdwarfs, and (3) F-type stars of normal metal abundance. The observed and computed profiles are in excellent agreement, and the line profiles can be used as temperature indicators independent of reddening. A comparison of the derived Hγ temperatures with those determined by fitting observed absolute energy distributions to fluxes computed from model atmospheres shows good agreement, provided allowance is made for interstellar reddening.

For the normal metal stars of spectral type later than F5, the continuum at Hγ is depressed by line blanketing to such an extent that a temperature determination by Hγ fitting is not possible. For late F- and G-type stars, however, Hα profiles remain a practicable temperature indicator, and computations of a grid of Hα profiles have been completed. In the late G-type stars, the Hα profile is dependent not only on temperature but also on metal-to-hydrogen ratio.

Weidemann has analyzed the hydrogen-line profiles and the colors of normal white dwarfs of spectral type DA. The Kolb-Griem-Shen theory was used, and allowance was made for the dependence of pressure on depth. The profiles so computed were essentially independent of the H/He ratio and covered a wide range of surface gravity and temperature. The maximum of the intensity of the hydrogen lines is shifted about 4000° hotter than in main-sequence stars, and the maximum in the Balmer discontinuity about 2500°. The effects of the lines on U, B, V colors and of the reemitted blanketed radiation were estimated. The temperature scale derived is close to an earlier estimate by Greenstein. Weidemann finds that the DA stars range from 18,000°K to 7300°K in effective temperature, log g from 7.3 to 8.5, and masses from 0.25 to 0.85$M_\odot$. The problem of the DB stars, which show only He I lines, was considered briefly; it was found that a very high He/H ratio is needed to alter the opacity source from pure H. Therefore, only if He/H $> 10^3$ will the H lines disappear and be replaced for hot white dwarfs by He I lines.

Wright has reported extraordinarily low excitation temperatures for normal A0 stars. Jugaku explored possible theoretical explanations for this phenomenon, which he found also to be present in Hunger's analysis of α Lyrae, which gives only 5350° for T_{exc}. Jugaku has computed the effects of stratification in depth of line formation, which corresponds to only 300° temperature change, and the model-atmosphere effects which predict T_{exc} = 8460°K for T_{eff} = 9500°—again too small a temperature change to account for the low observed T_{exc}. A possible explanation is a large deviation from local thermodynamic equilibrium, and another is increase of turbulent velocity for lines of low excitation potential.

In metal-poor stars of low T, Rayleigh scattering becomes important, as Traving pointed out for the analysis of globular-cluster red giants and as Greenstein and Wallerstein find for the metal-poor field red giants like HD 122563. Jugaku evaluated the contribution of metallic absorption continua, finding them to be small compared with H^- and Rayleigh scattering. There is no direct observational support for Rayleigh scattering, since it would produce a steeply wavelength-dependent depression of the blue and ultraviolet (i.e., a $U - B$ deficiency in metal-poor stars).

Nishida has studied the problems of the structure and evolution of the helium stars. A series of models for a helium star of 1 solar mass, both in the helium burning phase and in the carbon burning phase, have been constructed. Results show that the evolutionary track lies near the location of nuclei of planetary nebulae in the H-R diagram, but that it does not move to the right across the main sequence of the Population I stars. Calculations of the stellar models for the following problems are in progress using the IBM 7090: (1) Evolution along the

horizontal branch of the Population II stars. (2) Stellar models for stars with very small masses (less than $0.1M_{\odot}$). (3) Evolutionary tracks in the H-R diagram for stars that gravitationally contract to become white dwarfs.

G. Münch and Dr. R. Kippenhahn of the Max Planck Institut für Astrophysik in Munich, Germany, are studying the spectrum of late F-type supergiants to verify whether the Balmer lines can be explained on the basis of models with a unique temperature as function of depth in the atmosphere. Suggestions have been made in the past to explain a supposed increase in the strengths of the Balmer lines, as the surface gravity of the stars decreases at constant effective temperature, in terms of the temperature fluctuations produced by the strong turbulence observed in the line contours and curves of growth for such stars. If the absolute magnitude effect is confirmed, an attempt will be made to construct models nonhomogeneous in temperature and to relate the temperature fluctuations to the properties of the turbulent fields.

Star Formation

Theoretical work on star formation on a phenomenological basis was continued by Schmidt. The main assumption made in these considerations is the absence of systematic radial transport of stars or gas in the Galaxy. It appears that the average past rate of formation of stars around 1 solar mass is less than three times their present formation rate. The past formation history of bright, rapidly evolving stars can be deduced indirectly from the distribution of ultraviolet excesses in late G-type dwarfs. It can be shown that a time-independent birth luminosity function implies a certain distribution of excesses, independent of the past formation rates. Specifically, in this case 72 per cent of the late G-type dwarfs would be metal-deficient by a factor exceeding 2 relative to the Hyades. The actual distribution of excesses shows between 40 and 50 per cent of the late G-type dwarfs to be that deficient. Thus, the birth luminosity function in the solar neighborhood must have been different at earlier times, specifically such that in the early stages of the Galaxy the rate of formation of stars of 10 solar masses, relative to that of stars of 1 solar mass, was about 10 or 20 times larger than it is at present.

Stellar Dynamics

Theory predicts that the only exactly steady states of an unrelaxed stellar system which shows star streaming directed to and from the center are those of Eddington's type. These have potentials of the form $\psi = [\zeta(\lambda) - \eta(\mu)]/(\lambda - \mu)$, where λ and μ are spheroidal coordinates and ζ and η are arbitrary functions. Satisfactory models of this form have been discussed by Kusmin. An attempt was made by Lynden-Bell to discover whether the light distribution of NGC 4594 could arise from such a system. Plates taken by Münch were reduced to relative light intensities which were compared with the predicted functional form of the projected mass density. Results showed that NGC 4594 cannot be of Eddingtonian form unless the mass-to-light ratio varies very considerably and systematically as a function of distance from the center.

Two further stellar dynamical investigations were completed by Lynden-Bell. The first answers the question how long it takes a nonsteady stellar system to approach an unrelaxed steady state and how it does so in the absence of dissipation. The time is about 10 times the period of a typical star around the system, and the mechanism is Landau damping, well known in plasma physics. The second gives a method of solving the self-gravity equation for flattened steady-state systems, and as a result the first exact theoretical model is produced.

Cosmology

Previous reports have mentioned that a decision between the several proposed models of the expanding universe, such

as exploding world models compared with the steady-state model, can be made if the deceleration of the expansion can be measured. This is because the deceleration is caused by the gravitational attraction of matter on individual galaxies. The rate of deceleration measures the density of matter in space, which in turn determines the spatial curvature and the intrinsic geometry of space via the field equations of general relativity. Various ways of finding the deceleration parameter q_0, described in *Year Book 59*, depend on measurement of deviations from linearity of the redshift-magnitude relation or a similar relation such as that between redshift and apparent diameter. The principle of these measurements is that one looks back in time as one looks out in space and can therefore sample the expansion rate of the universe in past cosmic times. This indirect method is observationally very difficult, because galaxies with redshifts of the order of $\Delta\lambda/\lambda_0 = 0.5$ must be observed for significant answers. Furthermore, as was pointed out last year, uncertain corrections for the evolution of the stellar content of galaxies must be made to account for the change in absolute luminosity of these distant galaxies in the light travel time, which is of the order of 5×10^9 years.

Sandage looked into the theoretical possibility of detecting the deceleration directly, if a series of redshift measurements of a given galaxy were made over a suitable time interval. Exact predictions of the change of redshift in a given galaxy with time can be made using the various world models. It turns out that this test of world models is a powerful one in principle because the sign of the effect is different for exploding models and the steady-state model. However, the test is beyond our present technical capabilities, because the effect is extremely small. For a galaxy with a present redshift of $Z = \Delta\lambda/\lambda_0 = 0.4$, the change of redshift with time is only -5.9 km/sec (decelerating) in a million years for the Euclidean model. Similar numbers hold for the hyperbolic and elliptical exploding models. In the steady-state model, a galaxy with $Z = 0.4$ at the present epoch will experience an acceleration rather than deceleration, increasing its redshift at the instantaneous rate of $+9.2$ km/sec per million years. With present optical techniques there is no hope of detecting such small changes of redshift for time intervals of less than 10 million years. If radio techniques are used with observation of the 21-cm H I line, the detection of a frequency shift of 3×10^{-2} cps per year in a signal of frequency 2000 Mc/sec is required, which again appears to be impossible by present methods. To solve the problem in this way will require, at our present level of technology, a precision redshift catalogue to be stored away in a stable society for 10 million years.

Miscellaneous

The problem of the transfer of the radiation in the [O I] line $^3P_2 - {}^3P_1$ at 158.13 cm^{-1}, between the two lowest sublevels of the ground state, through the earth's ionosphere, has been studied by G. Münch by removing a number of restrictive assumptions introduced in previous attempts at a solution. The solution found has been numerically applied to a model atmosphere by computing the specific intensity and flux of radiation as a function of height. In a paper submitted for publication to the *Astrophysical Journal*, the possibility of measuring the flux emergent at the top of the atmosphere, which amounts to 0.1 erg/cm^2 sec, has been suggested as a method for the determination of the kinetic temperature and the oxygen concentration.

The eventual possibility of carrying out observations of galactic and extragalactic objects in Lyman-α radiation was investigated by Münch. It has been found that the decay of Lyman-α through 2-photon emission and dust absorption makes it quite unlikely that galactic diffuse Lyman-α radiation exists, unless the immediate neighborhood of the sun is an H II region, as is suggested by the

emission nebulosities excited by the nearby stars γ Velorum and ζ Puppis. Lyman-α emission from the H II regions in extragalactic systems not surrounded by neutral gas and with low dust content (as the coronas of M 31 and the Galaxy) may possibly reach the solar system when their redshift exceeds 1000 km/sec.

INSTRUMENTATION

The 10-inch ruling engine has been in operation with the new system of interferometric control. As was described in last year's report, this system employs intermittent spacing, with a fringe clamp and differential corrector. Gears are now on hand for ruling at 407, 610, and 915 grooves per millimeter. With water cooling of the mercury-198 source for the interferometer, the contrast of the fringes is more than ample for a path difference of 10 inches. The performance of the control system has been accurate and reliable, so that the average quality of the gratings has been raised and the productivity of the machine has been increased. Virtually theoretical resolving power is obtained in the higher orders of the gratings, and scattered light is held to very low levels.

Eleven plane gratings of high quality were produced by Roberts under the direction of H. W. Babcock in sizes ranging from 3 by 4 to 6 by 10 inches; most were 5 by 8 inches with a spacing of 610 grooves per millimeter.

Gratings have been delivered to the Kodaikanal, Sacramento Peak, Dominion Astrophysical, and David Dunlap Observatories and to the National Bureau of Standards. The records show that gratings produced here are now in use at 16 observatories throughout the world in addition to Mount Wilson and Palomar.

During the last year, a number of minor modifications have been made by Oke to the photoelectric coudé spectrum scanner on the 100-inch telescope. The seeing compensation is now found to be quite satisfactory, provided that the seeing is average or better and the zenith distance is not more than approximately 45°. A program has been begun to measure line profiles and equivalent widths in the spectra of selected stars. Accurate photoelectric profiles, such as those of Hγ in A stars, can be used to check the photographic calibration systems used at various observatories.

To facilitate the reduction of very small-aperture observations made with the solar magnetograph, digitizing equipment has been installed at the 150-foot tower telescope under the supervision of Howard. A shaft encoder digitizes the shaft position of a strip-chart recorder, and this information is punched on paper tape at the telescope. These data will be fed to a digital computer at a later time, and the autocorrelation analysis can proceed with no intermediate steps in the reduction. Thus it will be possible to accumulate a great number of observations with small apertures and greatly increase the accuracy of the existing autocorrelation functions.

GUEST INVESTIGATORS

The following programs have been carried out by guest investigators during the report year.

Dr. George O. Abell of the Department of Astronomy, University of California, Los Angeles, continued his study of rich clusters of galaxies. The luminosity functions for galaxies in six rich clusters, determined by a method of extrafocal photographic photometry, are now available. Except for scale factors that depend upon the richnesses of the clusters, the luminosity functions are all similar. That of the Coma cluster is representative. The number of galaxies brighter than m, $N(m)$, is given, approximately, by

$$\log N(m) = \text{constant} + s \log m$$

where $s = 0.78$ for $m \leq 14.7$, and lies in the range 0.23 to 0.29 for $14.7 < m \leq 18.3$, depending upon what correction is applied for the nonmember galaxies in the cluster field. The discontinuity in s is due to a maximum in the bright end of the luminosity function. If the luminosity functions of the different clusters are fitted together at this discontinuity, and the relative distance moduli so obtained are plotted against the known redshifts of the clusters, the root-mean-square velocity dispersion about a straight line is less than 600 km/sec. On the basis of the clusters investigated so far, therefore, it appears that the luminosity functions of clusters can provide good estimates of their relative distances.

In the course of this photometry of galaxies Abell made a detailed investigation of some properties of the U, B, V system. He has numerically integrated 25 stellar energy distributions published by Code and Melbourne against the response functions of the U, B, V cell-filter combinations, through various air masses. The computed variation of extinction with color (for a given air mass) is present but small for V, is in approximate agreement with the variation usually assumed for $B - V$, and is appreciable and nonlinear for $U - B$. Observed values of the extinction at Mount Wilson are, on the average, in agreement with those computed. In addition, the computed extinctions for a given color of star vary nonlinearly with air mass; as a consequence, the usual procedure for reducing photoelectric observations can introduce errors in $U - B$ colors of as much as 0.1 mag. Finally, accurate color equations were derived to transfer from instrumental to U, B, V colors, and improved colors of blackbodies have been obtained.

Dr. Lawrence Aller of the University of Michigan Observatory obtained a series of spectrograms of the planetary nebula NGC 7009 with exposures ranging from a few hours to two nights. They revealed a large number of recombination lines of C, O, Ne, and other elements. With the aid of photoelectric observations of the stronger emission lines, an attempt was made to reduce the intensities of the lines as measured on the spectrograms to a true relative scale for a study of recombination rates and ionic abundances in the nebula. Further spectrograms of shorter exposure and probably additional photoelectric measurements of weaker lines will be necessary to obtain an accurate wavelength-dependent intensity calibration.

Dr. Stanley J. Czyzak of the Aeronautical Research Laboratories at the Wright Patterson Air Force Base continued his calculations of accurate wave functions of various ions of P, S, Cl, and A, all of which are of astrophysical interest. The values for 25 ions of the $3p^q$ configuration were completed.

It was now possible to begin a detailed examination of the screening constants, spin-orbit, spin-spin, and spin-other-orbit calculation for the transition probabilities for the $3p^q$ configurations. Spectral data of various gaseous nebulae were examined for forbidden lines to determine which of the transitions had been observed. Also a study was made to determine other transitions which are significant. In addition, preliminary calculations of collision cross sections were carried out by Dr. Czyzak on ions with $q = 1$, since these calculations would be simpler than those for $q > 1$.

Dr. H. Gollnow of the Mount Stromlo Observatory of the Australian National University took spectra of about 20 stars with the coudé spectrographs of the 100-inch and 200-inch telescopes in a search for magnetic stars. Dispersions of 4.5 A/mm and 10 A/mm and a differential analyzer in front of the slit were used. The stars were selected between declinations $+25°$ and $-40°$, so that their observations can be continued at the Mount Stromlo Observatory. About 50 per cent of the stars show too large rotational broadening for the measurement of Zeeman displacements. Of the other stars, HD 24712 was studied in some

detail and a magnetic field varying between +574 and +997 gauss was found. The observation of this star will be continued.

During the report year, studies of velocity fields in the solar atmosphere have been continued by Dr. R. B. Leighton of the California Institute of Technology with the assistance of Robert W. Noyes and George W. Simon. Special emphasis was given to oscillatory motions and large-scale currents discovered with the Mount Wilson instruments in 1960.

The main results may be summarized as follows: (1) The small-scale velocity field (1000–5000 km linear dimension) in the upper photosphere exhibits a strong tendency to repeat itself in time with a 5-minute period. That is, each local region undergoes a quasi-sinusoidal motion which may persist for several cycles. (2) The period of the above oscillation does not seem to be strictly constant with altitude but tends to decrease by 10 to 20 per cent as one proceeds from the middle photosphere into the lower chromosphere. (3) The intensity variations in the lower chromosphere, as seen at the cores of such strong lines as Na λ5896, Mg λ5173, or similar lines, are also observed to fluctuate in time with the period of the velocity oscillations. This suggests that the oscillatory motions are connected with waves which transport energy into the chromosphere and liberate it as heat or radiation. (4) A network of horizontal currents, grouped into a system of large-scale (5000–30,000 km) "convective cells," is observed. These cells appear to be distributed rather uniformly over the solar surface, and their correlation properties suggest a strong tendency toward an ordered array over distances up to 50,000 or 100,000 km.

Dr. D. H. McNamara of North American Aviation investigated the rotational velocities of B5–B9 stars in the Orion association. The spectra from which the rotational velocities were determined were obtained with the 16-inch camera of the 100-inch coudé spectrograph. This study was an extension of an earlier investigation during the previous year of the rotational velocities of the B0–B3 Orion stars. The B5–B9 stars were found to rotate somewhat more slowly on the average than the general field stars of the same type. The maximum rotational velocities were found to occur in the B5–B7 spectral types. The observations also indicate that there is a smaller percentage of slow rotators among the B5–B9 group than among the B0–B3 group.

Narrow-band photometric observations of stars in the star clusters h and χ Persei and M36 have been obtained by Dr. McNamara with the 60-inch telescope. A narrow-band photometric study of eclipsing variables has also been initiated with the 20-inch telescope at Palomar Observatory. No results are yet available on these photometric programs.

Dr. Walter E. Mitchell, Jr., of the Perkins Observatory continued the solar observations with the Snow telescope during the summer of 1961 with the assistance of Mr. John C. Muster. Numerous improvements were made to the telescope and spectrograph. To reduce the scattered light due to Rowland ghosts, an arrangement of mirrors and intermediate slit was designed to deliver the beam twice to the grating, i.e., to have the spectrograph act as its own monochromator. A beam splitter consisting of a plane parallel plate of fused quartz was mounted just inside the entrance slit of the spectrograph. The fraction of the beam returned by this plate was used as a monitoring signal for ratio recording.

The double-pass system was employed to make photoelectric tracings of the following regions: Na D_1 and D_2, Mg 'b', Ca II H and K, Hβ, Hγ, Hδ, Ca I 4226, and λ3570. Throughout, there is a noticeable lowering of central intensities (by amounts up to 10 per cent of the continuum) both as compared with the *Utrecht Photometric Atlas of the Solar Spectrum* and with the Snow single-pass observations with the same grating. The region 6700–3900 A was recorded in first

order with full resolution and with band passes of 4, 8, and 16 A.

Infrared stellar photometric observations were obtained by Dr. Mitchell and Mr. Philip E. Barnhart with the assistance of Messrs. John C. Muster, Ronald E. Roll, and John H. Hill. Instrumental assistance was also provided by Messrs. Charles E. Gramm, Anthony J. Prasil, and Dr. William H. Haynie of the Eastman Kodak Company. Preliminary infrared magnitudes were measured for 31 G, K, and M giants, 6 red supergiants, and ϵ Aurigae using an improved Eastman Kodak–Ohio State University infrared stellar photometer on the 60-inch and 100-inch telescopes. The photometric system has the following characteristics:

Magnitude Designation	Effective Wavelength, μ	Band Pass, μ
X	2.2	0.24
Y	3.7	0.43

When the observed visual-infrared color indices of the measured stars are compared with the indices deduced theoretically for the stars considered as blackbody radiators, the following conclusions may be drawn: (1) Nonvariable giants and supergiants lie, in general, close to the theoretical relation; i.e., to a first approximation these stars behave as blackbody radiators. (2) A few nonvariable giants and supergiants fall unexpectedly far above or below the theoretical relation, suggesting large blanketing effects on visual magnitude or errors in temperature assignments. (3) When observed at visual magnitudes well below maximum, long-period variable stars, whose temperatures are derived from their spectrum characteristics at mean maximum light, show an excessive reddening compared with the theoretical relationship; that is, the variability occurs almost entirely at wavelengths shorter than 2 μ. (4) Epsilon Aurigae shows an infrared excess of approximately 1.2 mag, thus supporting the hypothesis that it has a large infrared component.

Attempts were made by Dr. Mitchell and Mr. Barnhart to operate a helium-cooled Ge:Cd detector for the 8- to 13-μ region, but no stellar signal was distinguishable from photometer and telescope signals.

Dr. Bruce C. Murray of the Lunar Research Laboratory at the California Institute has continued the program of photoelectric colorimetry of the moon using the spectrum scanner at the Cassegrain focus of the 60-inch. The scanning technique initiated during 1960–1961 has been perfected, including the successful implementation of "lunar rate" for the 60-inch, to a point where an accuracy of 0.01 to 0.02 mag has been achieved for the eleven independent color values obtained from each object examined. Approximately fifteen lunar areas of 15 by 15 km size have been observed as well as various planetary and stellar objects for comparison. The data are in the final phase of reduction preparatory to being submitted for publication.

The testing and development of a long-wavelength infrared photometer have continued during the year, two nights during 1961 having been devoted to this project at the 60-inch. Recently, however, a special 20-inch infrared telescope has been designed and built. This telescope with a novel optical and photometer system has been given preliminary trials at Mount Wilson but will later be placed in operation at a 13,000-foot site on White Mountain.

Dr. Robert L. Wildey and Mr. Howard A. Pohn of the Lunar Laboratory have initiated a U, B, V photometric program to investigate an apparent asymmetrical phase lag in the brightness versus phase curves of different localities on the moon. This phenomenon is apparent in the older photographic photometry of the moon; if confirmed photoelectrically, it represents a most surprising natural phenomenon of the moon.

Observations were continued by Dr. Daniel M. Popper of the University of California at Los Angeles on the program of establishing absolute dimensions of

stars of various kinds from the analysis of eclipsing binary systems. Relatively few spectrograms were obtained during the year. Reanalysis has been completed for three solar-type eclipsing binaries: VZ Hydrae, WZ Ophiuchi, and UV Leonis. The new spectrographic observations with higher dispersion lead to masses about 30 per cent smaller than those obtained previously for two of the systems. The revised values are more in accord with the values from visual binaries of the same spectral types. The photometric observations used in the analysis of WZ Oph are also new, having been obtained with the 20-inch at Palomar. A modern light curve is badly needed for VZ Hyd.

The following new results are based on incomplete observations. (1) RR Arietis is a sixth-magnitude K star found to be eclipsing by Archer. The velocity variation appears to be less than 5 km/sec. (2) Revised values of the masses of the K-type giants of RZ Cancri are 3.2 and 0.5. (Dr. Popper's earlier published values were 0.4 and 2.6.) (3) The D lines of the fainter components, not previously announced, have been observed in the following eclipsing systems: TW Draconis (difficult), WW Draconis, RR Lyncis (metallic-line star; observations complete), XY Puppis (difficult), and TX Ursae Majoris (difficult).

Dr. Jorge Sahade of the La Plata Observatory, Argentina, continued his spectroscopic observations, obtaining plates of the following objects: (1) The eclipsing star V453 Scorpii to supplement material previously obtained at Bosque Alegre with lower dispersion; (2) HD 188439, an early-type object which Lynds had announced as showing a photometric period of about 9 hours; (3) HD 207739 and AG Pegasi to detect spectral changes, if any, relative to observations of previous years; (4) the eclipsing system V367 Cygni to compare the spectral features with those of other systems already investigated; (5) HD 192281 to supplement observations made in 1960; and (6) 17 Leporis to be used with material already taken by Dr. G. Wallerstein for a joint investigation of the velocity curve of the *M* component of the system.

Dr. H. Spinrad of the Jet Propulsion Laboratory of the California Institute has analyzed infrared spectrograms of Venus in the plate files. Rotational temperatures have been derived from the intensity distributions of CO_2 rotational lines in the λ7820 band. The rotational temperatures vary from 214°K to 445°K. Total pressures have been obtained from measurements of the corrected widths of the CO_2 rotational lines; these pressures correlate quite well with the rotational temperatures in the sense that the high pressures correspond to observations of high rotational temperatures.

Dr. Spinrad has also found that the ammonia and methane rotational lines in the yellow-red region of the spectrum of Jupiter do not have the expected inclination on coudé spectra in which the spectrograph slit was placed along the planet's equator. This result is interpreted to mean that these gases are probably not rotating with the same velocity as the Jovian cloud layer. Examination of 100-inch and 200-inch coudé spectra indicated marked variations in the relative intensities of the Jovian NH_3 lines near λ6460.

Dr. Uli Steinlin continued his observations with the 48-inch schmidt camera to obtain material for the program on three-color photometry of the Observatory in Basel, Switzerland. Dr. W. Becker from Basel participated in the observations from February until April. The observing program was completed in April with 572 plates taken (416 of them after July 1961) in the following fields: eight Milky Way fields: NGC 1807/17, M37, Great Sagittarius cloud, Small Sagittarius cloud, Scutum, Aquila, Lacerta, Cassiopeia; ten fields in higher galactic latitudes: Selected Areas 51, 54, 57, 82, 94, 107, 133, 141, 158, and Hyades.

Plates have mostly been taken in *R*, *G*, and *U* for three-color photometry of some clusters and, above all, of field stars in the Milky Way as well as in higher

galactic latitudes. In some fields, plates have also been taken in B and V to permit three-color photometry in the U, B, V system as well, and to make possible a comparison of the effectiveness of the two systems. The limiting magnitude lies in general between 18^m and 19^m. The three-color photometry should provide: (1) density function and luminosity function in different directions from the sun; (2) color-magnitude diagrams of clusters and of clouds of stars within the Milky Way; (3) possibly a separation of disk and halo populations in higher galactic latitudes.

About 1500 stars in each of the following fields have already been measured: Selected Area 54, 57, 82, and 107. The reduction of these measurements and work in other fields is under way at the Basel Observatory.

Photoelectric U, B, V standards for the Basel Observatory program have been obtained with the 100-inch by Dr. A. Th. Purgathofer of the Vienna University Observatory. Observations of stars in the magnitude range from $V = 16^m$ to 18^m were obtained for most of the Selected Area fields.

Dr. A. Unsöld of the University of Kiel, Germany, in 14 nights of observing, obtained high-dispersion spectra of various groups of stars which might be suitable for studying the relations between chemical composition and evolution. Most of the plates were taken with the 32-inch camera of the coudé spectrograph of the 100-inch telescope, and they cover the photographic and the visual regions.

HD 161817, usually classified as sdA2, is most probably a horizontal branch star. Its huge space motion, according to Eggen, is shared by Wilson 10367 = LPM661, an 11-mag F8 main-sequence subdwarf, of which at least the photographic region could be obtained with the 16-inch camera. Both stars are obviously metal-poor. Quantitative comparison of their chemical composition should give most interesting indications about evolutionary events in the red giant or supergiant region of the H-R diagram. At present both spectra are being analyzed in Kiel. For comparison with HD 161817, the more or less normal stars δ Delphini A7V, α Ophiuchi A5III, and 111 Herculis A3V had been selected. Delta Del has quite sharp lines and has since been measured for wavelengths and identifications. The other two stars show strong rotational broadening. Although α Oph is an MK-type star for A5III, the three-dimensional Paris classification would place it under A5V. This is probably due to rotational broadening of the high members of the Balmer series simulating the Stark broadening in main-sequence stars. This problem is being further analyzed, with J. Kaler (Michigan), working at present in Kiel.

Spectra for investigating possible differences in composition connected with evolution were taken by Dr. Unsöld with the 32-inch camera in the photographic and visual regions. Five stars with spectral types F5 to G2V of Eggen's γ Leonis group, including 5β Virginis (F8), supposed to be metal-superrich, and two later-type (dK5) stars taken from O. C. Wilson's "red" and "violet" groups of the main sequence, HD 156026 and HD 192310, were observed.

Some visual test plates of α Cygni A2 Ia showed that the structure of its Hα emission component has changed considerably since the last visual plates were taken in 1957. The photometric analysis is being carried through by Dr. Comper in Kiel.

A considerable number of 100-inch coudé plates of γ Serpentis F6IV–V taken in 1957 by Unsöld, in 1959 by Traving, and in 1960 by Bonsack have been analyzed in detail by W. Kegel in Kiel. The variation of turbulence with depth turned out to be an essential feature. The relative abundances of the metals are the same as in the sun, but, relative to hydrogen, all the heavier elements are reduced by a factor of about 1.7. That, as well as the space velocity and the weak ultraviolet radiation, indicates that γ Ser is a member of the intermediate Population II.

Dr. George Wallerstein of the Astronomical Department of the University of California at Berkeley has been observing K giants in order to obtain abundances of the elements. The observations of G8–K2 stars in the general field at 6 A/mm in the yellow region are now complete. Stars with ultraviolet excesses from 0.20 mag to deficiencies of 0.10 mag will be compared with the K0 giants in the Hyades. Many of the stars included are high-velocity stars. Some of the other more interesting stars on the list are a few that Gyldenkern suspects to be metal-rich from his narrow-band photometry, and some "4150 stars" that show strong CN in the blue region. In addition, two K0 giants in Praesepe have been observed at 15 A/mm in the yellow. This work is in cooperation with Dr. Helfer of the University of Rochester.

The high-velocity A star HD 109995 has been observed in order to compare it with Sirius and another high-velocity A star, 7 Sextantis. A cursory examination of one 4 A/mm and two 10 A/mm plates shows that the lines in HD 109995 are very much weaker than in either of the other two stars.

Dr. Wallerstein obtained several plates of 31 Cygni that showed chromospheric Ti II lines as well as the K line. These plates will be reduced in cooperation with Dr. Wright of the Dominion Astrophysical Observatory.

Dr. R. v. d. R. Woolley and Mr. C. A. Murray of the Royal Greenwich Observatory carried out two programs with the coudé spectrograph attached to the 100-inch reflector. They exposed a number of plates with the 32-inch camera and with the 72-inch camera. Some of these were exposed to an intensity suitable for the measurement of radial velocity; others were more lightly exposed so that they would be suitable for spectrophotometry. The radial-velocity plates have been measured, and the results will be published; the remaining plates have been examined with a spectrophotometer. The spectra of τ Ceti, 107 Piscium, and o^2 Eridani obtained with these plates have been investigated by Dr. Pagel at the Royal Greenwich Observatory, and the results have been worked up for a determination of the abundances of elements in these stars by differential curve-of-growth analysis.

Dr. Woolley and Mr. Murray also carried out a program of direct photography at the Cassegrain focus of the 60-inch telescope. They took repeat plates of a number of cluster fields that had been observed by van Maanen, including the cluster M 67. In all, 15 fields were photographed; the plates, together with a selection of van Maanen's first epoch plates, are at the Royal Greenwich Observatory awaiting measurement for proper motion.

STAFF AND ORGANIZATION

The Observatories suffered a severe loss in the sudden death on December 26, 1961, of Don O. Hendrix, Superintendent of the Optical Shop. Mr. Hendrix joined the staff in 1931 and became Superintendent of the Optical Shop in 1947. He developed extraordinary skill in the hand figuring of large nonspherical surfaces required in many modern optical designs. Among the projects he carried out were the optics for the 48-inch schmidt telescope at Palomar and the corrector plates for the 15 schmidt cameras on the spectrographs at Palomar and Mount Wilson. He also did the final figuring of the 200-inch mirror after it had been moved to Palomar. While on leave from the Observatories he ground and figured the 120-inch mirror of the Lick Observatory. The high efficiency of the present optical equipment of the Observatories is to a large extent due to Hendrix' skill and ingenuity.

Drs. Robert Howard and Olin Eggen joined the staff of the Observatories in September 1961. Dr. Howard plans to investigate solar magnetic fields, and Dr. Eggen has undertaken an extensive photometric program. Dr. Otto Struve became a member of the staff in March 1962.

Research Division

Staff Members

Halton C. Arp
Horace W. Babcock, *Assistant Director*
William A. Baum
Ira S. Bowen, *Director*
Armin J. Deutsch
Olin J. Eggen
Jesse L. Greenstein
Robert F. Howard
Robert P. Kraft
Guido Münch
J. Beverley Oke
Allan R. Sandage
Maarten Schmidt
Otto Struve
Olin C. Wilson
Fritz Zwicky

Research Associates

Jan H. Oort
Kenneth O. Wright

Staff Members Engaged in Post-Retirement Studies

Harold D. Babcock
Milton L. Humason
Alfred H. Joy
Seth B. Nicholson

Senior Research Fellows

Rudolph Kippenhahn
Satoshi Matsushima
Minoru Nishida
Evry Schatzman
Leonard T. Searle
Volker Weidemann

Carnegie Research Fellows

Léo Houziaux
Charles R. O'Dell

National Science Foundation Fellows

John C. Brandt
Paul W. Hodge

Research Fellows

Jacques Berger
John Hazlehurst
D. H. P. Jones
Jun Jugaku
Donald Lynden-Bell
Luis Münch
Konrad Rudnicki
Wallace L. W. Sargent
Henrietta Swope

Research Assistants

Christine Arpigny
Jeanne Berger
Frank J. Brueckel
Sylvia Burd
Subhash Chandra
Jai H. Choy
Mary F. Coffeen, Librarian
Thomas A. Cragg
Donald S. Hayes[1]
Emil Herzog
Joseph O. Hickox[2]
Basil N. Katem
Charles T. Kowal
A. Louise Lowen
Joyce E. Sheeley
Merwyn G. Utter

Student Observers

James E. Gunn
Manuel E. Mendez
Dimitri M. Mihalas
Robert H. Norton
Robert A. R. Parker
Lewis L. Smith
Robert L. Wildey

Photographer

William C. Miller

Instrument Design and Construction

Lawrence E. Blakeé, Senior Electronic Technician
Eileen I. Challacombe, Draftsman
Floyd E. Day, Optician
Kenneth E. DeHuff, Machinist
Robert D. Georgen, Machinist
Don O. Hendrix, Superintendent of Optical Shop[3]
Melvin W. Johnson, Optician
Stuart L. Roberts, Instrument Maker
Bruce Rule, Project Engineer
Marlin N. Schuetz, Electronic Technician
Russell R. Van Devender, Jr., Designer and Superintendent of Instrument Shop
James S. White, Electronic Technician[4]

Maintenance and Operation

Mount Wilson Observatory and Offices

Paul F. Barnhart, Truck Driver

[1] Resigned March 23, 1962.
[2] Retired September 30, 1961.
[3] Died December 26, 1961.
[4] Resigned December 15, 1961.

Wilma J. Berkebile, Secretary
Herbert A. Cole, Laborer[5]
Hugh T. Couch, Carpenter
Helen S. Czaplicki, Editorial Typist
Stewart F. Frederick, Janitor[6]
Eugene L. Hancock, Night Assistant
Mark D. Henderson, Gardener
Margaret Higgins, Stewardess
Anne McConnell, Administrative Assistant
Leah M. Mutschler, Stenographer and Telephone Operator
Bula H. Nation, Stewardess
Alfred H. Olmstead, Night Assistant
Arnold T. Ratzlaff, Night Assistant
Glen Sanger, Janitor
John E. Shirey, Laborer
William D. St. John, Janitor and Relief Engineer
Wilma G. Totten, Stewardess[7]
Benjamin B. Traxler, Superintendent

[5] Resigned March 9, 1962.
[6] Resigned October 31, 1961.
[7] Resigned November 15, 1961.

Palomar Observatory and Robinson Laboratory

Audrey A. Acrea, Stewardess
Fred Anderson, Machinist
Jan A. Bruinsma, Custodian
Maria J. Bruinsma, Stewardess
Eleanor G. Ellison, Secretary and Librarian
Arlis R. Grant, Stewardess[8]
Leslie S. Grant, Relief Night Assistant and Mechanic
Byron S. Hill, Superintendent
Helen D. Holloway, Secretary
Charles E. Kearns, Night Assistant
J. Luz Lara, Laborer
Harley C. Marshall, Office Manager
Dwight M. Miller, Mechanic
George W. Pettit, Janitor[9]
Robert E. Sears, Night Assistant
William C. Van Hook, Electrician and Assistant Superintendent
Gus Weber, Assistant Mechanic

[8] Resigned October 13, 1961.
[9] Retired September 3, 1961.

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Geophysical Laboratory

Washington, District of Columbia

Philip H. Abelson
Director

Contents

INTRODUCTION

The Geophysical Laboratory continues its diversified program of studying the earth through application of physical science and mathematics. Superficially, the areas of effort during the report year appear to be very similar to those of the preceding period. There was activity in experimental petrology, statistical petrology, crystallography, ore minerals, meteorites, geothermal calculations, the ages of rocks and minerals, and organic geochemistry. There were, however, substantial shifts of emphasis within the program. For instance, much of the work this year in experimental petrology was focused on the pyroxenes, and greater emphasis was placed on phase equilibria at higher pressures. A substantial effort was expended on studies of the mineralogy of meteorites. New investigations of organic geochemistry were initiated, including analysis of Precambrian carbonaceous materials.

The Laboratory is continuing its broad program of studying the phase relations in basalts and their derivative rocks. The alkali-type basalts in particular have been examined from several viewpoints this year. The fundamental joins nepheline-diopside, acmite-diopside, and nepheline-acmite, bearing on alkali-type rocks, were worked out by Schairer, Yagi, and Yoder. Studies in the system Na_2O-Fe_2O_3-Al_2O_3-SiO_2 have been carried out by Schairer and Bailey in view of its importance to the peralkaline derivative rocks.

Work by Clark, Schairer, and de Neufville on the composition plane $CaMgSi_2O_6$-$CaAl_2SiO_6$-SiO_2 at low and high pressures represents the first extensive study of the effect of pressure, transmitted by an inert medium, on melting and subsolidus relations in a portion of a complex quaternary system. Some of the phase relations at 20 kb are totally different from those at atmospheric pressure. Anorthite melts incongruently at this pressure, and there are fields of sillimanite, corundum, and probably also "β alumina" on the liquidus near its composition. Pressure greatly increases the maximum amount of alumina that the pyroxene can accommodate in its structure; lime Tschermak's molecule ($CaAl_2SiO_6$) is stable up to 1500°C at 20 kb, and there is complete solid solution between this phase and diopside. This work clearly shows that important changes in melting relations are produced by pressure and that phase diagrams determined at atmospheric pressure cannot be applied to the production of magma at great depths in the earth.

Boyd and Schairer present final results on the system $MgSiO_3$-$CaMgSi_2O_6$. Most mafic rocks contain two pyroxenes, and this binary system is fundamental to an understanding of the mineralogy and genesis of these rocks. It was found that the solvus intersects the solidus over a composition interval of 35 weight per cent, so that solid solution between the Ca-rich and Mg-rich pyroxenes is much more restricted than was previously thought. Evidence for a hitherto unrecognized form of Mg-rich pyroxene was found in runs at temperatures above 1385°C.

Yoder and Tilley continued with Schairer their heating experiments at 1 atmosphere on natural basalts and natural coexisting pyroxenes, and completed the preparation of their extensive monograph, "The origin of the basalt magmas: An experimental study of natural and synthetic rock systems."

One of the crucial systems in petrology, diopside-forsterite-silica, has been reinvestigated in part by Schairer and Yoder. The revisions are of exceptional import to the general differentiation of magmas and the nature of pyroxenes that crystallize in them. Many refinements occur within a very small temperature interval, and by close temperature control and calibration seven isothermal

sections in 5° intervals were mapped out. Turnock has surveyed melting relations of synthetic pyroxenes on the join diopside-hedenbergite, using controlled atmospheres with fixed partial pressures of oxygen.

In his studies of metamorphic reactions Greenwood has made two contributions, one theoretical and the other experimental. He has derived equations for equilibrium boundaries between reacting phase assemblages in systems that contain two volatile components and are subject to variation of pressure and temperature. These equations have the same form as the usual expressions for crystal-liquid equilibria but do not carry the restriction that the relative proportions of the two volatile components are limited by the proportions of the other components. The effect of removing this restriction is to make stable many reactions that would normally be regarded as metastable. He has also been able to delineate the upper and lower stability limits of anthophyllite and observe the nucleation kinetics of the mineral.

Many of the principal mineral assemblages found in low-grade metamorphic rocks include chlorite and quartz. Previous synthesis studies had indicated that these are not compatible minerals. New studies by Fawcett and Yoder, involving experiments of long duration, have successfully resolved this apparent conflict with nature.

Lindsley has shown that synthetic titaniferous magnetites in equilibrium with ilmenite contain little or no ilmenite in solid solution at temperatures below 1000°C; compositions of these magnetites lie close to the magnetite-ulvöspinel join. The titanium content of the magnetites is strongly dependent on oxygen fugacity as well as on temperature and hence cannot be used as a simple geologic thermometer. Hydrothermal oxidation of magnetite-ulvöspinel solid solutions yields ilmenite-magnetite intergrowths texturally similar to natural occurrences, supporting the hypothesis that many natural ilmenite-magnetite intergrowths may form by oxidation.

The most important natural compound of boron is tourmaline. The stability range of one of the end members of tourmaline, dravite, has been outlined under hydrous conditions, special care being taken to retain the sodium and boron. The work was carried out mainly by Robbins, of the National Bureau of Standards, working during his free time at the Laboratory, with the help of Yoder.

Boyd and England have found that pyrope garnet melts incongruently to spinel and liquid in the same pressure range as that in which basalts are believed to form in suboceanic areas. Spinel contains no silica, and so the liquid that forms by this reaction is oversaturated. Hence, the incongruent melting of pyrope may explain the development of oversaturated basalts from mantle rocks believed to be undersaturated. The classic reaction that has been used to explain the development of oversaturated lavas is the incongruent melting of enstatite, discovered by Bowen in runs at atmospheric pressure. However, Boyd and England showed last year that enstatite melts congruently at pressures greater than those present at moderate depths in the earth's crust.

In statistical petrography, studies of the phenocryst-groundmass relations in peralkaline lavas (Zies and Chayes) and of the relations between two-mica and biotite-hornblende granites in the Port Clyde peninsula (Suzuki and Chayes) have been continued, with results of considerable interest. Chayes has now fully substantiated his suggestion of last year that in the typical Harker array the variance of silica is approximately an order greater than any other variance; in 23 of 25 published arrays for which calculations have been completed the variance of silica is larger than the *sum* of all other variances. This great excess of silica variance is the most important single influence on the correlations that characterize a Harker array.

J. D. H. Donnay (Johns Hopkins University) and G. Donnay have applied their second generalization of the law of Bravais to the elucidation of the external forms of ionic crystal structures. Their study of the mineral barite, the morphology of which had heretofore remained unexplained, leads to the new concept of "centers of charges." These turn out to be the equipoints of the bond assemblages. This punctualization of charges is apparently the key to the morphologies of ionic crystals, the interpretation of which is a generalization of that of the NaCl morphology.

Burnham has developed a least-squares technique for refinement of lattice constants of crystals, which has been programmed for the IBM 7090 digital computer. The procedure, already employed successfully by other staff members, has the following features. It is applicable to crystals of any symmetry and will accept data, from cards or tape, either as angle measurements for any wavelength or in the form of calculated *d* values. Observations may be weighted according to any scheme, and up to nine systematic correction terms may be included with each observation.

Morimoto has studied the transition mechanisms by which the three polymorphic forms of bornite are interconverted.

Studies of many facets of the ore minerals continue. Increased emphasis is being placed on application of laboratory findings to ores. However, the program is still predominantly a laboratory investigation of phase equilibria. The Mo-S system was studied by Morimoto and Kullerud, who found that the "Mo_2S_3" phase is only stable above 605°C. Very important results have been obtained on the Fe-Ni-S system, in which at high temperatures Kullerud found liquid immiscibility over a large region extending across the sulfur-rich part of the system. In other studies he also showed that pentlandite breaks down at 610°C and that bravoite is only stable below 137°C. In the Fe-Mo-S system Kullerud and Buseck found that the minerals pyrite and molybdenite are stable together below 726°C. The Cu-Ni-S system is being investigated by Moh and Kullerud, who have finished the 600°C isothermal section. Buseck studied the Fe-Ni-As system and has synthesized the new mineral oregonite. The solid solutions in the univariant region containing pyrite, pyrrhotite, chalcopyrite, and vapor were examined by von Gehlen and Kullerud. They found that at 600°C application of the pyrite-pyrrhotite thermometer gives temperatures about 50°C lower when chalcopyrite is present than when it is absent. Brett studied exsolution textures from solid solutions involving bornite (digenite-bornite, chalcocite-bornite, chalcopyrite-bornite). His results indicate that textural evidence alone does not permit drawing of reliable conclusions about the thermal history of the minerals that form these solid solution pairs.

Two studies were directed at problems involving meteorites. Clark, studying the system Fe-Ni-P, has demonstrated that the Fe/Ni ratio of the schreibersite, $(Fe,Ni)_3P$, in equilibrium with both kamacite and taenite changes measurably with temperature. The phosphide is a common constituent of iron meteorites, and its composition, along with the compositions of the alloy phases, will help to trace the history of these extraterrestrial bodies. A curious, but simple, relationship that has emerged from the synthetic system is that the ratio Ni/(Fe + Ni) is the same in the schreibersite as in the taenite (γ alloy) with which it is in equilibrium. Ramdohr has examined polished sections of more than a hundred stony meteorites. He has identified more than twenty minerals, half of which had not been seen previously in stony meteorites. Native gold was seen in specimen. In addition, he observed twelve new minerals that have not been fully identified but whose composition can be partially inferred from associations with known substances. Noteworthy

structural and textural relations were also seen, including localized droplets of fused troilite and iron.

In a continuation of the theoretical geothermal studies of the past few years, the effect on heat flow at the surface produced by very high thermal conductivity at depth in the earth has been investigated by Clark. He has found that under certain circumstances high conductivity at depth reduces the surface flux. Under other conditions the opposite effect is produced. The possibility of variable conductivity in the earth introduces an ambiguity in the interpretation of heat-flow measurement in addition to ambiguities caused by lack of precise knowledge of the distribution of radioactivity and the initial temperature. A second geothermal investigation concerns the cooling of the deep mantle. It has been found that appreciable cooling could take place if the initial thermal gradient were sufficiently steep, but it is not yet clear whether such a steep gradient is tolerable on other grounds.

Zircon age studies have been made by Davis on the ancient igneous and sedimentary rocks at Rainy Lake, Ontario. The results indicate that all the zircons crystallized about 2750 million years ago. The rocks were eroded to form sediments, which were subsequently metamorphosed about 2600 million years ago.

Additional age determinations by Tilton and Kouvo in Finland show that the Karelian and Svecofennian orogenies occurred at about the same time, although geological evidence suggests that the Svecofennian orogeny is the older.

A geochronological map of the United States and southern Canada, based on several hundred mineral age determinations, has been constructed. The Precambrian rocks occur in belts or zones, with younger rocks on either side of an old central belt.

Abelson and Parker have isolated saturated fatty acids including stearic, palmitic, and myristic from rocks as old as 500 million years. Very recent sediments contain these same entities and virtually no unsaturated types. This relationship differs sharply from that noted in algae, which ostensibly are the major source of organic matter in sediments. Parker has isolated pure fatty acids from algae and found that their C^{13}/C^{12} ratios differed from the C^{13}/C^{12} ratio of total cell. Different types of fatty acids from the same organism have the same C^{13}/C^{12} ratio.

Hoering has studied geochemical evidence for the existence of life in Precambrian rocks. The fractionation of the stable isotopes of carbon into a C^{13}-rich carbonate fraction and a C^{13}-depleted reduced fraction, which is characteristic of sedimentary rocks of known biological association, was found to exist in some of the oldest known sedimentary rocks, including the Bulawayan limestone, which has a minimum age of 2.7 billion years. Hoering also has isolated a number of organic compounds from these Precambrian rocks. The chemicals are similar to those that have been found in coal. Both these results are consistent with the existence of life and photosynthesis during early Precambrian times.

EXPERIMENTAL PETROLOGY

Pyroxenes

The Join Diopside-Ca Tschermak's Molecule at Atmospheric Pressure

John de Neufville and J. F. Schairer

In order to study the extent of the substitution of Al_2O_3 in diopside, Hytönen (*Year Book 60*) prepared three series of compositions on the plane enstatite-wollastonite-corundum. During the past year his work on the diopside-Ca Tschermak's molecule ($CaAl_2SiO_6$, henceforth abbreviated CTs) series has been extended to the CTs composition. Figure 1 is a temperature-composition section at at-

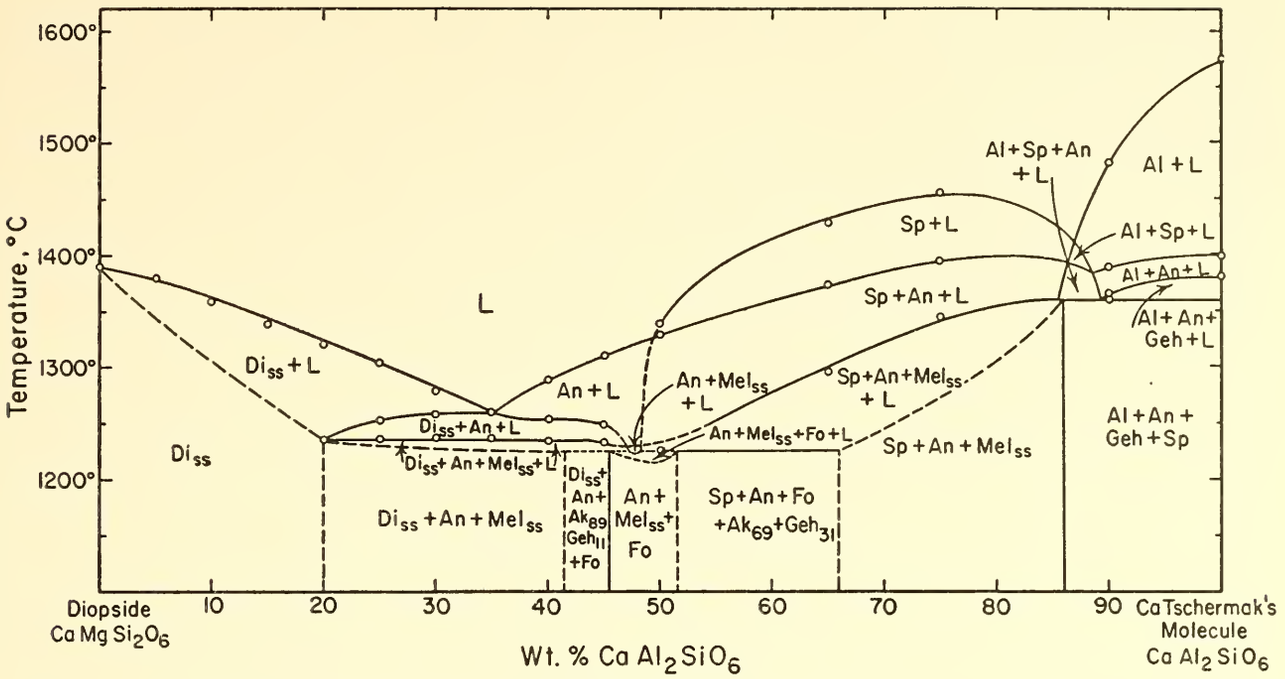

Fig. 1. Temperature/composition plot of data obtained on the join diopside-Ca Tschermak's molecule ($CaAl_2SiO_6$) at 1 atmosphere. Abbreviations for phases encountered: Di_{ss}, diopside solid solution; Mel_{ss}, melilite solid solution; $Ak_{89}Geh_{11}$, etc., specific melilite composition in terms of weight per cent akermanite and gehlenite; Sp, spinel; Fe, forsterite; An, anorthite; Al, "β alumina" and/or corundum; *L*, liquid.

mospheric pressure along this join. It has been constructed using Hytönen's unpublished data on eleven compositions and new data on four more aluminous compositions. Hytönen's X-ray determinative work on diopsidic pyroxenes has been extended to more aluminous pyroxenes and melilite solid solutions in polyphase assemblages. The Di-CTs join intersects at a high angle the "grossularite"-"pyrope" join studied under similar conditions by Chinner and Schairer (*Year Book 59*). Thus it continues the assault on univariant lines, invariant points, and solidus volumes in the silica-poor part of the CaO-MgO-Al_2O_3-SiO_2 quaternary system. Quenching experiments on the Di-CTs compositions at temperatures between the liquidus and the solidus have given new data on the temperatures of three quaternary invariant points and on the positions of critical planes separating the seven solidus volumes encountered.

The maximum degree of stable pyroxene solid solution is estimated indirectly to be greater than 40 mole per cent AlAl for (Ca,Mg)Si. Sakata (1957) observed a continuous shift in pyroxene lattice parameters in day-long runs at 1200°C on compositions between diopside and $Di_{60}CTs_{40}$. We find that the same extreme pyroxene solid solution phases form and persist indefinitely at somewhat higher temperatures in the presence of a small amount of liquid. The pyroxene crystallizing in $Di_{60}CTs_{40}$ at 1 atmosphere must lie off the Di-CTs join, probably on the diopside-$MgAl_2SiO_6$ (Mg Tschermak's molecule)-$CaAl_2SiO_6$ plane, because it coexists with two relatively magnesium-poor phases, anorthite and akermanite. Lattice parameters determined by Sakata (1957) closely fit the lattice parameter curves (fig. 4, p. 63) at $Di_{60}CTs_{40}$ for pyroxenes crystallized directly on the Di-CTs join at 20 kb. Present evidence indicates, accordingly, that the $Di_{60}CTs_{40}$ pyroxene crystallized at atmospheric pressure has approximately the same degree of AlAl for MgSi substitution as if it lay on the Di-CTs join at that bulk composition.

In molar coordinates the Di-CTs join

is parallel to the akermanite-gehlenite series of melilites within the tetrahedron defined by the four oxides, both series involving the exchange of AlAl (gehlenite and $CaAl_2SiO_6$) for MgSi (akermanite and diopside). Melilites are encountered in six of the seven solidus volumes cut by the Di-CTs join, and they range in composition from pure gehlenite to pure or nearly pure akermanite. Ervin and Osborn (1949) have determined the d values of several X-ray reflections as a function of composition in this series. Only the strongest peak, (211), may be used for X-ray determinative work in polyphase assemblages. Although the change in $d_{(211)}$ from akermanite (2.871 Å) to gehlenite (2.846 Å) is linear, it is very small. Thus, an estimated possible measuring error of $\pm 0.02°$ in 2θ corresponds to about ± 7 weight per cent akermanite. Nine melilite compositions determined by this method are shown in figure 2.

The melilite composition changes as a function of bulk composition in the three-phase assemblages Mel_{ss} + An + Sp, Mel_{ss} + An + Fo, and Mel_{ss} + Di_{ss} + An. It is invariant in the four-phase assemblages Geh + Sp + An + "alumina," $Ak_{69}Geh_{31}$ + An + Sp + Fo, and $Ak_{89}Geh_{11}$ + An + Di_{ss} + Fo. The melilite composition can be calculated as a function of bulk composition in the three-phase regions where melilite coexists with phases of fixed compositions, that is in Mel_{ss} + An + Fe and in Mel_{ss} + An + Sp. It cannot be uniquely calculated in three-phase assemblages containing Mel_{ss} + An + Di_{ss}. To calculate the melilite composition in four-phase solidus assemblages, the critical planes bounding the tetrahedron must be precisely located; only for Geh + Sp + An + "alumina" was it possible to do this. Where these compositions could be calculated as a function of bulk composition they are shown on figure 2 as solid lines. In all other assemblages the melilite compositions have been estimated from the approximate position of critical planes, and are dotted.

At least three invariant points have been encountered in the phase-equilibrium studies of the Di-CTs series compositions. All of them lie outside their respective four-phase volumes and are reaction points. The Geh + "alumina" + Sp + An + L reaction point lies

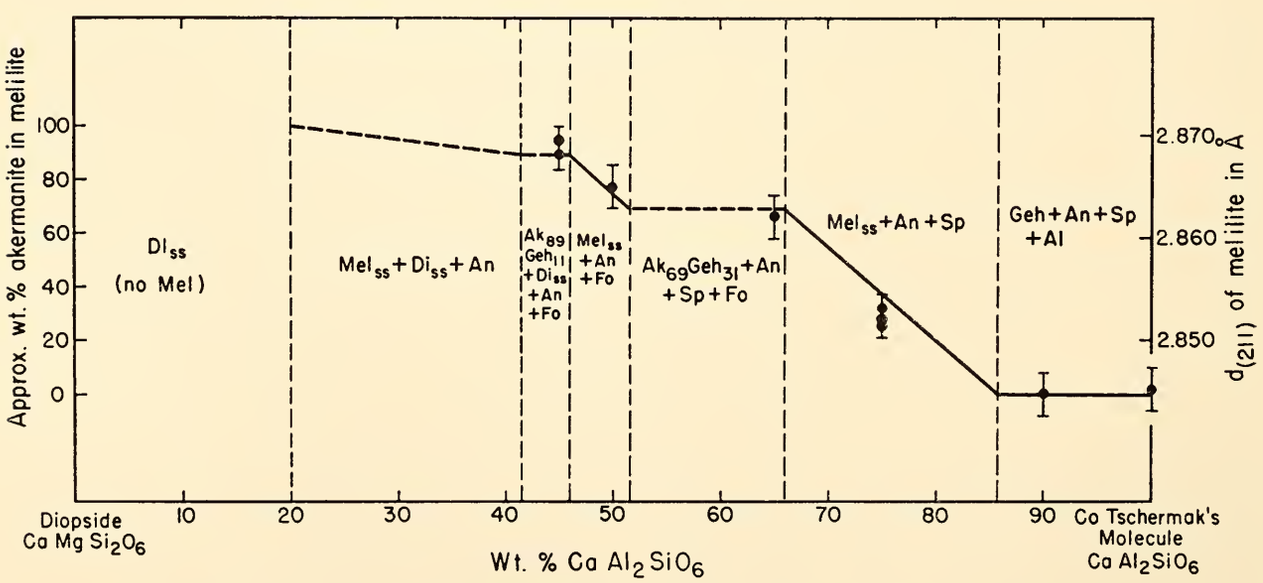

Fig. 2. Plot of melilite compositions in solidus assemblages versus bulk composition along join diopside-Ca Tschermak's molecule ($CaAl_2SiO_6$). Solid lines, calculated theoretical melilite compositions; dashed lines, estimated theoretical melilite compositions; dots, compositions actually determined by measurement of $d_{(211)}$ of the melilites; bars, estimated possible error in determinations ($\pm 0.02°$ 2θ = ± 7 weight per cent akermanite). Abbreviations as in figure 1.

within the Mel_{ss} + An + Sp volume at a temperature of 1360° ± 5°C. This is only in fair agreement with De Vries and Osborn (1957), who measured a value of 1350°C for this point. "Alumina" refers to corundum and/or "β alumina." These phases often occur together, although "β alumina" is predominant near liquidus temperatures, and only corundum peaks are observed in X-ray patterns of solidus assemblages.

The Sp + An + Fo + $Ak_{69}Geh_{31}$ + L invariant point lies within the Mel_{ss} + An + Fo volume, and its temperature is 1225° ± 5°C. The Di_{ss} + An + Fo + $Ak_{89}Geh_{11}$ + L invariant point also lies within the same volume. Its temperature is not known precisely. It is drawn in figure 1 at 1225°C, which probably represents a maximum value. Since these two invariant points have closely similar temperatures and compositions, it is difficult to decipher their mutual relationship. If they have the same temperature and different compositions, it is likely that some compositions along the Di-CTs join will pass through neither point and will crystallize directly to Mel_{ss} + An + Fo without forming any pyroxene or spinel. This possibility is depicted on figure 1. Another, equally likely, possibility is that the reaction point at which pyroxene is consumed occurs at a slightly lower temperature than the reaction point at which spinel is consumed. If this is so, some compositions along the join may pass through both points as they crystallize, before winding up as a mixture of melilite, anorthite, and forsterite. Since compositions along the "grossularite"-"pyrope" join (Chinner and Schairer, *Year Book 59*) appear to raise but not answer the same questions, the answers can be supplied only by the study of compositions lying off these joins in the Ak-$Ak_{70}Geh_{30}$-An-Fo volume.

Chinner and Schairer observed that several compositions on the "grossularite"-"pyrope" join crystallized an aluminous pyroxene that reacted with liquid at lower temperatures to produce a melilite-anorthite-forsterite assemblage. They suggested that extensive fractionation of diopside crystallized from a basaltic melt as the result of limestone syntexis would enrich the melt in Al_2O_3. Aluminous pyroxene could then store up CaO and Al_2O_3, which would contribute to the formation of melilite as pyroxene redissolved in the magma during the final stages of crystallization. This mechanism is in complete qualitative agreement with the classic contamination sequence described by Tilley and Harwood (1931) at Scawt Hill. It also receives excellent qualitative confirmation by the relations observed on the Di-CTs join. The analogous reaction point in this system is Di_{ss} + $Ak_{89}Geh_{11}$ + An + Fo + L, at which melts do indeed consume pyroxene and form melilite and other phases. This is presumably the same reaction point encountered by Chinner and Schairer. Their mechanism, however, has at least one serious limitation in the application to the melilite rocks of Scawt Hill. The pyroxene at this reaction point in the synthetic system has been shown to contain about 40 mole per cent AlAl for (Ca,Mg)Si. This is more than twice as much Al_2O_3 as is found in Scawt Hill aluminous pyroxenes (Tilley and Harwood, 1931). Thus the analogy between the synthetic and the natural pyroxene reaction point is less direct than Chinner and Schairer inferred.

Phase Relations in the System $CaMgSi_2O_6$-$CaAl_2SiO_6$-SiO_2 at Low and High Pressure

Sydney P. Clark, Jr., J. F. Schairer, and John de Neufville

There is substantial indication that basaltic magmas are generated in the mantle, perhaps at considerable depths. Some of this evidence is seismic, some geothermal, some geologic. With the exception of the seismic activity associated with Hawaiian eruptions, it is indirect and perhaps capable of other

interpretations. But enough evidence points in the same direction to make a study of the effect of pressure on melting relations in systems of petrological interest worth while. Furthermore, an upper limit to the temperature in the mantle is set by the liquidus of whatever material is down there, for superheated liquid must either move relatively rapidly toward the surface or lose its superheat by reaction with surrounding solid material. Effects of pressure on liquidus relations must be studied in systems of moderate complexity before inferences about melting in the mantle can be drawn with any confidence. It has been found that a pressure of 20 kb produces large effects on the liquidus that could not have been predicted from data obtained at atmospheric pressure alone.

The quaternary system CaO-MgO-Al_2O_3-SiO_2, which contains the join $CaMgSi_2O_6$ (diopside)-$CaAl_2SiO_6$ (lime Tschermak's molecule)-SiO_2, is of great importance, because it is sufficiently complicated to represent qualitatively the phase relations of the basic igneous rocks, particularly basalts, and rocks arising from their metamorphism. The main constituents commonly present in such rocks and absent from this quaternary system are iron in both its valence states, soda, water, and to a lesser extent K_2O, TiO_2, and MnO. In this simplified system it is impossible to study such important relationships as the effects of the fugacities of water and oxygen or changes in composition of feldspars. Experimental difficulties occasioned by the various possible oxidation states of transition elements and the volatility of alkalies and water at high temperatures, however, are avoided. Despite the simplifications, a number of reactions of petrological importance take place in this system; because of this and its relative chemical tractability the system is well suited for a beginning to the study of complex chemical equilibria at high pressures.

Among the important phases lying in this system are the oxides spinel and corundum, forsterite, the melilites akermanite and gehlenite, wollastonite, diopside, enstatite and its polymorphs, the aluminosilicates andalusite, kyanite, sillimanite, and mullite, the garnets pyrope and grossularite, cordierite, anorthite, and the polymorphs of silica. During the report year a new phase, with the composition of lime Tschermak's molecule ($CaAl_2SiO_6$), was synthesized for the first time at a pressure of 20 kb.

Because of the importance of this quaternary system, much previous work has been done to elucidate phase relations in it. Most were investigations of lines and planes joining two or three of the phases listed above. In this way the tetrahedron is crossed in many directions, and, given enough such studies, it should be possible to deduce with high precision the quaternary equilibrium relations at atmospheric pressure.

For initial study in this system at high pressure we selected the join diopside-anorthite. As Bowen recognized, this is a simple, pseudobinary representation of many basalts and diabases. The system was first shown not to be truly binary by Osborn (1942). We expected that the nonbinary behavior would be accentuated by pressure, and this has proved to be so. Part of the join is quaternary because of the incongruent melting of anorthite at high pressures. We have, however, only studied compositions lying in the plane diopside-lime Tschermak's molecule-silica, which contains the join diopside-anorthite.

For purposes of orientation it is helpful to consider the composition plane enstatite-wollastonite-corundum (fig. 3). All phases shown in the figure lie precisely in this plane; none are projected. Of particular interest are the intersecting joins diopside-lime Tschermak's molecule and grossularite-pyrope. The pyroxene join is characterized by complete solid solution at 20 kb; the garnet join, by complete solid solution above 30 kb. Magnesian Tschermak's molecule, shown on the diagram, has never been synthe-

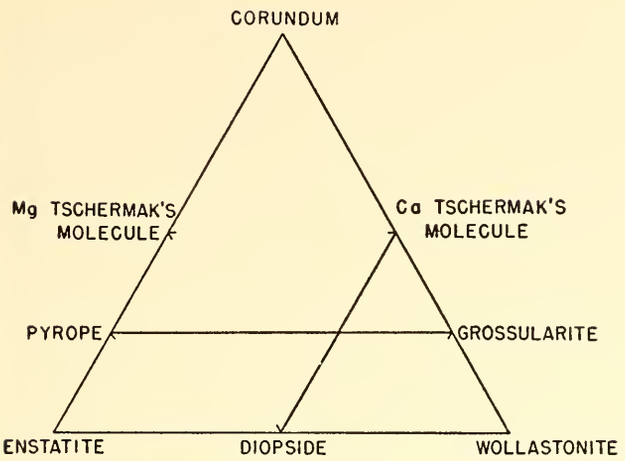

Fig. 3. Solid phases in the plane $CaSiO_3$-$MgSiO_3$-Al_2O_3.

sized, although Boyd and England (*Year Book 59*, p. 49) have made enstatites with at least 15 mole per cent Al_2O_3 in solid solution.

The plane in figure 3 contains a number of phases with a striking variety of crystal structures, all characterized by a metal-to-oxygen ratio of 2:3. Their densities are closely correlated with the structure. Densities of diopsidic pyroxenes mentioned in the ensuing paragraphs have been calculated from X-ray data discussed below. Cell edges of the grossularite-pyrope series of garnets were given by Chinner, Boyd, and England (*Year Book 59*, p. 77), and the densities of other phases were taken from the literature, using X-ray data whenever possible.

Wollastonite and the pyroxenes are chain-type silicates. The lightest, wollastonite, has a density slightly greater than 2.9 g/cm^3. The density of enstatite is 3.212 g/cm^3, that of diopside is 3.281 g/cm^3, and that of lime Tschermak's molecule is 3.437 g/cm^3. The garnet structure is composed of isolated silica tetrahedra, connected by irregularly coordinated cations. The density of pyrope is 3.566 g/cm^3; that of grossularite is 3.603 g/cm^3. The densest structure is that of the closely packed oxide corundum, 4.02 g/cm^3.

It has been suggested that pyroxenes might undergo transitions to the corundum structure at very high pressures, and this inversion has been reported in $MgGeO_3$ (Ringwood and Seabrook, 1962). It does not seem to have been remarked that garnets also have the metal-to-oxygen ratio appropriate to undergo a transition to a corundum structure. Such an inversion may take place deep in the transition zone in the mantle.

Two other comparisons of density are interesting to make. The first is between the density of crystalline lime Tschermak's molecule ($CaAl_2SiO_6$) and the densities of its low-pressure breakdown products gehlenite (ρ = 3.038 g/cm^3), anorthite (ρ = 2.765 g/cm^3), and either "β alumina" or corundum. The density of "β alumina" is not well known, but neither alumina phase is present in large amounts. The mean density of the breakdown products cannot be far from 2.9 g/cm^3. Lime Tschermak's molecule is 18 per cent denser than this. The density change between the pyroxene and garnet at the intersection of the two joins shown in figure 3 is 6 per cent (3.368 versus 3.592 g/cm^3).

X-ray data for diopsidic pyroxenes. In order to set up suitable determinative procedures for complex solid solutions such as those shown by diopsidic pyroxenes, careful crystallographic work must be done. The fine-grained nature of synthetic crystals precludes single-crystal studies, and care must be taken that determinative peaks on powder patterns can be unambiguously indexed. Otherwise errors from effects of preferred orientation may influence measurements of unresolved multiple reflections.

In crystals of low symmetry it is all but impossible without the aid of a high-speed computer to be sure that all indexing allowed by the space group has been compared with the observed reflections. Only by being certain that all possibilities have been considered can one be sure that a reflection is not multiple. Such precautions have not always been taken in the past.

All data processing was carried out on

an IBM 7090 digital computer using programs written by Charles W. Burnham. His program for calculating unit-cell parameters by least squares is described elsewhere in this report. His program for calculating d values permitted by the space group from the parameters of the unit cell was used in indexing powder patterns.

TABLE 1. Miller Indices and d Values of Reflections Used in Calculating Unit-Cell Parameters of Diopsidic Pyroxenes

hkl	d Value	
	Diopside	Lime Tschermak's Molecule
221	2.9897	2.9412
310	2.9492	2.8997
311	2.8924	2.8613
$\bar{3}11$	2.3009	2.2739
330	2.1546	2.1062
331	2.1322	2.0902
421	2.1067	2.0752
132	1.9679	1.9468
150	1.7535	1.7026

The starting point in our investigation was a carefully indexed powder pattern of pure diopside. This pattern was compared with patterns obtained on material prepared by completely crystallizing glasses on the join diopside-lime Tschermak's molecule at 20 kb. The positions of the peaks were found to shift smoothly as a function of composition from one end of the join to the other. No peaks appeared that could not be traced into their counterparts in the diopside pattern; this plus optical examination provides evidence that only one phase, a pyroxene, was present in these runs. As a check, the complete pattern for the composition 50 per cent diopside, 50 per cent lime Tschermak's molecule, was calculated. No unexpected interferences between peaks were found. The reflections used and their d values for diopside and lime Tschermak's molecule are given in table 1. These reflections were chosen because they can be indexed unambiguously and are sharp and strong—an important feature if they are to be used for determinative purposes in mixtures of phases that do not contain very much pyroxene.

The first three reflections listed in table 1 fall at 2θ angles less than 31° for copper radiation. Hence the d values cannot be determined with high accuracy. The parameters of the unit cells were calculated by least-squares adjustment both with and without these peaks. The resulting parameters do not differ significantly, but the standard errors are usually smaller if the low-angle peaks are rejected.

The unit-cell parameters of lime Tschermak's molecule and diopside are given in table 2, along with parameters for diopside from other observers. The agreement is good. The change of parameters along the joins diopside-lime Tschermak's molecule and diopside-enstatite is shown in figures 4 and 5. The data in figure 5 were obtained by applying the procedures described above to a series of glasses that had previously been crystallized at 1 atmosphere. Compositions containing more than 40 per cent

TABLE 2. Unit-Cell Parameters of Lime Tschermak's Molecule and Diopside

	Lime Tschermak's Molecule (present)	Diopside (present)	Diopside (Sakata, 1957)	Diopside (H. H. Hess, unpublished)
a, Å	9.615 ± 0.003	9.745 ± 0.001	9.743	9.741
b, Å	8.661 ± 0.002	8.925 ± 0.001	8.923	8.924
c, Å	5.272 ± 0.003	5.248 ± 0.001	5.251	5.247
β, deg	73.88 ± 0.03	74.13 ± 0.01	74.07	74.15
V, Å^3	421.79 ± 0.28	439.08 ± 0.07	438.98	438.77

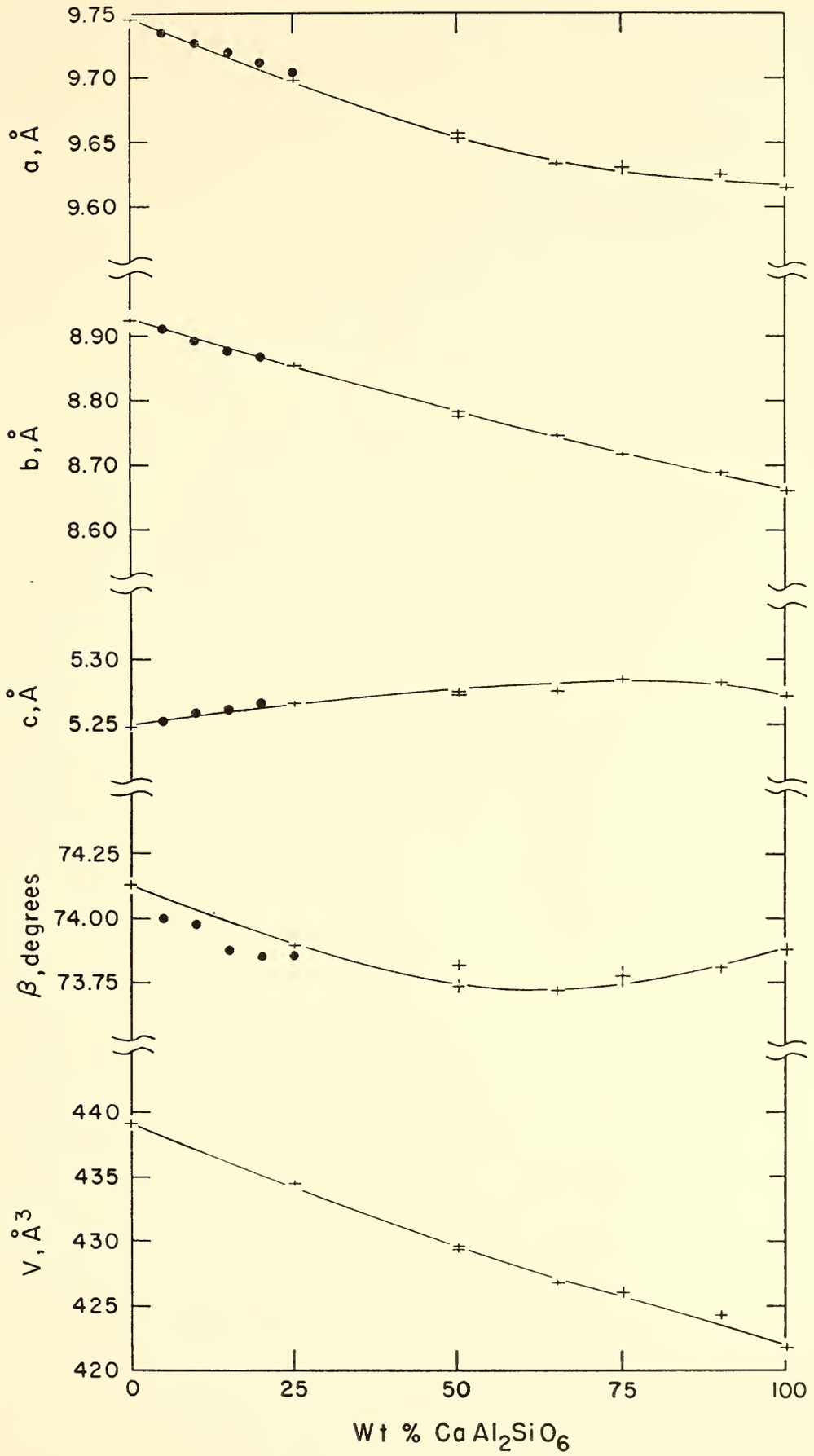

Fig. 4. Unit-cell parameters along the join diopside-lime Tschermak's molecule.

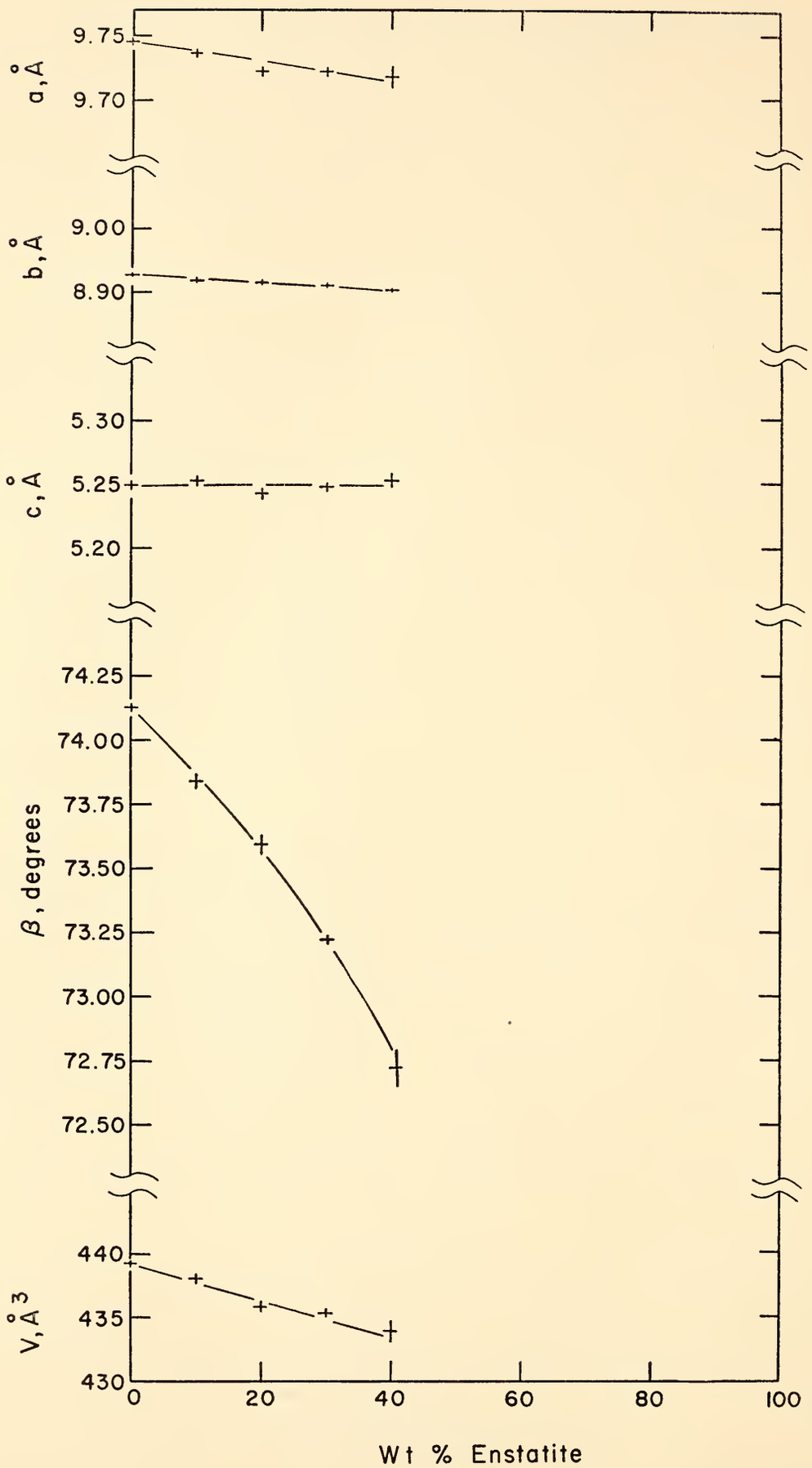

Fig. 5. Unit-cell parameters along the join diopside-enstatite.

enstatite do not crystallize to a single phase under these conditions.

Along the join lime Tschermak's molecule-diopside, the substitution is Al-Al for Mg-Si. One would expect that replacing an Mg atom with a relatively small Al would cause a and b to decrease. Likewise, replacing an Si atom with a relatively large Al atom in the silica chains would cause a slight increase in c. These are the observed effects. β changes little in this series. The internal consistency of the data for this parameter, i.e., the lack of scatter of the points about the curve in figure 4, is remarkable considering the scale of the diagram. The parameter that changes most is b, and hence reflections with large k are most satisfactory for determinative purposes along this join.

Volumes in this solid solution series depart systematically from a straight line connecting the end members in a way that implies that they are nonlinearly related to composition. The departure from linearity, although apparently real, is not large. A straight line would fit the data within 0.5 per cent.

Edges of the unit cell change little along the diopside-enstatite join; the most conspicuous feature of figure 5 is the large decrease in β with increasing content of enstatite. The volume is essentially linear with composition over the limited range of the data.

There is a systematic difference between our results and those of Hytönen and Schairer (*Year Book 60*, p. 136). They based a determinative procedure for diopsidic pyroxenes on the positions of the (150) and (510) reflections. We did not read (510) because of possible interferences with (422) and (332), but we can calculate its position from our data. For both reflections our 2θ angles are about 0.1° larger than those reported by Hytönen and Schairer. By assuming a value for β, it is possible to calculate a and b for diopside from their data. Using the extreme values of β in table 2, it is found that a = 9.751 to 9.755 Å and b = 8.937 Å. These values are sufficiently larger than those found by other workers to cast serious doubt on the determinative curves given by Hytönen and Schairer.

Melting relations in the system diopside-anorthite-silica. Liquidus data for this system at atmospheric pressure are shown in figure 6. Dots indicate the compositions studied by the quenching method. Except for compositions near the diopside-silica join, the figure has approximately the appearance of the simplest type of ternary diagram, that is one in which only three pure solid phases exist and liquid miscibility is complete. That this is only approximately true was first shown by Osborn (1942), who demonstrated that the join diopside-anorthite is not binary owing presumably to solid solution of alumina in the pyroxene. This result has been confirmed by Hytönen and Schairer (*Year Book 60*). To obtain more precise information on the composition of the pyroxene, careful X-ray work was done on a composition lying on the diopside-anorthite join that was equilibrated with liquid at 1260°C and on a composition lying in the ternary plane that was equilibrated with liquid at 1220°C. In both, the departure of the unit-cell parameters from those of pure diopside was small; it was greater for the composition crystallized at the higher temperature. Hytönen and Schairer (*Year Book 60*, p. 137) indicate that at 1135°C in this system (a temperature well below the solidus) the pyroxene contains about 3 per cent lime Tschermak's molecule. They considered it probable that this amount of solid solution was metastable, and our results suggest the same. Because of the small shift in properties relative to experimental error, it is not possible to determine the direction in which these pyroxenes differ from pure diopside.

In all the sixteen compositions within the triangle diopside-anorthite-silica, the third solid phase first appeared on cooling at temperatures between 1218° and 1225°C. This implies that the system diopside-anorthite-silica is very nearly ternary, and that the stable pyroxene

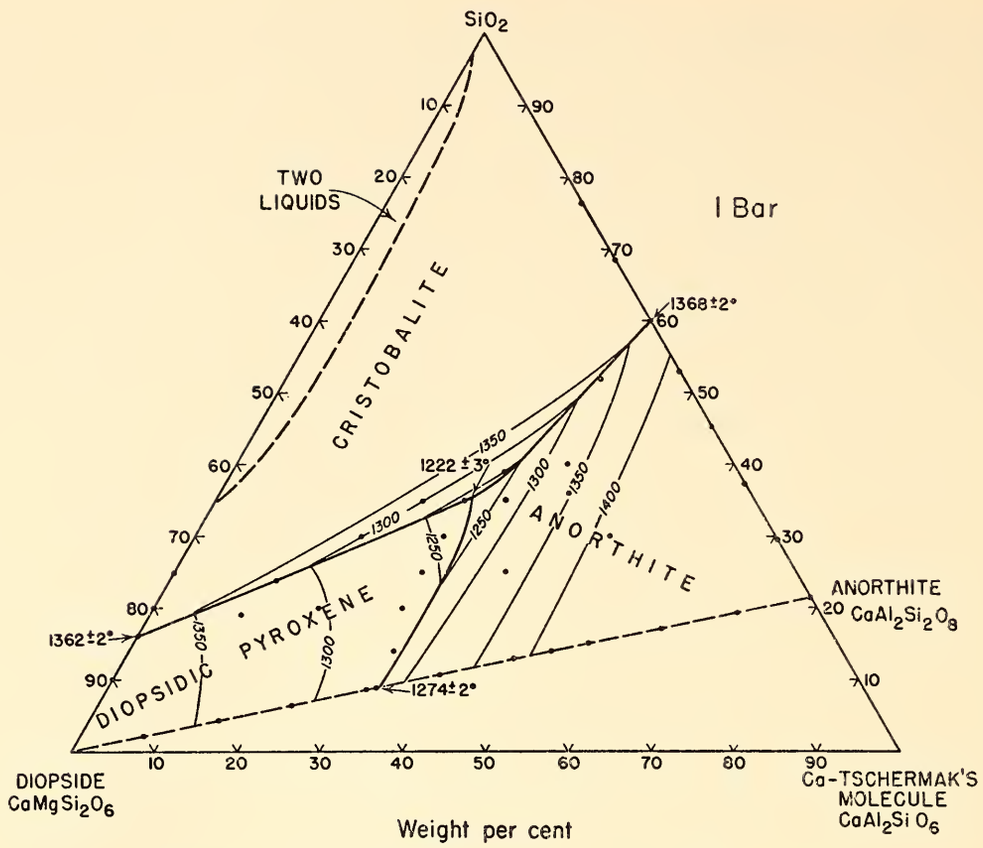

Fig. 6. Equilibrium diagram for the system diopside-lime Tschermak's molecule-silica at 1 atmosphere.

must lie close to the plane of figure 6 at 1222°C. The X-ray evidence implies that it is essentially pure diopside. The piercing point, or ternary eutectic, must be close to or at the thermal maximum on the quaternary univariant line connecting two quaternary eutectics. At one, wollastonite, diopsidic pyroxene, anorthite, and a silica phase coexist with liquid, and at the other enstatitic pyroxene, diopsidic pyroxene, anorthite, and a silica phase coexist with liquid. Determination of the composition of the latter eutectic is of great geologic significance, since it represents the goal of crystallization of a simplified silica-saturated basalt at low pressures.

High-pressure studies of the liquidus in this system have been carried out in a "single-stage" type of apparatus similar to that described by Boyd and England in *Year Book 60*. Results at 20 kb are shown in figure 7; a large number of runs have also been made at 30 kb, but this work is not yet ready for presentation. In all the work described the load pressure has been decreased by 3 per cent to allow for the effect of friction.

The accuracy with which temperature can be measured is much lower at high pressures than at atmospheric pressure. At high pressures the uncertainty in temperature ranges from ±10°C in favorable cases to ±20°C or so. These estimates are based on the internal consistency and the reproducibility of some of our results. There is in addition a correction for the systematic effect of pressure on the emf of a thermocouple; this has been omitted because the elusive problem of quantitative determination of the correction remains to be successfully attacked. In contrast, at atmospheric pressure an accuracy of ±2°C can be achieved with care.

The eutectic temperature in the binary system diopside-silica is raised by slightly more than 200°C by a pressure of 20 kb.

This is essentially the same as the change in melting point of diopside itself. The composition of the eutectic is not measurably affected by pressure. In this system, as in all the work at 20 kb, quartz is the silica phase stable on the liquidus. The effect of pressure on the two-liquid region in this system has not been investigated.

The system anorthite-silica is not binary at 20 kb because of the incongruent melting of anorthite, probably to corundum + liquid (Boyd and England, *Year Book 60*, p. 119). Between the fields of corundum and quartz on the liquidus there is a field of sillimanite. The temperature of lowest point on the liquidus, between the quartz and sillimanite fields, is 1540°C, and the composition is 48 weight per cent SiO_2. At atmospheric pressure the binary eutectic lies at 1368°C and 59 weight per cent SiO_2. (Both silica contents are determined relative to lime Tschermak's molecule.)

Changes produced by pressure in the system diopside-anorthite are greater than in the other limiting systems. Not only does anorthite melt incongruently at high pressures but also the amount of alumina in the pyroxene increases dramatically. At compositions near anorthite, corundum and "β alumina" both appear at high temperatures, with and without other crystalline phases. One of these alumina phases must be metastable on the liquidus, but it is not clear which. There is some evidence that, although corundum is stable at the anorthite composition, "β alumina" is the stable liquidus phase at neighboring magnesian compositions. It will be difficult to work out the correct relationship between these phases because of the stubbornness with which they both persist metastably.

The nature of the minimum on the liquidus in this system has not yet been determined. It may be a cusp, resembling a eutectic, or it may be a smooth trough, depending on whether the minimum lies within the pyroxene field or at its boundary. Figure 7 is drawn as if this

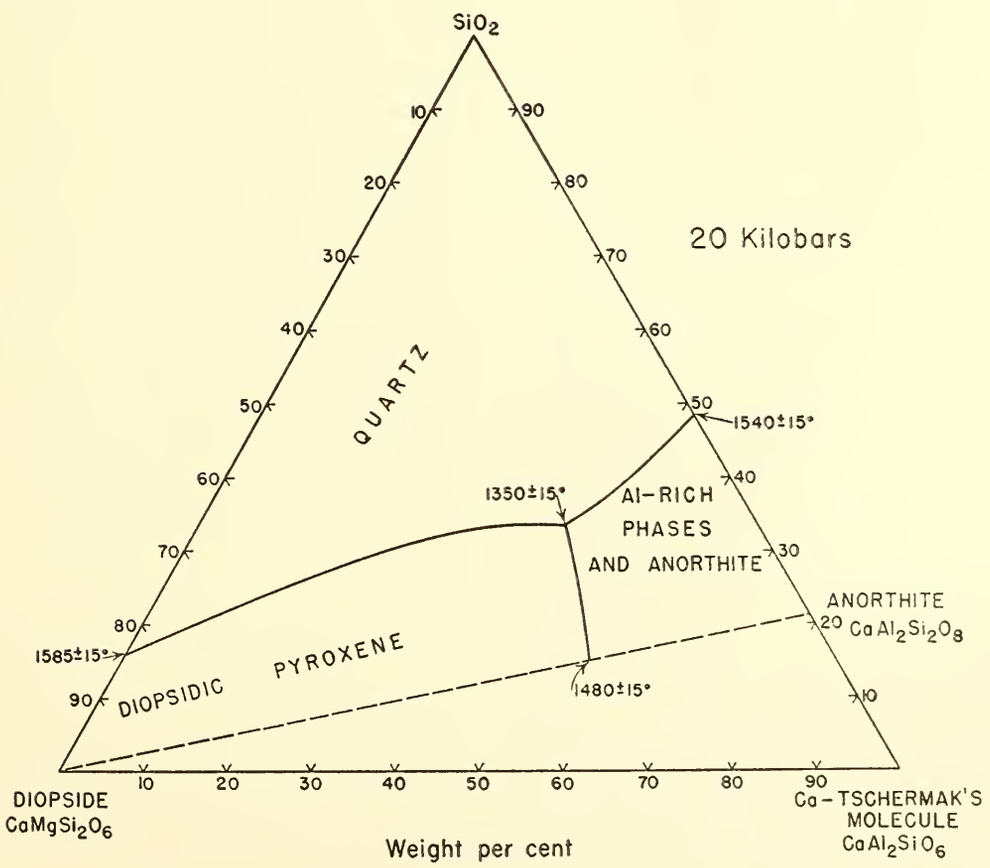

Fig. 7. Equilibrium diagram for the system diopside-lime Tschermak's molecule-silica at 20 kb.

minimum were a cusp at the boundary of the field, but future work may indicate the need for modification of this feature of the diagram. The temperature and composition of this point are 1480°C and 71 weight per cent anorthite. At atmospheric pressure this point lies at 1274°C and 43 weight per cent anorthite.

The complex relations at high pressures found in the systems diopside-anorthite and anorthite-silica continue into the triangle of figure 7. The fields adjacent to the anorthite composition have not yet been fully delineated. There must be a field of sillimanite, one of corundum, probably one of "β alumina," and, near the piercing point, one of anorthite itself. Although pyroxene, anorthite, and quartz are the solid phases present at the piercing point, the relationship there is not ternary. There is a melting interval of about 30°C. This point contains about 10 weight per cent more anorthite than its counterpart at atmospheric pressure, and its temperature is raised about 125°C by 20 kb. This is somewhat less than the increase in the minima in the diopside-anorthite and anorthite-silica systems.

That pyroxenes grown in this system at 20 kb do not lie on the join diopside-lime Tschermak's molecule is shown by the failure of compositions, as determined by X rays, to bear the relations to each other demanded by principles of phase equilibria, and by the fact that different parameters of the unit cell have values that would correspond to different amounts of lime Tschermak's molecule in solid solution. Correction for enstatite in solid solution, determined from β, improves the internal consistency of the data, but the remaining discrepancies are probably large enough to be considered real. Presumably there is also magnesian Tschermak's molecule (or corundum) in solid solution in the pyroxene.

These results should dispel any doubts that pressure, even in the absence of volatile constituents, can profoundly affect phase diagrams. In part of the range of compositions, the system at 20 kb is not even qualitatively similar to the system at atmospheric pressure, and quantitative differences in melting behavior occur at all compositions. The most striking new features caused by pressure are the incongruent melting of anorthite, the appearance of sillimanite on the liquidus, the appearance of quartz on the liquidus above 1000°C, and extensive solid solution in the pyroxene. None of these effects occurs at atmospheric pressure, and none of them could have been inferred without high-pressure experimentation.

There is an interesting possible geological consequence of the shift in composition of the piercing point with pressure. If a small amount of liquid were formed by fractional fusion at 20 kb in this system, it would have the approximate composition diopside$_{22}$-lime Tschermak's molecule$_{42}$-quartz$_{36}$. If this liquid were then decompressed suddenly, perhaps by rapid upward intrusion, it would arrive in a superheated condition and the composition of the liquid would be well inside the anorthite field at low pressure. The liquid would crystallize large quantities of feldspar before other solid phases appeared, which suggests a mechanism for the origin of anorthosites. It is to be expected that in the system albite-diopside-silica the piercing point will behave in a similar way because of solid solution of jadeite in the pyroxene and the eventual disappearance of albite from the liquidus. An important unexplored question is the behavior of intermediate plagioclases; it is not yet known whether the mechanism outlined can produce feldspars of the compositions found in anorthosites.

The System $MgSiO_3$-$CaMgSi_2O_6$

F. R. Boyd, Jr., and J. F. Schairer

Mineral assemblages containing two pyroxenes are of almost ubiquitous occurrence in mafic and ultramafic igneous rocks. The two pyroxenes are usually a calciferous pyroxene, augite or ferro-

augite, and a lime-poor hypersthene or pigeonite. Such pyroxenes show a wide variation in Mg/Fe ratio together with a more limited variation in Ca/(Mg + Fe). Understanding of the equilibria between such pyroxene pairs is of great petrologic interest, and the simplest system through which the problem can be approached is the join $MgSiO_3$-$CaMgSi_2O_6$.

Liquidus-solidus relations along this join were determined many years ago by Bowen (1914). Atlas (1952) was the first to study the subsolidus equilibria, and by means of fluxes he located the solvus and showed that two pyroxenes coexist at all temperatures below 1350°C. The crest of the solvus as determined by Atlas was shown to be about 50° below the solidus curve; within this 50° interval a complete solid solution between $MgSiO_3$ and $CaMgSi_2O_6$ seemed to exist. Atlas showed that orthorhombic $MgSiO_3$ was stable at temperatures up to 985°C. Although clinoenstatite is commonly obtained in runs on $MgSiO_3$ composition quenched from above 1000°C, Atlas argued that protoenstatite was the stable form in this range and that clinoenstatite formed in the quench. High-temperature X-ray studies by Foster (1951) showed that both orthorhombic $MgSiO_3$ and clinoenstatite could be inverted to protoenstatite at temperatures above 1275°C. These studies proved that protoenstatite has a stable field at high temperature.

Boyd and Schairer (*Year Book 56*) determined the solvus on this join by both dry and hydrothermal techniques. We found that the solvus intersected the solidus over a composition interval of about 15 weight per cent, so that a complete solid solution between $CaMgSi_2O_6$ and $MgSiO_3$ does not exist at any temperature.

Scatter of our preliminary results along the part of the solvus curve that defines the limit of solubility of $CaMgSi_2O_6$ in protoenstatite led to a further investigation of this part of the system. Results obtained this year indicate that the composition interval over which the solvus intersects the solidus is much wider than was previously indicated. Also evidence was found for an additional form of Mg-rich pyroxene, stable above 1385°C.

Figure 8 shows the liquidus and subsolidus equilibria for the system $MgSiO_3$-$CaMgSi_2O_6$. Quenching data for an extensive series of compositions along the join locate the liquidus temperatures and the equilibrium between crystals and liquid. Small circles indicate the temperatures as determined. Liquidus temperatures and the temperature of appearance of a Mg-rich pyroxene were determined for some compositions by Bowen (1914). Our results are in complete agreement with these data within the error of measurement. Bowen's data are given in figure 8 as triangles.

Several of the crystal-liquid fields on the diopside side of the equilibrium diagram are so small that they cannot be shown on the scale of figure 8. This part of the diagram is expanded in figure 9. There is a series of solid solutions with a temperature minimum on the melting and freezing curves. These relations are interrupted by the incongruent melting of pyroxenes to forsterite and liquid. The composition of the binary reaction point forsterite + diopside + liquid was determined as $En_{23.25}$ $Di_{76.75}$ (weight per cent). The temperature is 1389° ± 2°C. Bowen (1914, p. 233, fig. 18) inferred these relations. Our data also tie in very closely with those of Schairer and Yoder on the system forsterite-diopside-silica given elsewhere in this report (pp. 75–82).

Compositions on the join $MgSiO_3$-$CaMgSi_2O_6$ that are crystallized dry in the two-phase field form cryptoperthitic intergrowths of protoenstatite and diopside. X-ray methods are therefore necessary to fix the composition of individual phases and to locate the solvus boundaries. Since protoenstatite inverts to clinoenstatite in the quench, the X-ray data are given for clinoenstatite. The shift of several reflections with composition is sufficiently large to fix the

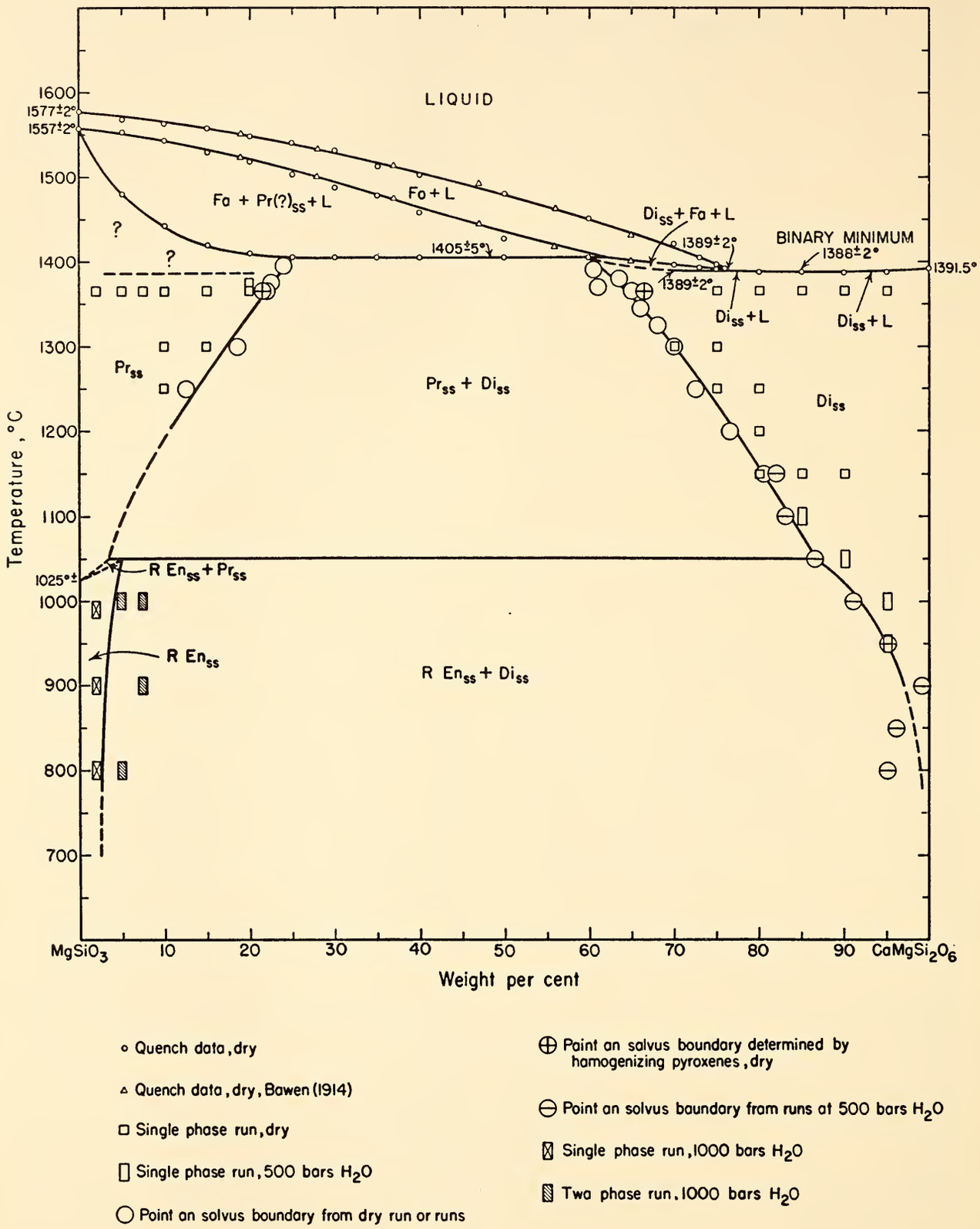

Fig. 8. Liquidus and subsolidus equilibria in the system $MgSiO_3$-$CaMgSi_2O_6$. Some liquidus points determined by Bowen (1914) are shown along with the data obtained by the authors. The value of 1025°C given for the inversion of rhombic $MgSiO_3$ to protoenstatite is based largely on extrapolation of preliminary high-pressure results. See figure 9 for an expanded view of the phase relations on the diopside side of the diagram.

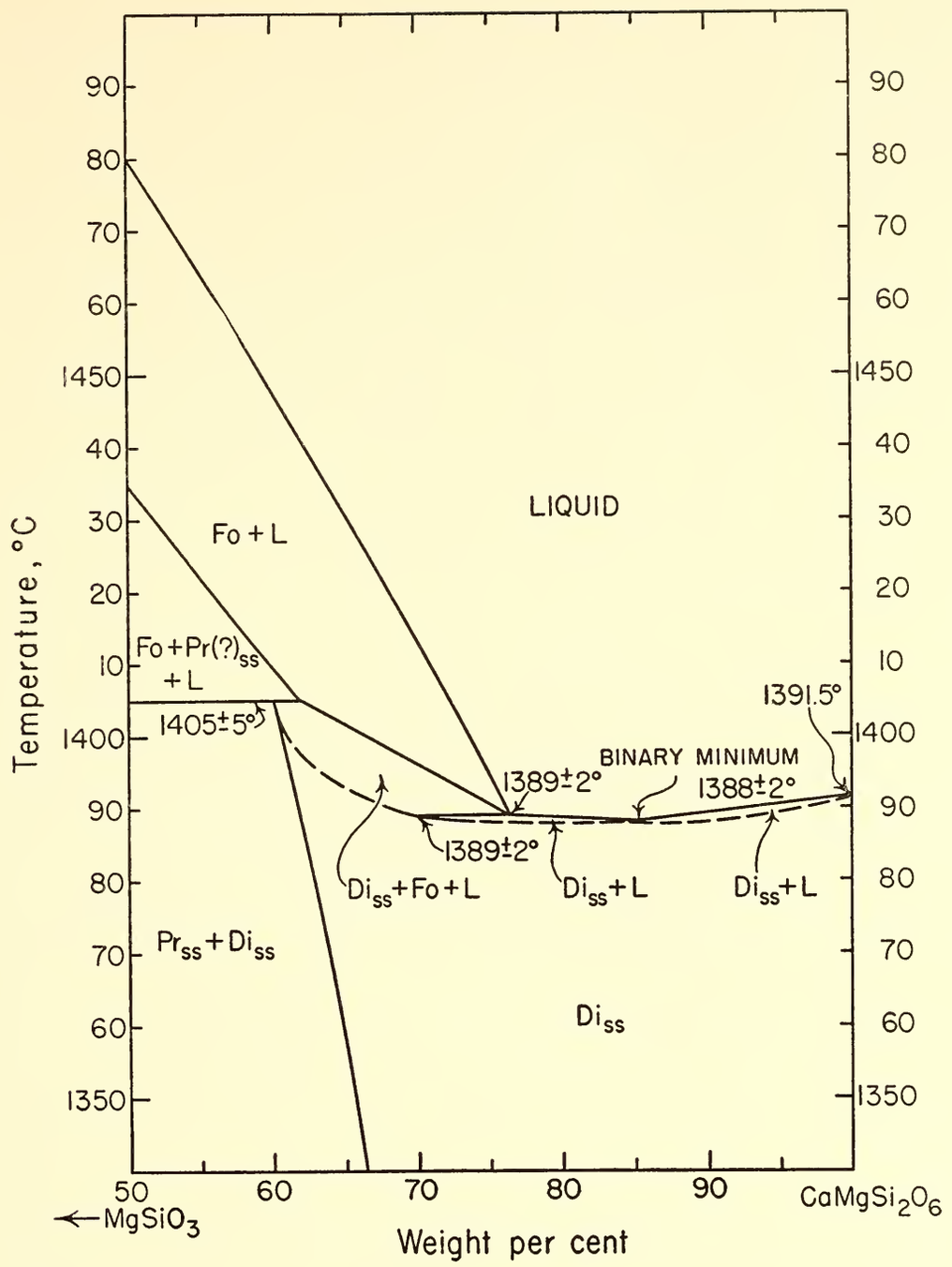

Fig. 9. Expanded view of the phase relations in a part of the system $MgSiO_3$-$CaMgSi_2O_6$. See figure 8 for the entire diagram.

composition of a phase within ±2 per cent. Figure 10 shows the diffractometer patterns of the 220 peaks for a series of compositions across the solvus. These runs were made dry, without flux, and were held at 1365°C for 2 weeks. In the single-phase regions, the 220 reflection forms a sharp, single peak, but compositions within the two-phase field show a double reflection, indicating the presence of two intimately intergrown pyroxenes.

Figure 11 shows measurements of the 220 reflection for a series of compositions from pure $MgSiO_3$ to $En_{65}Di_{35}$. Silicon was used as an internal standard for these measurements. The points for runs at 1365°C shift progressively with composition from pure $MgSiO_3$ to a bulk composition of $En_{80}Di_{20}$. For compositions richer in diopside than $En_{80}Di_{20}$, the composition of the enstatite is fixed at $En_{78}Di_{22}$ independent of the bulk compo-

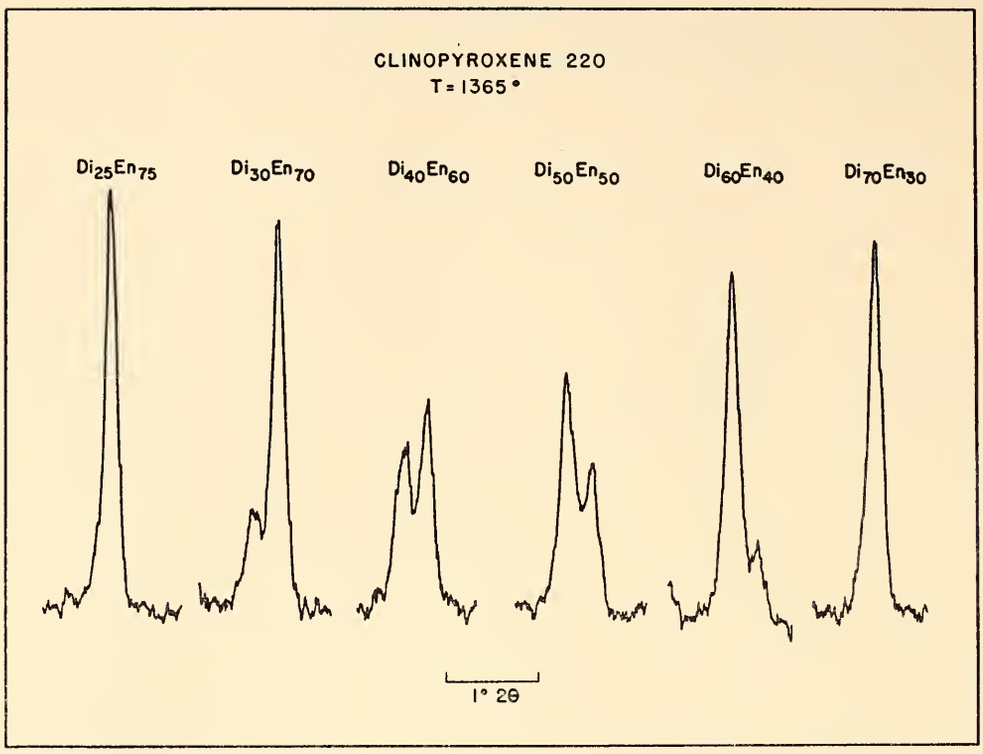

Fig. 10. Tracings of the 220 reflection from X-ray diffractometer patterns of a series of runs across the solvus in the system $MgSiO_3$-$CaMgSi_2O_6$. The 220 reflection is a sharp, single peak in the single-phase regions of solid solution bordering the solvus. Within the solvus the 220 reflection splits into a doublet indicating the presence of a cryptoperthitic intergrowth of a Ca-rich diopsidic pyroxene and a Mg-rich clinoenstatite.

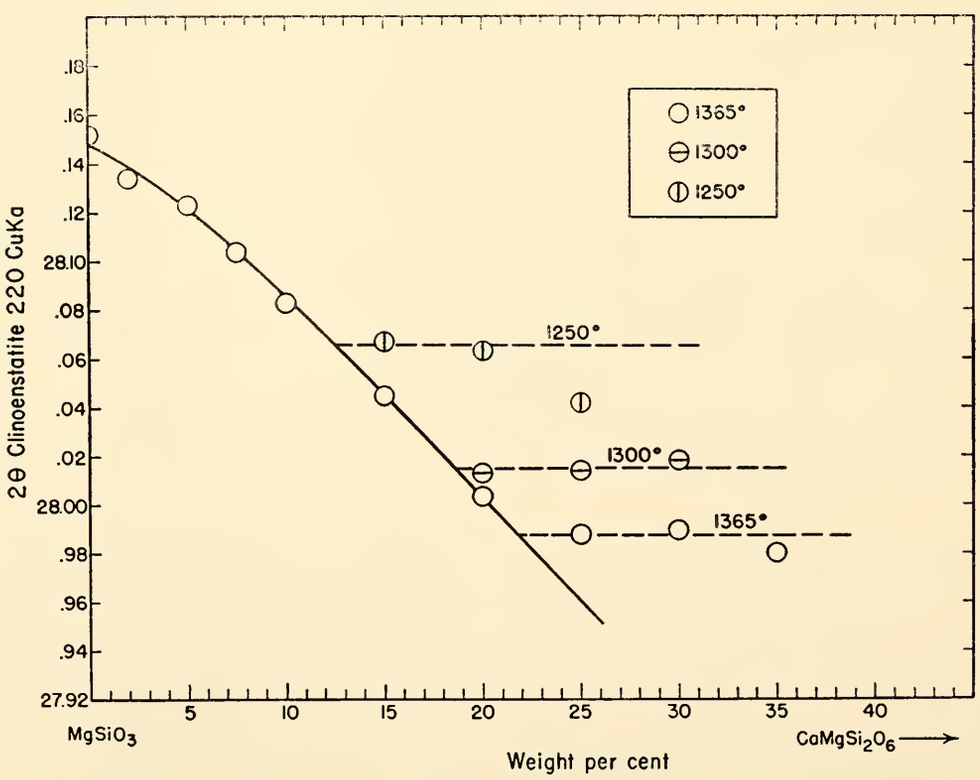

Fig. 11. Shift of the 220 reflection in clinoenstatites in the system $MgSiO_3$-$CaMgSi_2O_6$. Runs in the single-phase field at 1365°C fall on a smooth curve when plotted against the bulk compositions of the runs. Runs in the two-phase field at various temperatures fall off this curve, indicating that diopside is present as well as clinoenstatite and that the composition of the clinoenstatite is fixed at constant temperature, independent of the bulk composition of the run.

sition; diopside is present as a phase in these runs, and the Mg-rich pyroxene is saturated. Two-phase runs at 1300° and 1250°C are also shown in figure 11, and the points on the solvus determined by these data are plotted in the equilibrium diagram, figure 8.

Reversals have been obtained at 1365°C for both sides of the solvus. At 1365°C the points on the solvus obtained by unmixing solid solutions are $En_{78}Di_{22}$ and $En_{35}Di_{65}$. Values obtained by homogenizing pyroxenes previously unmixed at lower temperatures are $En_{78.5}Di_{21.5}$ and $En_{34}Di_{66}$. It has not proved possible to reverse the solvus at temperatures below 1365°C. At most temperatures, however, two or more bulk compositions within the two-phase field gave the same value for the solvus. This is a strong presumption of equilibrium though not a proof of it.

The solvus curve defining the limit of solubility of $MgSiO_3$ in diopside is little changed from our preliminary diagram (*Year Book 56*), although additional data have been incorporated. Present results, however, show that the solubility of $CaMgSi_2O_6$ in protoenstatite is much more restricted than was indicated by our preliminary data. The intersection of the solvus curve with the solidus on the $MgSiO_3$ side is at a composition of $En_{75}Di_{25}$ rather than $En_{55}Di_{45}$. The error in our preliminary results developed chiefly because runs were too short. The time required to reach equilibrium on the $MgSiO_3$ side of the solvus is 2 to 4 times as long as on the diopside side. Present results on the $MgSiO_3$ side are based on runs of 2 to 6 weeks.

A curious phenomenon has been found in the temperature interval between 1365° and the solidus at 1405°C. Measurements of the 220 reflections for a series of compositions from $MgSiO_3$ to $En_{60}Di_{40}$ crystallized at 1395°C are shown in figure 12. These points show a progressive shift of 220 with composition out to a bulk composition of $En_{75}Di_{25}$. Points for compositions richer in diopside fall off the curve and indicate that the solvus for this temperature is at $En_{76}Di_{24}$. This point fits well with the points on the solvus obtained in the range 1250° to

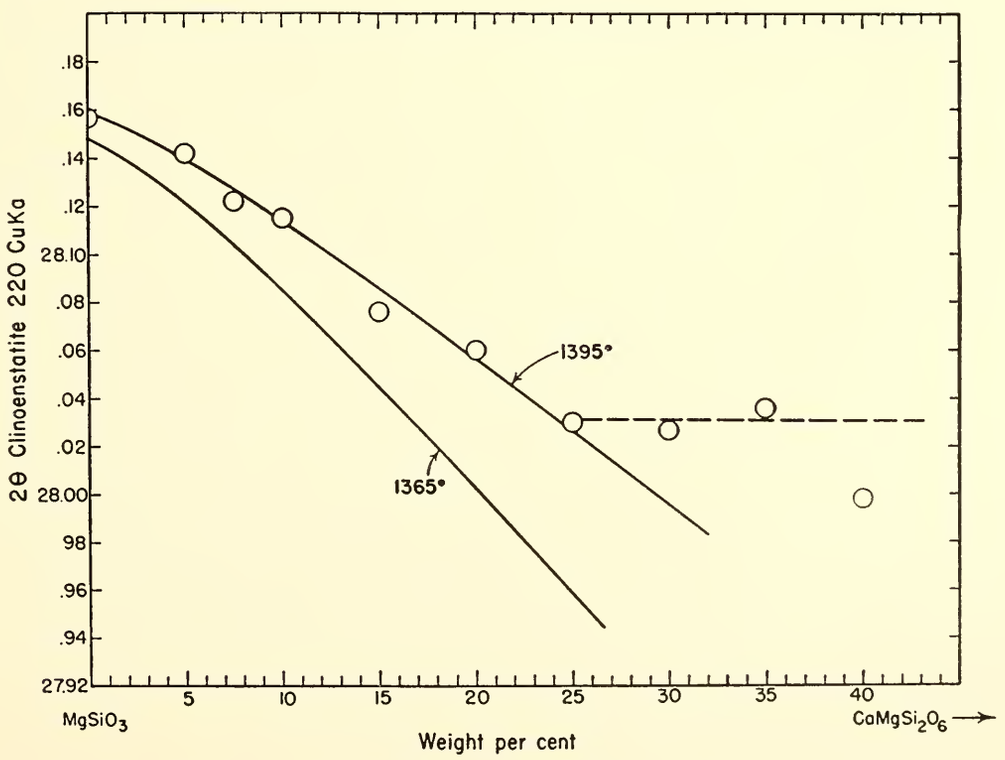

Fig. 12. Shift of the 220 reflection in Mg-rich pyroxenes crystallized at 1395°C. The curve for 1365°C runs is reproduced from figure 11.

1365°C (fig. 11). However, 220 measurements for pyroxenes in the single-phase field at 1395°C fall on a curve different from the curve obtained for 1365°C runs. The 1365°C curve is reproduced in figure 12, and the difference can be seen to be insignificant for pure $MgSiO_3$ but appreciable for bulk compositions containing diopside.

Full X-ray patterns of these 1395°C runs show that most of them are clinoenstatite but that some of them are *orthorhombic enstatite*. The position of the 220 reflection does not seem to be significantly influenced by whether the crystal form is orthorhombic enstatite or clinoenstatite. There can be no doubt that the orthorhombic enstatite in these runs formed in the quench, inasmuch as the orthorhombic form has been proved to be unstable at temperatures above approximately 1000°C (see below). We have obtained orthorhombic enstatite in the quench in a considerable number of runs over a range of bulk compositions in this system but always at temperatures above 1385°C. We have, however, never observed it to form in the quench in runs on pure $MgSiO_3$ composition.

Dry runs at all temperatures below 1365°C in the single-phase field have 220 spacings that fall on the 1365°C curve. These runs are normal clinoenstatite. A limited number of runs made above 1395°C seem to fall on the 1395°C curve, whereas runs at 1385°C scatter in between. These data suggest that there is a hitherto unrecognized form of Mg-rich pyroxene, stable above 1385°C. This form inverts in the quench to a distorted clinoenstatite or, sometimes, to orthorhombic enstatite.

High-temperature X-ray studies are needed to confirm or disprove this suggestion. It is difficult to reach temperatures above 1350° to 1400°C with diffractometer heating stages, but the problem is being investigated in the laboratory of J. V. Smith, of the University of Chicago.

The orthorhombic enstatite $\rightleftharpoons$ protoenstatite inversion is extremely sluggish at atmospheric pressure. Pure orthorhombic enstatite, prepared hydrothermally, has been heated for more than 2 months at 1080°C without change. Partial conversion to protoenstatite was observed in a dry run for 3 months at 1100°C. Addition of Na_2WO_4 as a flux lowers the temperature at which orthorhombic enstatite will invert to protoenstatite. With Na_2WO_4, partial inversion of the orthorhombic form was observed at temperatures as low as 1025°C. However, we were unable to convert clinoenstatite or protoenstatite to orthorhombic enstatite at any temperature at atmospheric pressure, even with the use of Na_2WO_4 as a flux.

The presence of H_2O increases the reaction rate somewhat. Orthorhombic enstatite can readily be prepared from $MgSiO_3$ glass under hydrothermal conditions, but not from clinoenstatite or protoenstatite. A reversal of the transition over a temperature interval of about 50° was accomplished in runs at 500 bars H_2O which were also fluxed with Na_2WO_4. In evaluating the hydrothermal data, however, account must be taken of the effect of pressure on the inversion.

Experiments made in single-stage apparatus (Boyd and England, *Year Book 60*) have shown that the orthorhombic enstatite $\rightleftharpoons$ protoenstatite inversion is very sensitive to pressure. Pressure favors the orthorhombic form, and preliminary data indicate that the slope of the transition curve is about 75°/kb. A reversed bracket on the transition was obtained at 1525°C and 6.7 $\pm$ 0.6 kb. Extrapolation of preliminary hydrothermal and high-pressure data indicates an inversion temperature at atmospheric pressure of about 1025°C, in agreement with the runs at atmospheric pressure that were fluxed with Na_2WO_4. This value is in rough agreement with the inversion temperature of 985°C determined by Atlas (1952) with LiF flux.

The solubility of diopside in orthorhombic enstatite was determined by hydrothermal runs at 1000 bars H_2O in the temperature range 800° to 1000°C. It

proved impractical to use X-ray methods on this part of the solvus. The runs were made long enough so that the presence or absence of diopside could be established by microscopic examination.

Attempts to locate the solvus in the range 1000° to 1250°C on the $MgSiO_3$ side of the diagram by crystallization of runs with Na_2WO_4 flux were not successful. The flux differentially dissolves SiO_2 and CaO, and so the products of these runs were usually pyroxenes + forsterite. As long as the two pyroxenes are on the join $MgSiO_3$-$CaMgSi_2O_6$ their mutual solubility should not be influenced by the presence of the forsterite. However, the 220 spacings of the clinoenstatites in these runs indicated that they contain virtually no diopside. The results of the fluxed runs are inconsistent with the dry data at 1250° to 1400°C and inconsistent with the usual form of a solvus curve. A check on these results was attempted by making a hydrothermal run at 1150°C and 500 bars H_2O. In spite of a quartz buffer around the run it was severely desilicated, and the products were clinoenstatite + diopside + forsterite. Again the 220 spacing of the clinoenstatite indicated that it contained virtually no diopside. Hydrothermal runs on the $CaMgSi_2O_6$ side of the solvus gave results in excellent agreement with dry runs, but for the most part these runs were shorter and at lower temperature, and desilication was not a problem. Various explanations are possible for the failure of the fluxed runs to give consistent results. It may be that a variation of the (Mg + Ca)/Si ratio in the pyroxene is responsible. Evidence for the existence of such a variation in orthorhombic enstatite in high-pressure runs has been described (Boyd and England, *Year Book 59*).

The System Diopside-Enstatite-Silica

J. F. Schairer and H. S. Yoder, Jr.

New studies on the join diopside-enstatite (see pp. 68–75) indicate that the solid solution of these pyroxenes is not complete and that a large solid immiscibility gap exists at the solidus. Additional relations on the pyroxene liquidus of the diopside-enstatite-silica system are thereby introduced that were not distinguishable by Bowen with the techniques available to him in 1914. Difficulties arose in the new studies of diopside-enstatite near the solidus because of the complex changes within a small temperature interval, and it was realized that some advantage was to be gained by studying the pyroxene relations in the presence of the additional component silica. For these reasons a revision of the system diopside-enstatite-silica was undertaken.

The revised liquidus diagram of the diopside ($CaMgSi_2O_6$)-forsterite (Mg_2SiO_4)-silica (SiO_2) system of which diopside-enstatite-silica is a part is shown in figure 13. The data in the diopside-forsterite-enstatite ($MgSiO_3$) portion are those of Bowen (1914) with a suggested revision on the diopside solid solution (Di_{ss})-forsterite (Fo) boundary curve. A minimum relation is proposed instead of the continuous drop of temperature to the $CaMgSi_2O_6$-Mg_2SiO_4 join, which has been shown to be binary. The temperatures in the region of the proposed minimum are within experimental error, and the exact relations cannot be elucidated with present techniques. The two-liquid region is taken from the work of Greig (1927). The significant additions to Bowen's study are the realization of a boundary curve separating the fields of Di_{ss} and Pr_{ss} (protoenstatite solid solution) and a minimum on the Di_{ss}-Tr (tridymite) boundary curve. At point *A* the reaction Fo + *L* = Di_{ss} takes place at a temperature of 1405° ± 2°C. The Fo-Pr_{ss} boundary curve at temperatures higher than point *A* is the well known reaction curve involving Fo + *L* = Pr_{ss}. The boundary curve Fo-Di_{ss} at temperatures below point *A* is also a reaction curve for a part of its traverse to the minimum in the $CaMgSi_2O_6$-Mg_2SiO_4-$MgSiO_3$ composition triangle and involves Fo + *L* =

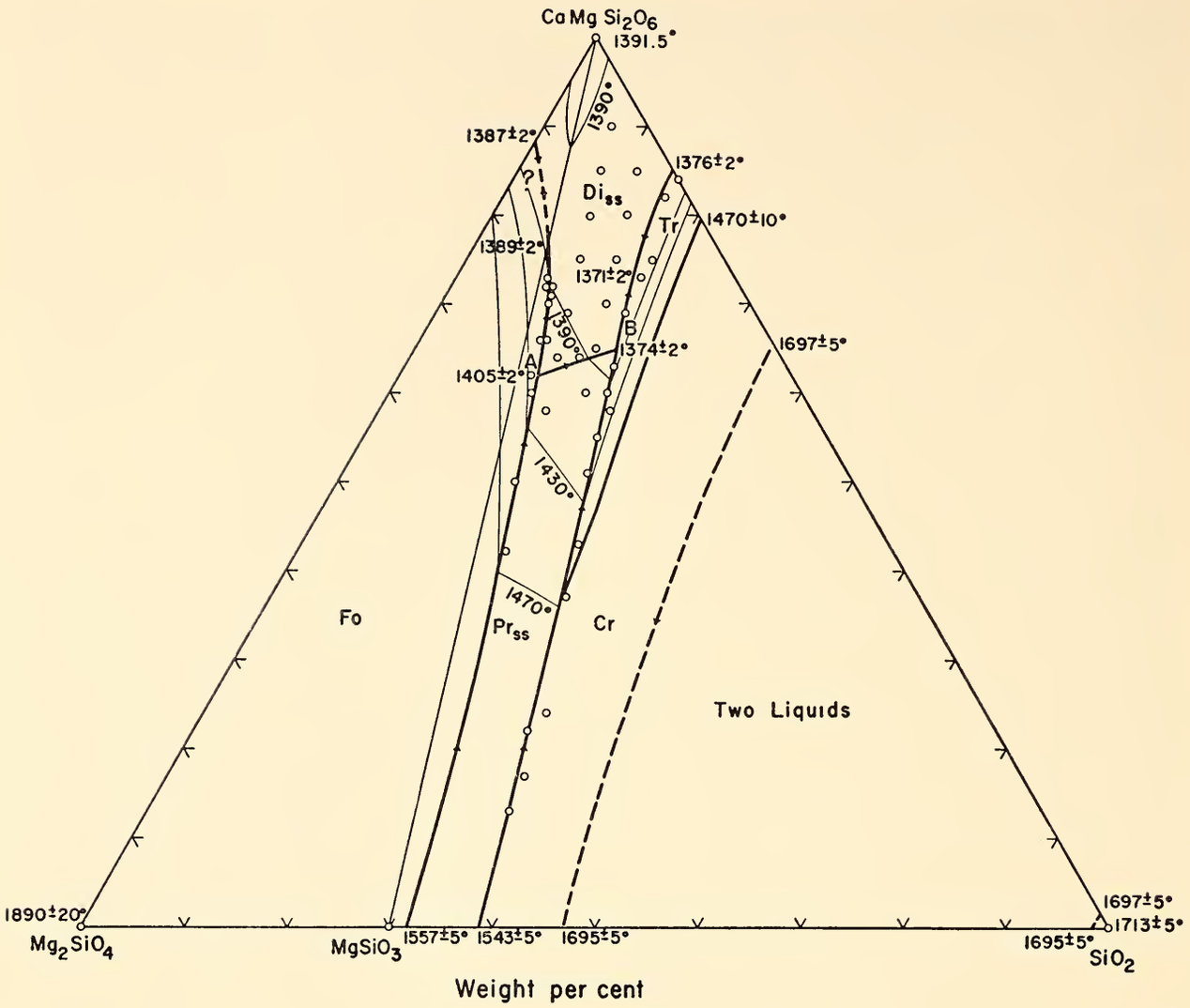

Fig. 13. Revised liquidus diagram at atmospheric pressure of the diopside-forsterite-silica system. Data in diopside-forsterite-enstatite portion are those of Bowen (1914); two-liquid region based on work of Greig (1927).

Di_{ss}. At point *B* the reaction $Pr_{ss} + L = Di_{ss} + Tr$ proceeds at a temperature of 1374° ± 2°C. There are reasons (see pp. 68–75) to believe that protoenstatite is not the correct crystal structure of the solid solutions crystallizing on the liquidus labeled Pr_{ss}. The powder X-ray diffraction patterns of $MgSiO_3$-rich pyroxenes quenched from above about 1370°C have unique characteristics that cannot be specifically assigned to the now recognized forms of $MgSiO_3$. They may be related to, but are different from, what Glasser and Osborn (1960) referred to as "high enstatite." It is to be understood that until more definitive data are at hand the crystal structure of the $MgSiO_3$-rich pyroxene crystallizing in the fields labeled "Pr_{ss}" is open to question.

Because of the many significant changes that take place between the temperatures 1410° and 1370°C, isothermal sections were studied in 5° intervals. Most of these are presented in figures 14 to 20, on which are plotted the bulk compositions of runs carried out. Runs on other bulk compositions, at slightly higher and lower temperatures, of course, contribute to fixing the relationships.

The relations at 1410°C, shown in figure 14, represent a temperature immediately above the first critical change. Only $MgSiO_3$-rich pyroxenes, Pr_{ss}, are

stable. Tridymite is believed to be the stable phase of SiO_2, but cristobalite is most often obtained metastably. Points *C* and *D* lie on the Fo-Pr_{ss} and Pr_{ss}-Tr boundary curves of figure 13, respectively. The dashed crystal-liquid tie line in the field marked $Pr_{ss} + L$ in figure 14 and similar tie lines in fields involving solid solutions in subsequent figures are estimated.

At 1405°C, figure 15, the intersection of the pyroxene solvus and the solidus takes place. The reaction is Fo + $L \rightarrow Di_{ss}$. The composition of Di_{ss} is marked by the letter *G*, about $Di_{58}Pr_{42}$, and is the maximum content of $MgSiO_3$ that Di can contain in solid solution. The maximum amount of $CaMgSi_2O_6$ held in solid solution by Pr, *H*, is also reached at this temperature; it is estimated to be about 24 weight per cent Di. The point *E* lies at the junction of the Fo-Pr_{ss}, Fo-Di_{ss}, and Pr_{ss}-Di_{ss} boundary curves of figure 13. Point *F* marks a position on the Pr_{ss}-Tr boundary curve of figure 13. All bulk compositions in the triangle Mg_2SiO_4-*G*-*H* become crystalline at essentially this temperature.

Lowering the temperature to 1400°C, figure 16, gives rise to five new fields: Fo + Di_{ss}, Di_{ss} + *L*, Fo + Di_{ss} + *L*, Pr_{ss} + Di_{ss} + *L*, and Fo + Pr_{ss} + Di_{ss}. Points *I*, *J*, and *K* lie respectively on the boundary curves of Fo-Di_{ss}, Pr_{ss}-Di_{ss}, and Pr_{ss}-Tr of figure 13.

In figure 17 are given the relationships found at 1390°C. A second field of Di_{ss} + *L* has evolved. Neither the extent of solid solution in the very $CaMgSi_2O_6$-rich pyroxenes nor the precise limits of the crystal + liquid field were determined. The points *M*, *N*, and *O* lie respectively on the boundary curves Fo-Di_{ss}, Pr_{ss}-Di_{ss}, and Pr_{ss}-Tr of figure 13.

In the temperature interval 1390° and 1385°C (compare figs. 17 and 18) the

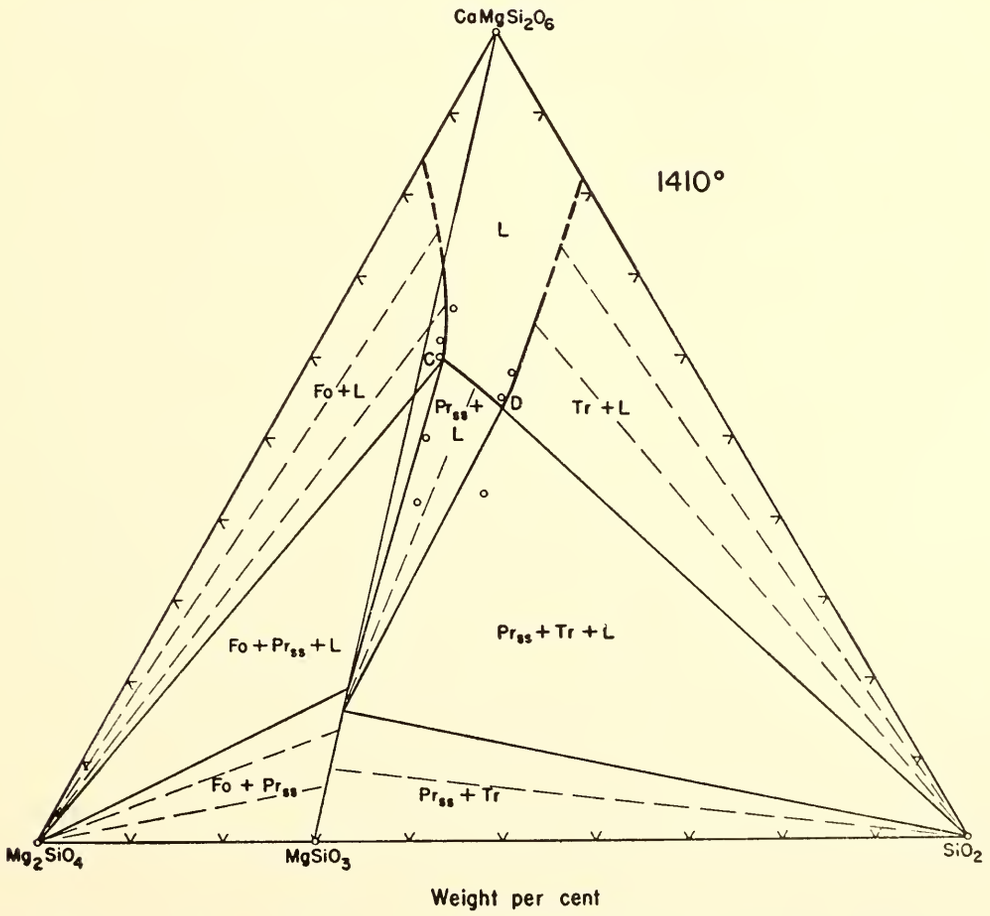

Fig. 14. Phase relations of diopside-forsterite-silica system at 1410°C.

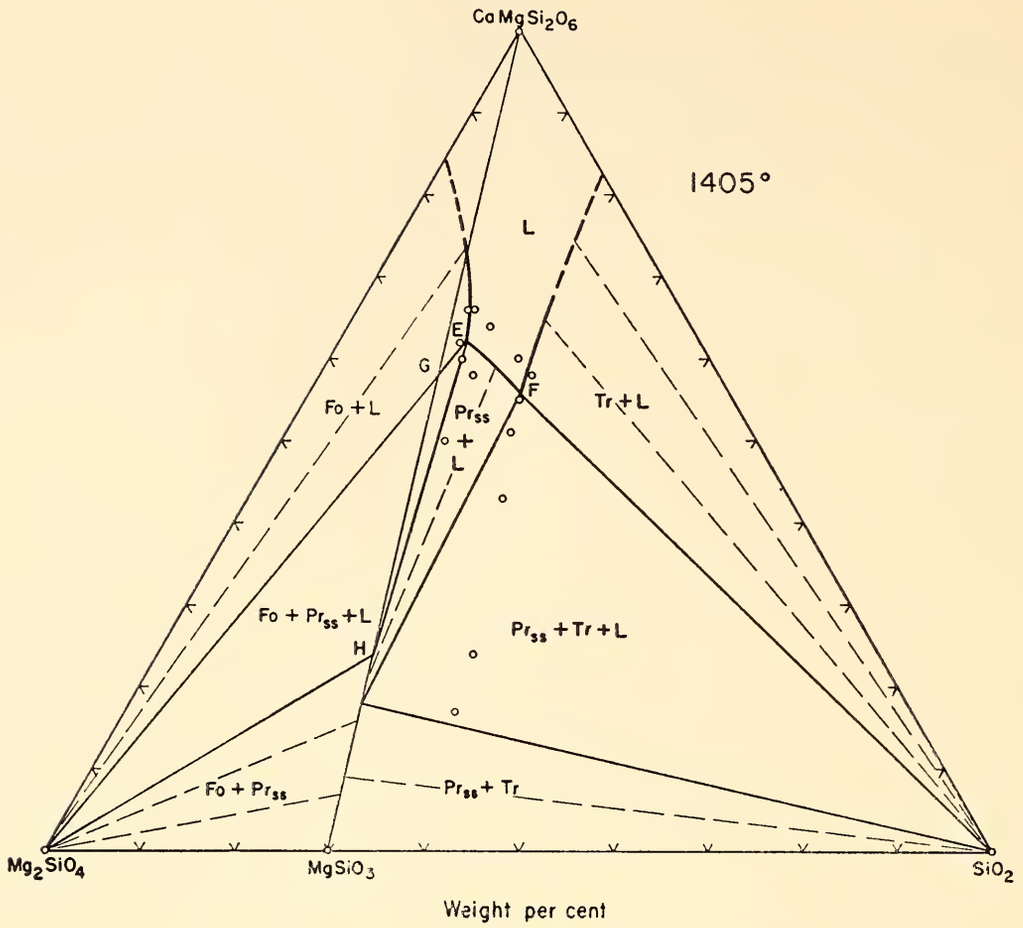

Fig. 15. Phase relations of diopside-forsterite-silica system at 1405°C.

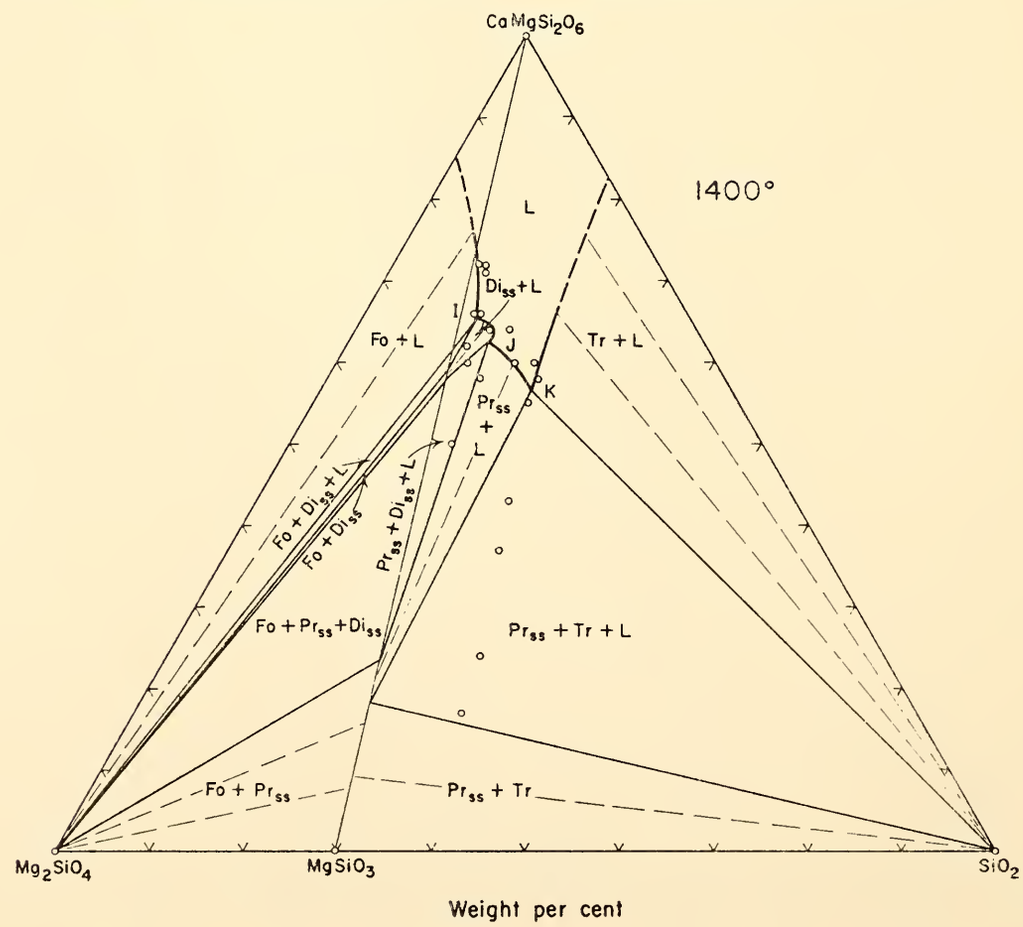

Fig. 16. Phase relations of diopside-forsterite-silica system at 1400°C.

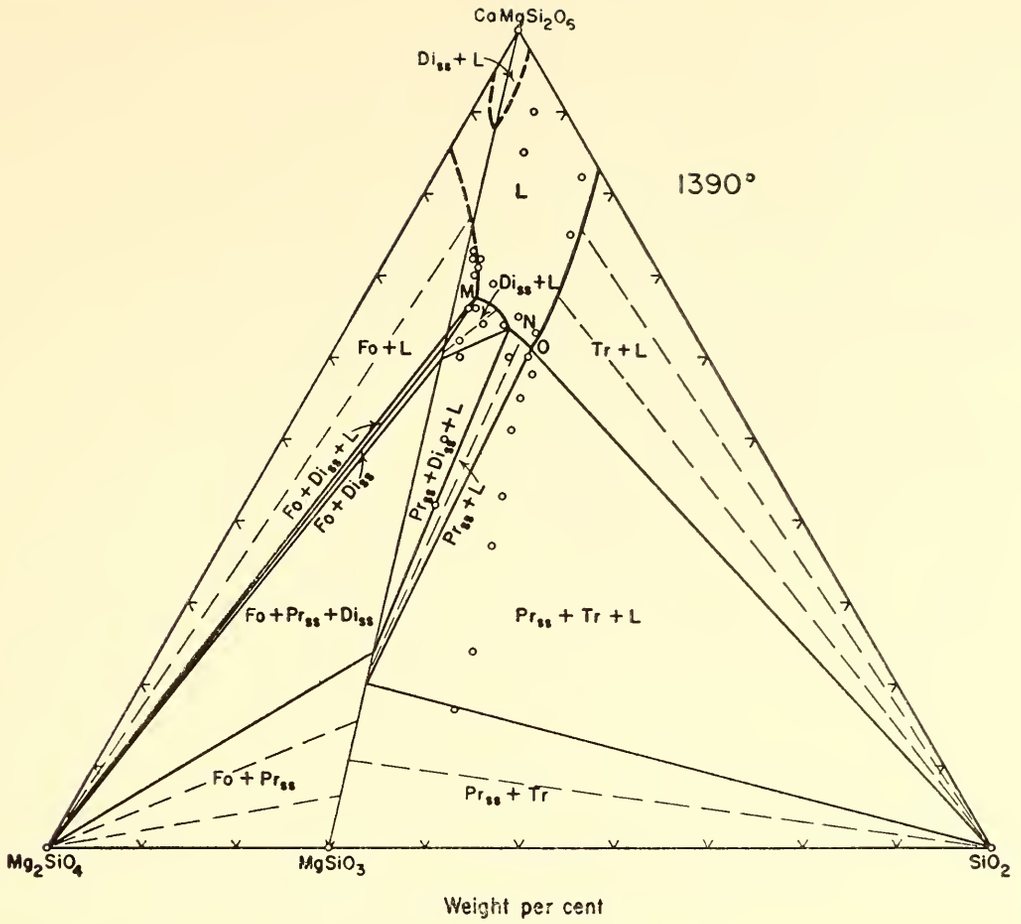

Fig. 17. Phase relations of diopside-forsterite-silica system at 1390°C.

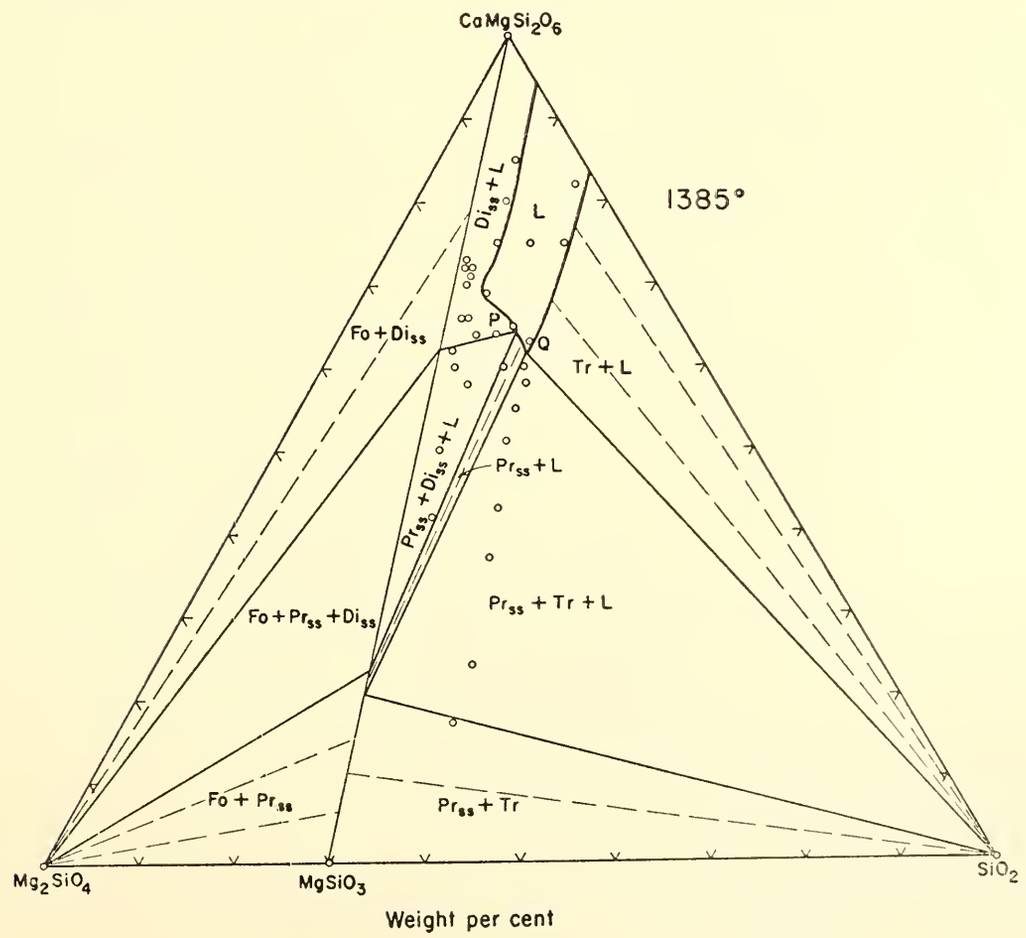

Fig. 18. Phase relations of diopside-forsterite-silica system at 1385°C.

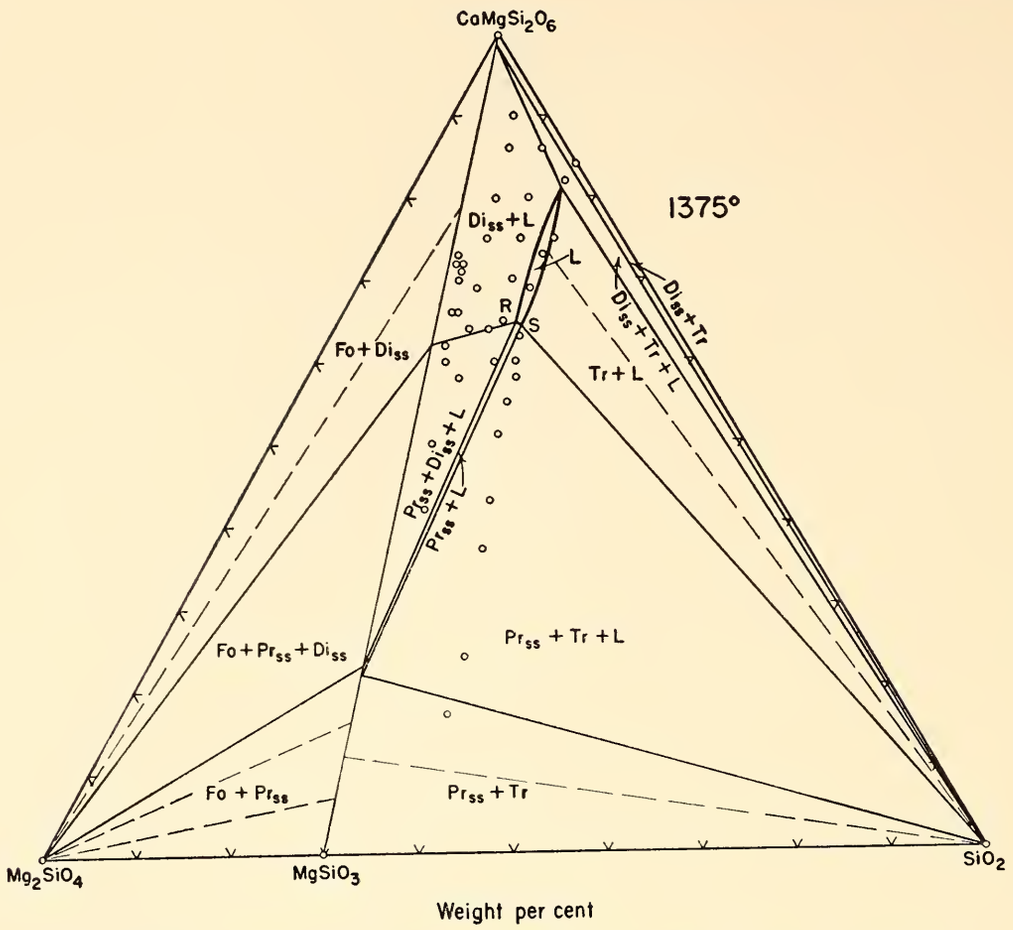

Fig. 19. Phase relations of diopside-forsterite-silica system at 1375°C.

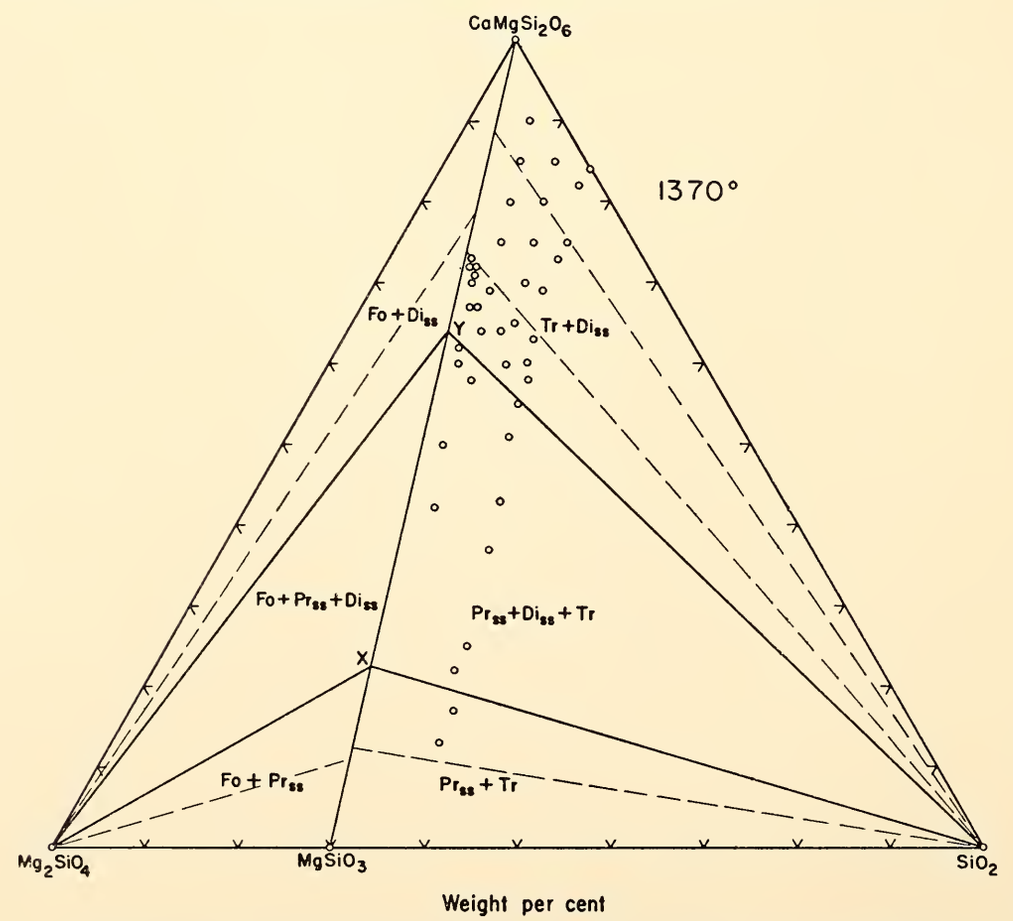

Fig. 20. Phase relations of diopside-forsterite-silica system at 1370°C.

remaining liquids in the $CaMgSi_2O_6$-$MgSiO_3$-Mg_2SiO_4 part of the system crystallize; presumably the last liquid is consumed at a minimum on the Fo-Di_{ss} boundary curve. In addition, all bulk compositions on the join $MgSiO_3$-$CaMgSi_2O_6$ become crystalline. The reaction relationship of Fo + L → Di_{ss} terminates at various temperatures along the Fo + Di_{ss} + L curve, depending on the bulk composition. The points P and Q of figure 18 are on the Pr_{ss}-Di_{ss} and Pr_{ss}-Tr boundary curves, respectively, of figure 13. No attempt was made to show the tie lines in the Di_{ss} + L region because of the wide spread in possible orientations.

Figure 19 portrays the relations at 1375°C, indicating the nature of the closure of the lowest temperature liquids and the precursory conditions of the Pr_{ss} + L → Di_{ss} + Tr reaction. The points R and S lie respectively on the boundary curves Pr_{ss}-Di_{ss} and Pr_{ss}-Tr of figure 13. Attention is called to the new fields Di_{ss} + Tr + L and Di_{ss} + Tr that result from the complete crystallization of compositions on the $CaMgSi_2O_6$-SiO_2 join at 1376° ± 2°C.

All compositions in the system $CaMgSi_2O_6$-$MgSiO_3$-SiO_2 are completely crystalline at 1371° ± 2°C, and the nature of the system is shown in figure 20 for a temperature of 1370°C. The important points X and Y are approximately $Di_{24}Pr_{76}$ and $Di_{64}Pr_{36}$, respectively. With the exception of polymorphic transitions

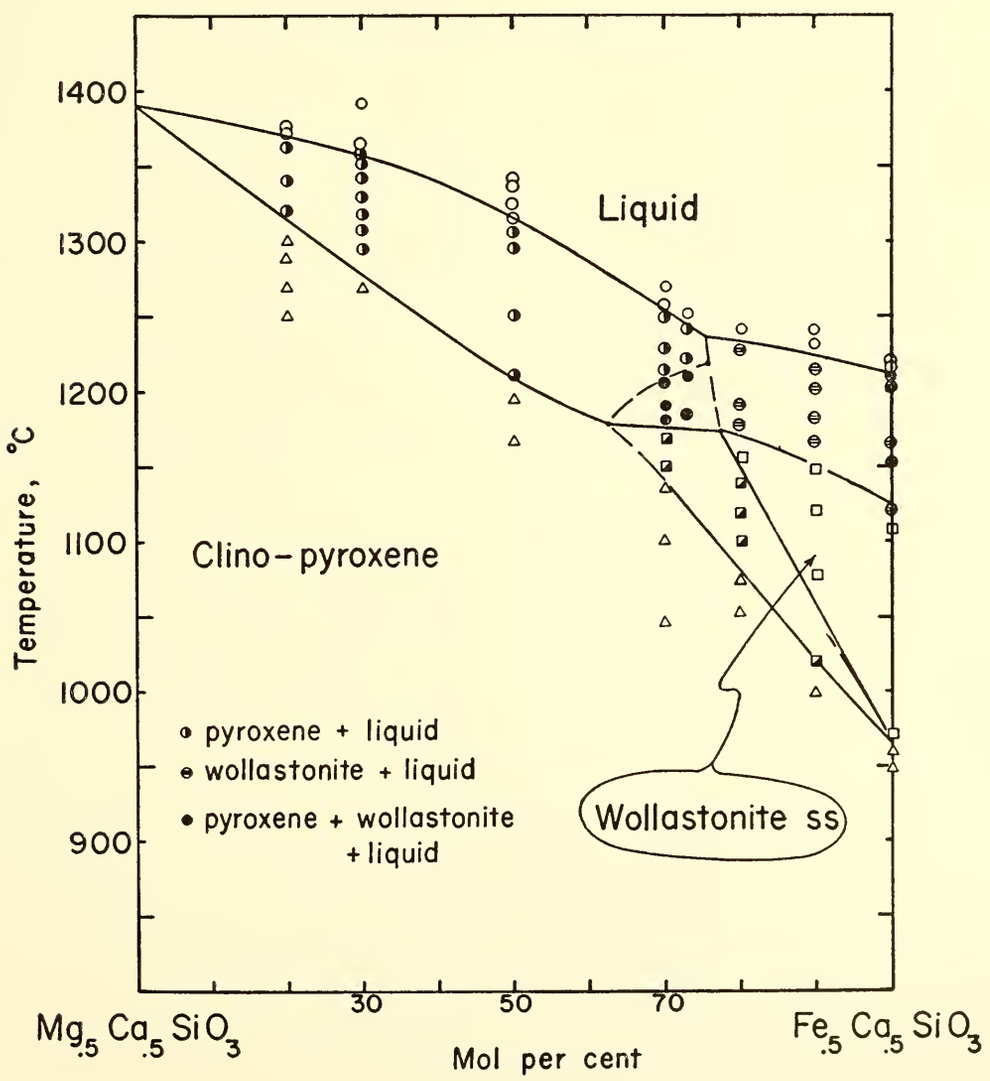

Fig. 21. Preliminary T-X section across the join $Mg_{0.5}Ca_{0.5}SiO_3$ (diopside)-$Fe_{0.5}Ca_{0.5}SiO_3$ (hedenbergite). Total pressure = 1 atm. Partial oxygen pressure is in equilibrium with iron + wüstite.

and exsolution phenomena little change in the character of the isothermal sections takes place with further lowering of the temperature.

The application of the revisions of the diopside-forsterite-silica system to petrological problems is of exceptional import. No attempt will be made here to evaluate the new implications. Light is cast on the presence or absence of hypersthene in natural rocks (see Tilley, 1961), the reaction relations of olivine with liquid to produce hypersthene in some cases and augite in others, the phenocryst-groundmass relations of hypersthene, pyroxene zoning and exsolution, and many aspects of fractionation in magmas and the evolution of derivative magmas.

Preliminary Results on Melting Relations of Synthetic Pyroxenes on the Diopside-Hedenbergite Join

A. C. Turnock

A study of melting relations of the Mg-Fe-Ca pyroxenes, with compositions in the three-component system $MgSiO_3$ (En)-$FeSiO_3$(Fs)-$CaSiO_3$(Wo), has been started with compositions along the join diopside ($MgCaSi_2O_6$)-hedenbergite ($FeCaSi_2O_6$) using a controlled-atmosphere quenching furnace with a total pressure of 1 atmosphere and a partial pressure of oxygen that would be in equilibrium with Fe + $Fe_{1-x}O$. The oxygen pressure was regulated by mixing carbon dioxide and carbon monoxide (Darken and Gurry, 1945).

A diagram of the experimental results is presented in figure 21. There is a complete series of monoclinic pyroxenes from diopside to hedenbergite, but the Fe-Mg substitution causes important changes in the stability of the pyroxenes. The Mg-rich pyroxenes, as shown on the left-hand side in figure 21, melt through a temperature range given by the solidus and liquidus curves, and this part of the diagram is essentially binary. The effect of iron content in lowering the temperatures of the solidus and liquidus is pronounced. Pyroxenes richer in the hedenbergite molecule than about 60 per cent, however, will not melt but convert to wollastonite solid solution. These two phases may be polymorphs across the range Hed 76 to Hed 100. The wollastonite solid solution persists metastably at lower temperatures, and in the diagram the two curves that define its subsolidus conversion to pyroxene are based on the reverse reaction, pyroxene → wollastonite solid solution. Time studies of this reaction satisfactorily showed that the transition interval was not occasioned by incomplete reaction, and the two curves intersect the liquidus at positions that satisfy boundary points for the field "pyroxene + $wollastonite_{ss}$ + liquid."

Wollastonite solid solution melts through an interval of about 90°C. In the low-temperature part of the field "$wollastonite_{ss}$ + liquid" there is probably another field, "$wollastonite_{ss}$ + liquid + tridymite." Small amounts of a silica phase have been observed, but there is not yet enough information to draw in a field boundary.

Metamorphic Petrology

Metamorphic Reactions Involving Two Volatile Components

H. J. Greenwood

Many metamorphic reactions involve more than one volatile or mobile component and are therefore influenced by pressure, temperature, and the composition of the coexisting fluid phase. The equilibrium relationships may be portrayed in a variety of ways, for example, by plotting the chemical potentials of the mobile components against one another at constant temperature and pressure (Korzhinskii, 1959; Zen, 1961). Alternatively, the situation may be represented on an isobaric T-x diagram, on which are plotted the temperature and the composition of the coexisting fluid phase, the other components being regarded as

nonvolatile or immobile. This kind of diagram has some advantages over the chemical potential, or μ_i versus μ_j, diagram, not the least of which is its direct use of the measurable variables temperature, pressure, and composition.

Equations have been derived for the equilibrium boundaries between reacting phase assemblages in such systems. These have the same form as the usual expressions for crystal-liquid equilibria, but they do not carry the restriction that the relative proportions of the two volatile components are limited by the proportions of the other components. The effect of removing this restriction is to make stable many reactions that would normally be regarded as metastable.

The slope of an equilibrium boundary for a reaction taking place at constant pressure in the presence of a one-phase binary fluid having zero enthalpy of mixing is

$$\left(\frac{\partial T}{\partial x_2}\right)_P = \frac{RT}{\Delta S}\left(\frac{\nu_2}{x_2} - \frac{\nu_1}{x_1}\right)$$

where x_2 is the mole fraction of component 2 in the fluid and ν_2 is the stoichiometric coefficient of component 2 in the reaction. All reactions that take place in such systems can be expressed by the general relation

$$aA \rightarrow bB + \nu_1 + \nu_2$$

in which a moles of solid phases A react to give b moles of solid phases B and ν_1 and ν_2 moles of the volatile components 1 and 2, respectively. The equation of the reaction should be written so that

$$|\nu_1| + |\nu_2| = 1 \quad \text{and} \quad \nu_1 + \nu_2 \gtrless 0$$

to make the stoichiometric coefficients equivalent to the mole-fraction composition of the gas given off in the reaction.

Inspection of these equations reveals several points of interest to metamorphic petrology. If $\nu_2 = 0$,

$$\left(\frac{\partial T}{\partial x_2}\right)_P = \frac{RT}{\Delta S}\left(-\frac{1}{x_1}\right) \qquad (1)$$
$$< 0$$

If $\nu_1 = 0$,

$$\left(\frac{\partial T}{\partial x_2}\right)_P = \frac{RT}{\Delta S}\left(\frac{1}{x_2}\right) \qquad (2)$$
$$> 0$$

If $\nu_1 > 0$ and $\nu_2 > 0$,

$$(\partial T/\partial x_2)_P = 0 \quad (T_{max}) \qquad (3)$$

where $\nu_1 = x_1$, $\nu_2 = x_2$.

If $\nu_1 = -\nu_2$, equal amounts of components 1 and 2 appear on opposite sides of the reaction, and their entropies tend to cancel, making ΔS for the reaction small and $(\partial T/\partial x_2)_P$ correspondingly large. Accordingly, as

$$\Delta S \rightarrow 0, \quad (\partial T/\partial x_2)_P \rightarrow \infty \qquad (4)$$

If $-1 < \nu_1 < 0$, $1 > \nu_2 > 0$ $(|\nu_1| < |\nu_2|)$,

$$+\infty > \left(\frac{\partial T}{\partial x_2}\right)_P > \frac{RT}{\Delta S}\left(\frac{1}{x_2}\right) \qquad (5)$$

If $1 > \nu_1 > 0$, $-1 < \nu_2 < 0$ $(|\nu_1| > |\nu_2|)$,

$$-\infty < \left(\frac{\partial T}{\partial x_2}\right)_P < \frac{RT}{\Delta S}\left(-\frac{1}{x_1}\right) \qquad (6)$$

The importance of these rather terse statements to metamorphic petrology can best be appreciated by examining some geologically interesting reactions that lend themselves to this treatment. Equation 1 describes a reaction in which only component 1 is given off (H_2O in fig. 22). As an example of such a reaction we might take

$$\underset{\text{Tremolite}}{Ca_2Mg_5Si_8O_{22}(OH)_2} \rightarrow 2\underset{\text{Diopside}}{CaMgSi_2O_6} + 3\underset{\text{Enstatite}}{MgSiO_3} + \underset{\text{Quartz}}{SiO_2} + H_2O$$

Equation 2 describes a reaction in which only component 2 is given off (CO_2 in fig. 22). Example (see fig. 22):

$$\underset{\text{Magnesite}}{MgCO_3} \rightarrow \underset{\text{Periclase}}{MgO} + CO_2$$

Equation 3 describes a reaction in which both volatile components are given off, such as

$$\tfrac{1}{4}\underset{\text{Tremolite}}{Ca_2Mg_5Si_8O_{22}(OH)_2} + \tfrac{3}{4}\underset{\text{Calcite}}{CaCO_3} +$$

$$\underset{\text{Quartz}}{\tfrac{1}{2}SiO_2} \rightarrow \underset{\text{Diopside}}{\tfrac{5}{4}CaMgSi_2O_6} + \tfrac{3}{4}CO_2 + \tfrac{1}{4}H_2O$$

(See fig. 22, T_{max} at $x_{CO_2} = 0.75$.)

Equation 4 describes a reaction such as

$$\underset{\text{Brucite}}{Mg(OH)_2} + CO_2 \rightarrow \underset{\text{Magnesite}}{MgCO_3} + H_2O$$

(See fig. 22, vertical boundary.)

Equation 5 describes a reaction such as

$$\underset{\text{Dolomite}}{\tfrac{5}{8}CaMg(CO_3)_2} + \underset{\text{Quartz}}{\tfrac{7}{8}SiO_2} + \tfrac{1}{8}H_2O \rightarrow$$

$$\underset{\text{Tremolite}}{\tfrac{1}{8}Ca_2Mg_5Si_8O_{22}(OH)_2} + \underset{\text{Calcite}}{\tfrac{3}{8}CaCO_3} + \tfrac{7}{8}CO_2$$

Equation 6 describes a reaction such as

$$\underset{\text{Serpentine}}{4H_4Mg_3Si_2O_9} + \underset{\text{Calcite}}{9CaCO_3} + 5CO_2 \rightarrow$$

$$\underset{\text{Tremolite}}{Ca_2Mg_5Si_8O_{22}(OH)_2} +$$

$$\underset{\text{Dolomite}}{7CaMg(CO_3)_2} + 7H_2O$$

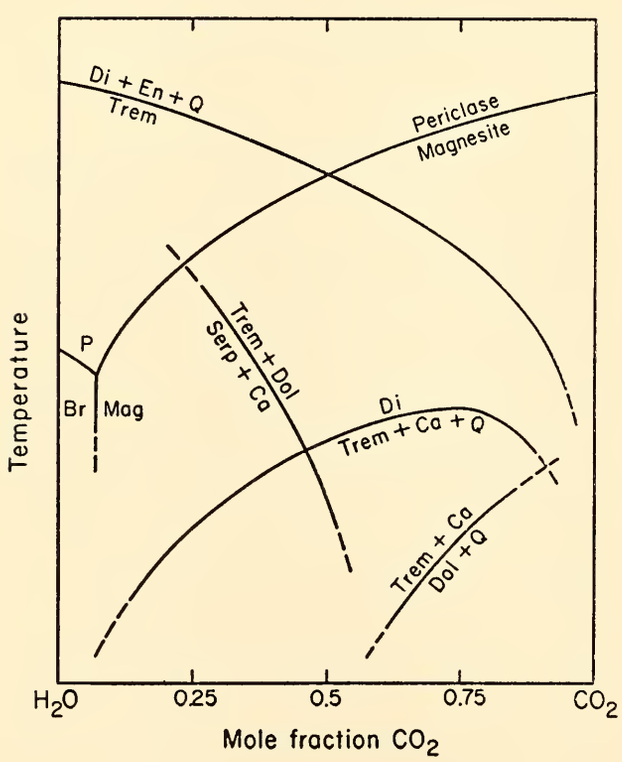

Fig. 22. Diagrammatic sketch illustrating the six types of crystal-vapor equilibrium reactions in binary gas mixtures. H_2O is component 1, and CO_2 is component 2, of the equations.

We may, for the sake of discussion, regard these reactions as models of metamorphic isograds. The most obvious feature is that an isograd defined on the basis of a reaction that evolves one volatile component may cross an isograd defined on the basis of a reaction that evolves the other volatile component. In addition, a plot like figure 22 may be regarded as a map of an area that has a gradient in the proportions of CO_2 and H_2O across it at a large angle to the thermal gradient. If such an area could be found in the field, containing rocks of suitable compositions, it should be possible to demonstrate the crossing of isograds. In reactions like the formation of diopside from tremolite, calcite, and quartz, it is clearly of great importance to know something of the composition of the fluid phase in equilibrium with the minerals before coming to any conclusion about the temperature of metamorphism, even assuming some knowledge of the total pressure.

Reactions like those described by equations 4, 5, and 6 are perhaps the most interesting of all when they are regarded as isograds. Their steep slopes in T-x plots like figure 22 show that the progress of many such reactions is affected more by the composition of the coexisting fluid phase than by either temperature or pressure. This observation leads directly to the concept of an isograd that is essentially neither isotherm nor isobar but that provides a firm limit on the composition of the fluid with which the minerals of the rock could have been in equilibrium. It cannot be too strongly urged, therefore, that when an isograd is under discussion the chemical reaction be precisely defined.

Experiments are now under way that will fix the positions of reactions of the sort just discussed in the system MgO-CaO-SiO_2-H_2O-CO_2. The apparatus is essentially the same as was used in an earlier investigation of the system $NaAlSi_2O_6$-H_2O-argon (Greenwood, 1961) in which the solid phases are held in open capsules in a bomb containing a mixture of CO_2 and H_2O. Pressure and temperature are measured, and the composition of the gas is analyzed at the end of each

run. The stability of wollastonite has been studied rather fully, and preliminary data are now available on a number of other equilibria. Figure 23 shows the stability relations of wollastonite in mixtures of CO_2 and H_2O at 1000 and 2000 bars. All the reactions shown represent reversals of the equilibrium. The data are in good agreement with those of Harker and Tuttle (1956), assuming that the CO_2 and H_2O mix ideally. This apparent close approach to ideal mixing is probably illusory, because it seems likely that the gas mixture contains three

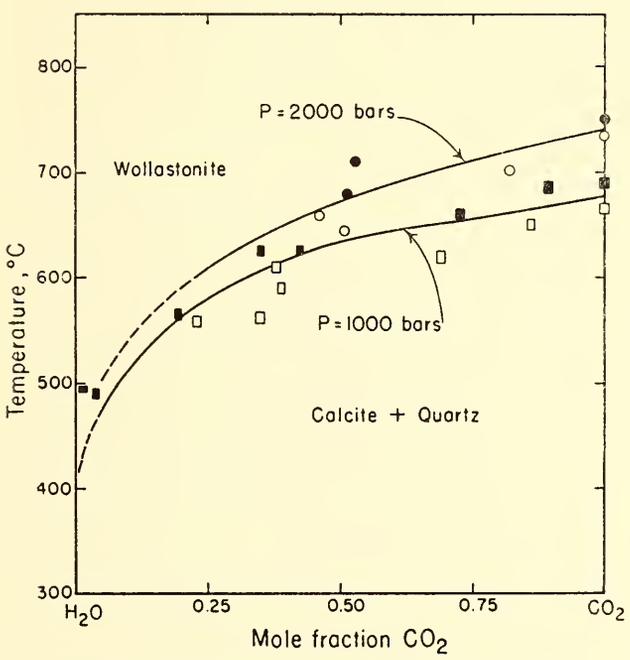

Fig. 23. Stability relations of calcite, quartz, and wollastonite in mixtures of H_2O and CO_2. Circles, 2000 bars; rectangles, 1000 bars.

molecular species rather than two. Reaction between CO_2 and H_2O to produce H_2CO_3 could easily produce the same effect as ideal mixing of CO_2 and H_2O on a solid-gas equilibrium. The accumulation of more data on mineral equilibria in the mixtures will allow direct estimation of the extent of reaction between H_2O and CO_2. In addition to the wollastonite reaction, preliminary runs indicate that the reaction of talc, calcite, and quartz to give diopside occurs at a lower temperature than the wollastonite reaction.

$$\underset{\text{Talc}}{\tfrac{1}{4}Mg_3Si_4O_{10}(OH)_2} + \underset{\text{Calcite}}{\tfrac{3}{4}CaCO_3} + \underset{\text{Quartz}}{\tfrac{1}{2}SiO_2} \rightarrow 3CaMgSi_2O_6 + \tfrac{1}{4}H_2O + \tfrac{3}{4}CO_2$$

According to equation 3 this reaction curve must pass through a maximum in temperature where $x_{CO_2} = 0.75$. At a total pressure of 1000 bars the temperature of this maximum has been determined to be 600° ± 25°C, at least 25° lower than the wollastonite curve at this composition, confirming the field observation that diopside can be formed at lower temperatures than wollastonite.

Synthesis and Stability of Anthophyllite

H. J. Greenwood

Pure magnesian anthophyllite, though of limited natural occurrence, has been the subject of considerable attention in the geological and geochemical literature. A significant part of this interest may be traced to the classic paper by Bowen and Tuttle (1949) on the system MgO-SiO_2-H_2O, describing experiments in which they were unable to demonstrate the stability of this mineral. Since that time there have appeared a number of papers, both theoretical and experimental, agreeing with their conclusion that the mineral is not stable in the presence of excess H_2O. Anthophyllite has been under study at this Laboratory for more than three years. Last year (Greenwood, *Year Book 60*, p. 105) the existence of a stability range in the presence of excess H_2O was indicated. Continuation of the study has produced enough data to allow detailed discussion of the upper and lower stability limits and nucleation kinetics of the mineral. Fyfe (1962) has recently given an account of some experiments with unanalyzed natural materials, which also indicate that the mineral has a range of stability in the presence of excess H_2O, but which do not define the reactions by

which anthophyllite may form from lower-temperature assemblages including talc.

Synthesis of anthophyllite is not easy. Most of the hydrothermal experiments that have failed to produce the mineral have failed because of its extreme reluctance to nucleate, even well within its own field of stability. Glasses, oxide mixes, and mixtures of the other minerals in the system MgO-SiO_2-H_2O in various proportions do not crystallize directly to anthophyllite, even when maintained within the anthophyllite stability field for periods as long as 4 months. The use of solutions of $MgCl_2$ and of HCl did not seem to facilitate the nucleation. The only way in which anthophyllite could be produced in the absence of preexisting nuclei was by the metastable decomposition of talc at 1000 bars and 830°C for a period of 20 hours. This procedure for obtaining starting materials with which to test the stability of anthophyllite was employed throughout most of the investigation. The materials so obtained are known to be of the pure magnesian anthophyllite composition; they have a refractive index of $n_Z = 1.615$ and give all the characteristic X-ray reflections of natural anthophyllite.

This metastable nucleation of anthophyllite about 80°C above its upper thermal stability limit at 1000 bars is readily explained on the basis of crystal structure. It seems unlikely that the mineral could form in the complete absence of any nuclei so far above its point of thermal breakdown. The requirement that talc be used as the starting material suggests that the talc is supplying nuclei having the anthophyllite structure. Disintegration of the sheets of tetrahedra in the talc structure into strips could provide the necessary structural units having $a_{anth} = c_{talc}$ and $c_{anth} \wedge b_{talc} = 30°$. Rate studies support this conclusion. Talc held under these conditions breaks down rapidly (half-life 7½ hours) to anthophyllite, protoenstatite, and quartz. Anthophyllite increases faster than the other reaction products until 16½ hours have elapsed, after which time it decreases, becoming undetectable after 120 hours. The final products are the phases stable at 830°C and 1000 bars, quartz and enstatite. Von Gehlen (1962) has shown that talc heated at 1300°C at atmospheric pressure is transformed into protoenstatite and quartz, with the protoenstatite oriented in the same way with respect to the parent talc structure as is postulated here for anthophyllite.

Rate studies on the decomposition of an analyzed natural anthophyllite (Al_2O_3, 1.94; FeO, 11.12; CaO, 0.64 weight per cent) at 1000 bars have shown that for times up to 40 days at 800°C (50°C above its stability limit) the amount of breakdown is barely perceptible, although at 850°C it is complete in 5 days. The excellent crystallinity and relatively coarse grain size of the natural material, perhaps together with its departure from the pure magnesian end member, evidently make it very slow to react, and extrapolation indicates that 4 or 5 months would be required to decompose the mineral near the equilibrium curve.

Starting materials for the runs used to define the limits of stability of the amphibole were prepared in the manner described. Oxide mixes on each of the bulk compositions $MgO \cdot SiO_2$, $7MgO \cdot 8SiO_2$, and $3MgO \cdot 4SiO_2$ were separately crystallized well inside the stability field of talc, and then given the heat treatment to produce anthophyllite, protoenstatite, and quartz from the talc, together with the other phases inherited from the crystallization in the talc field. These starting materials consisted of various mixtures of forsterite, enstatite (proto and clino), quartz, cristobalite, anthophyllite, and talc. This is an obvious disequilibrium mixture on the requisite bulk composition containing as nuclei all the phases to which the mixture could finally crystallize at equilibrium. Runs are from 3 to 4 months in duration, at the end of which time the reactions are from about 30 to 100 per cent complete.

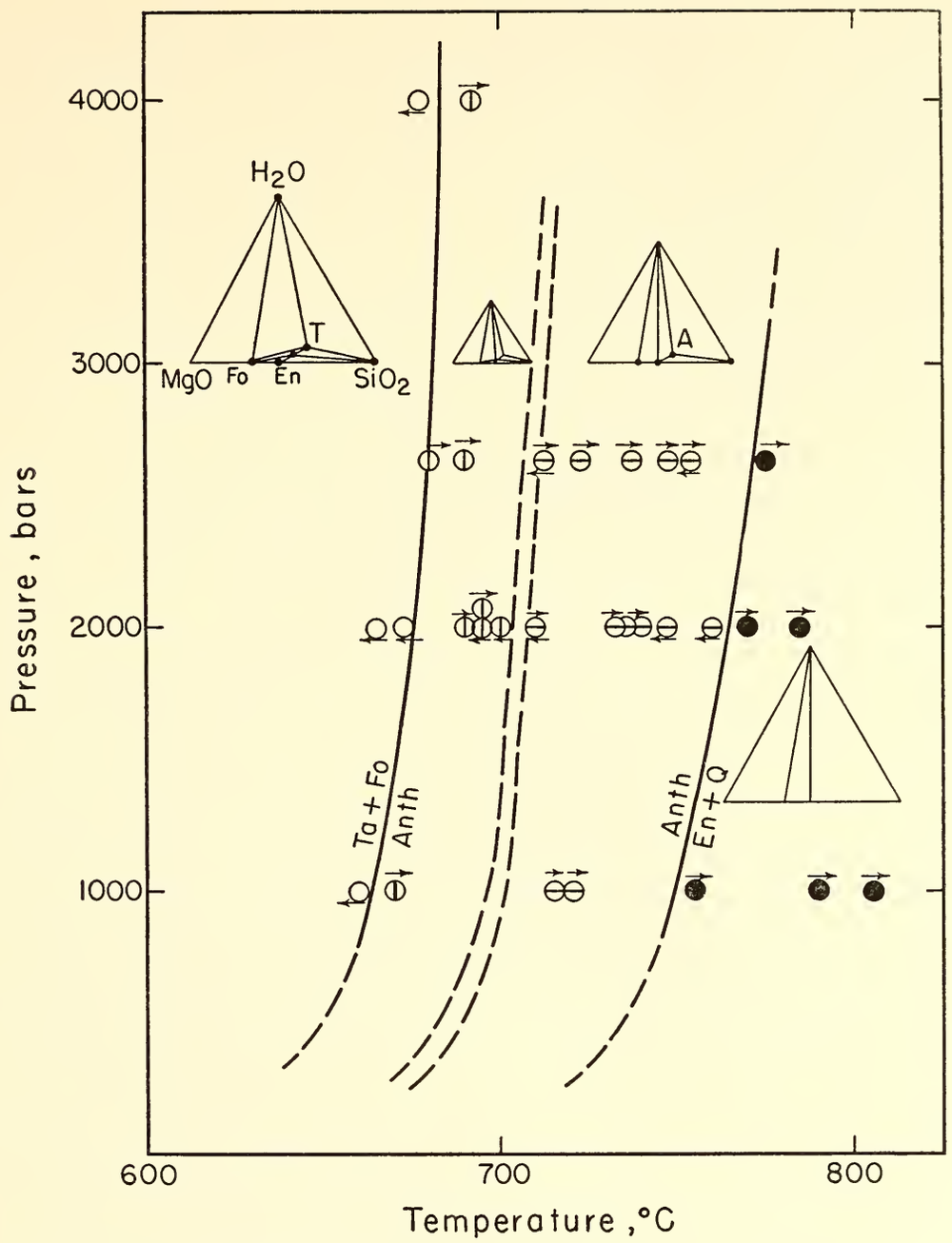

Fig. 24. Stability relations of talc, anthophyllite, and enstatite.

Depending on the bulk composition, the final products consist of mixtures of any pair of the phases forsterite, orthoenstatite, anthophyllite, talc, quartz. Protoenstatite, clinoenstatite, and cristobalite disappear.

The experimental results are shown in figure 24. All runs shown represent reversals of the reactions represented by the equilibrium curves. The arrows beside the run symbols indicate the direction from which the equilibrium was approached. Shorter runs in which no reaction occurred are not considered significant and are not reported. Both the upper and the lower stability limits of anthophyllite have been determined by reversing the reactions at several points, and they are considered to represent stable reversible equilibria. The upper stability limit of talc and the lower limit of enstatite in equilibrium with H_2O are indicated by the two dashed curves. Both reactions must occur in this narrow

interval, but it has not been possible to determine their relative positions.

In summary, anthophyllite has a range of stability in the presence of excess H_2O; and talc, anthophyllite, and H_2O can coexist in stable equilibrium over a narrow temperature interval.

Quartz-Chlorite Assemblages in the System MgO-Al_2O_3-SiO_2-H_2O

J. J. Fawcett and H. S. Yoder, Jr.

Investigation of synthetic systems closely related to low-grade metamorphic rocks offers many opportunities to the experimental petrologist. Experimental study of the low-grade metamorphic rocks is handicapped by the slow rates of reaction, which are probably due to the absence of a liquid silicate phase so that diffusion of ions must take place either in the solid state or, more likely, through a gas phase. To understand chemical reactions in low-grade metamorphic rocks and quantitatively evaluate prevailing physical conditions it is important to study in the laboratory the phase relations of the minerals and groups of minerals that play a significant role.

Chlorite and quartz are two of the most common minerals present in low-grade metamorphic rocks. Indeed, they characterize the chlorite zone of progressive metamorphism and often persist into the biotite and even garnet zones (Barrow, 1893; Tilley, 1925; Mason, 1962). In terms of the facies concept of metamorphism the quartz-chlorite assemblage characterizes in particular the quartz-albite-muscovite-chlorite subfacies of the greenschist facies (Fyfe, Turner, and Verhoogen, 1958, p. 218). The large volume and wide distribution of these rocks on the earth's surface suggest that these two minerals may exist together in equilibrium over a considerable, though as yet unspecified, range of temperature and pressure.

Hutton (1940) suggested that the chlorites of low-grade metamorphic rocks are dominantly iron rich, but a preliminary literature survey reveals only a small number of chemical analyses of chlorites from low-grade metamorphic rocks. Turnock (*Year Book 59*) showed that iron chlorite may exist in equilibrium with quartz up to almost 600°C at a total pressure of 2000 bars. The magnesium analogues of these chlorites may be represented in the system MgO-Al_2O_3-SiO_2-H_2O, studied by Yoder (1952) and Roy and Roy (1955); neither of these studies, however, shows chlorite and quartz as a compatible mineral pair in the temperature range 450° to 900°C at 15,000 psi (Yoder) or 130° to 1300°C between 5000 and 30,000 psi (Roy and Roy).

As natural occurrences indicate that chlorite and quartz may constitute a stable assemblage, a series of experiments have been performed in an attempt to define a stability field for Mg chlorites and quartz. Starting materials for the experiments were glasses whose compositions, shown in figure 25, for the most part lie on the anhydrous join anthophyllite ($7MgO \cdot 8SiO_2$)-Mg gedrite ($5MgO \cdot 2Al_2O_3 \cdot 6SiO_2$); the glasses were prepared under the supervision of Dr. Schairer in connection with a study of these amphiboles. Other glasses were supplied by Schairer and by Yoder. The compositions plot between the composition of the chlorite solid solution series and quartz as projected from H_2O onto the face MgO-Al_2O_3-SiO_2, in the system MgO-Al_2O_3-SiO_2-H_2O. Determinative runs were made at pressures of 2 and 5 kb in cold-seal hydrothermal bombs, the duration of the runs varying from 1 to 6 weeks. At 2 kb and temperatures of 400° and 600°C talc was found to grow readily, in the early stages of the runs, with charges containing less than 15 per cent Al_2O_3. Some of the talc reacts only very slowly to produce a more stable mineral assemblage. Talc also grows from compositions containing more than 15 per cent Al_2O_3, but the smaller amounts produced from these compositions are consumed completely by reaction in less than 3 weeks.

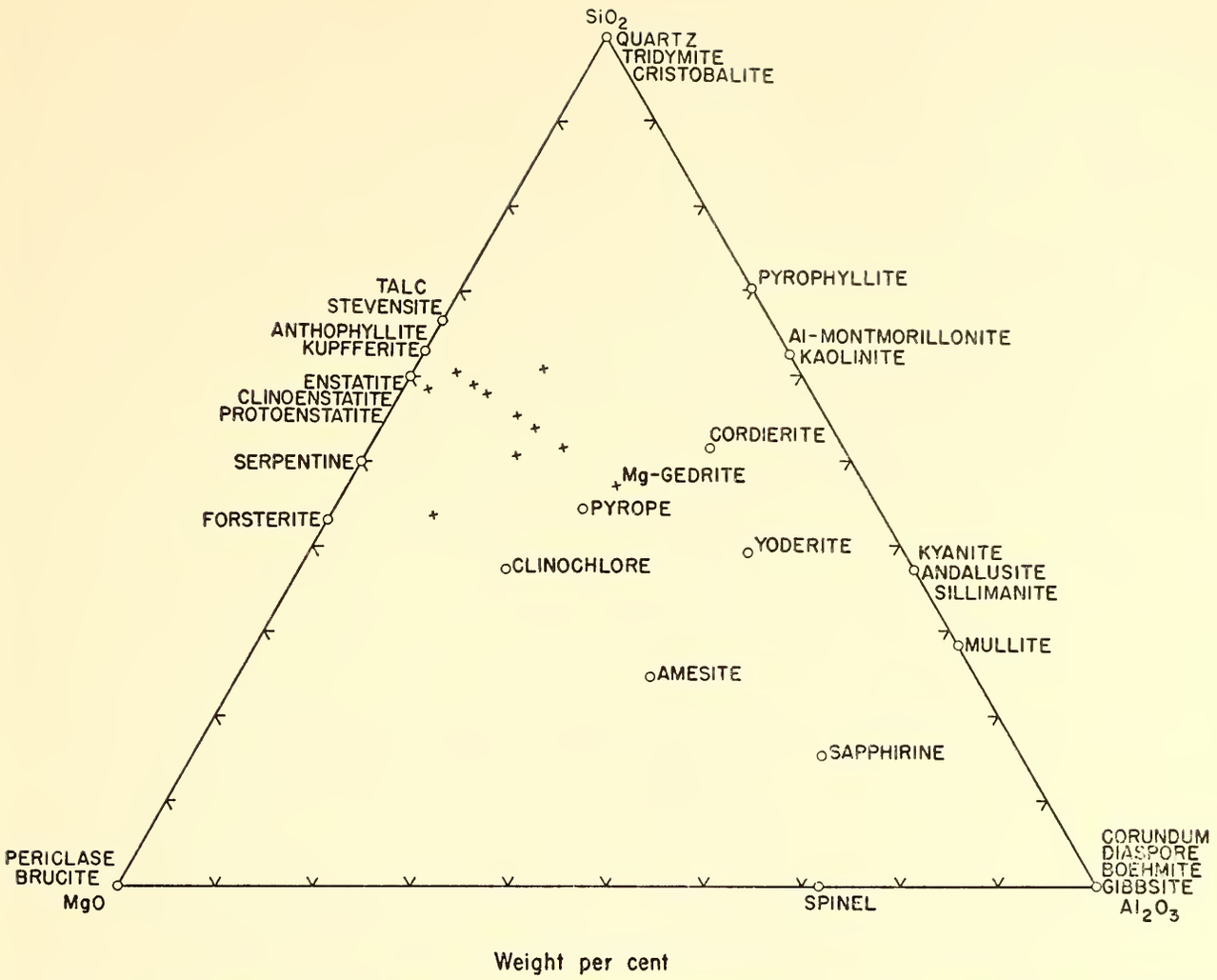

Fig. 25. Composition of glasses (+) used in the determination of the quartz-Mg chlorite stability field. The projected composition of Al montmorillonite should plot on top of pyrophyllite and not kaolinite, as shown.

Preliminary results are illustrated in figure 26. Quartz and chlorite are stable together up to almost 600°C, but the projected shape of the chlorite-quartz field is a reflection of the stability of the chlorite solid solution series. At lower temperatures (450°C) a wide range of chlorite compositions is stable; with increasing temperature the range becomes narrower. The chlorite coexisting with quartz at the maximum temperature of the quartz-chlorite field contains about 20 weight per cent Al_2O_3. An increase or decrease in the Al_2O_3 content of the chlorite results in a reduction of the maximum temperature of the quartz-chlorite stability field. Montmorillonite crystallized at temperatures below 450°C. Talc formed metastably in the less aluminous compositions in the quartz-chlorite field, but in the longer runs (6 weeks) quartz and chlorite represent a more stable assemblage. Many runs produced the 7 Å polymorph of the chlorites (aluminous serpentine, Yoder, 1952; septechlorite, Nelson and Roy, 1958), but most runs of 3 weeks or longer produced the 14 Å chlorite.

Chlorite and quartz coexist, together with a third phase (talc or cordierite) on either side of the quartz-chlorite stability field. These two small fields are limited at higher temperatures by the field of talc + chlorite + cordierite and, for more SiO_2-rich compositions than those in the section of figure 26, by talc + cordierite + quartz.

At temperatures in the 700° to 850°C range a careful search has been made for the phases in the anthophyllite

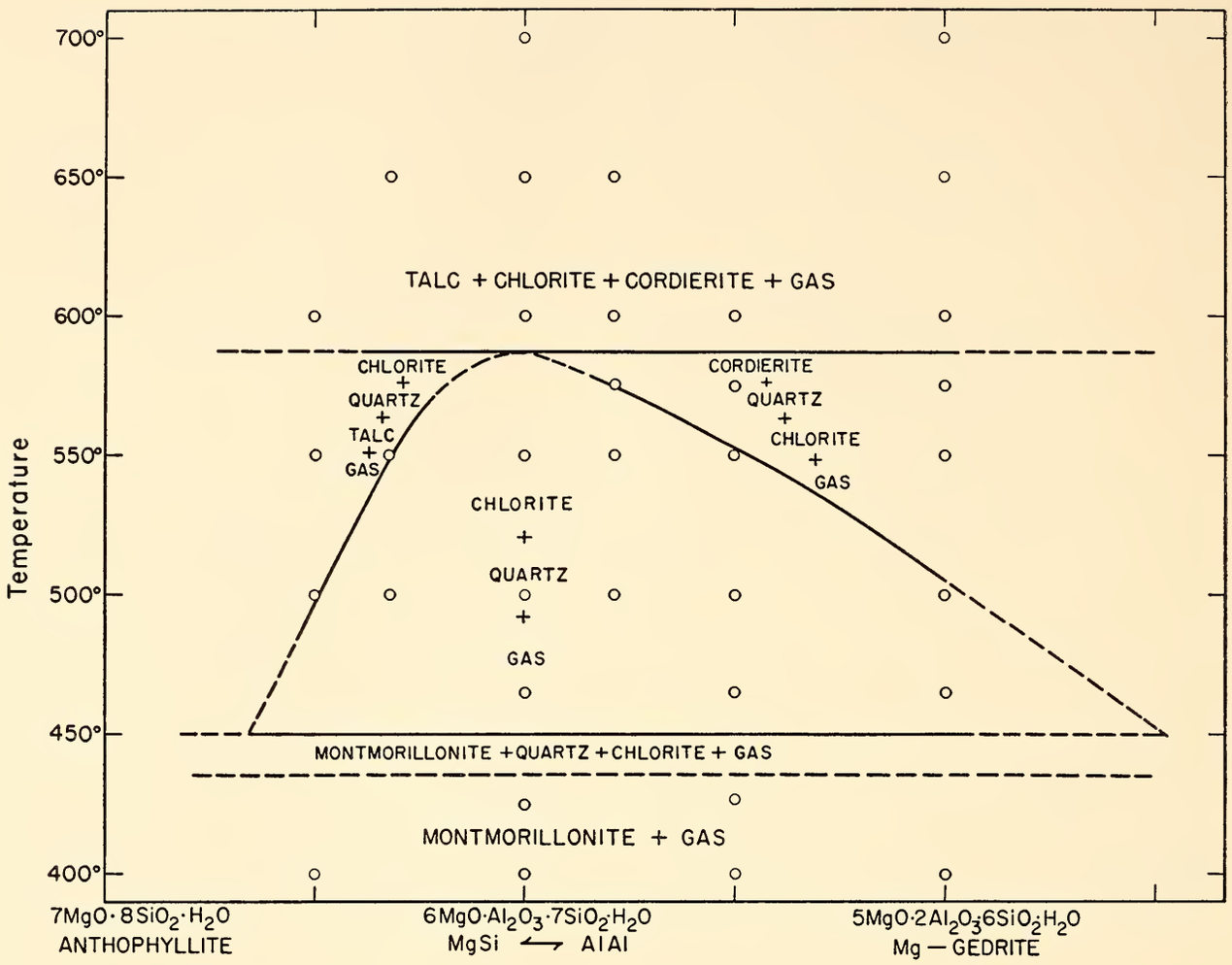

Fig. 26. Phase relations along the extended join anthophyllite ($Mg_7Si_8O_{22}(OH)_2$)-Mg gedrite ($Mg_5Al_4Si_6O_{22}(OH)_2$) in the system MgO-Al_2O_3-SiO_2-H_2O.

($Mg_7Si_8O_{22}(OH)_2$)-Mg gedrite ($Mg_5Al_4Si_6O_{22}(OH)_2$) group. Amphiboles of unknown composition have been synthesized, but they have been shown to be unstable. In view of the existence of a stability field for pure anthophyllite (Greenwood, this report), work on the aluminous anthophyllites will be continued in the coming year.

To test the results obtained with synthetic starting materials, naturally occurring minerals have been used as starting materials for some experiments; mixtures of talc + cordierite have been converted to chlorite + quartz + minor talc within the quartz-chlorite field of the synthetic materials, and similarly mixtures of quartz + chlorite have been converted to cordierite + talc + quartz within the stability field of that assemblage, as indicated by runs using glasses.

An increase in pressure from 2 to 5 kb raises the temperature of maximum stability of the quartz-chlorite assemblage from about 600° to 625°C. It is of interest to note that the quartz-chlorite reaction curve is about 100°C below the maximum stability of clinochlore at 2000 bars, whereas the analogous muscovite-quartz curve is only 15°C below the upper stability limit of muscovite (Yoder and Eugster, 1955). The effect of pressure on the lower stability limits of the quartz-chlorite stability field has not yet been determined.

All runs below 450°C produced a montmorillonite phase, but there is, on theoretical grounds, a narrow temperature interval between the montmorillonite field and the quartz-chlorite field in which

the stable assemblage is quartz or talc + montmorillonite + chlorite. The precise phase relationships have not yet been worked out at lower temperatures.

Nelson and Roy (1958) determined the maximum stability of the chlorite solid solutions in the absence of quartz to be 710°C at 1000 atm. The maximum stability of the chlorite + quartz assemblage was found in the present work to be 675°C at 2 kb. Although the pressures are not equivalent, the data are in accord with the general rule that heterogeneous reactions must take place within the stability fields of the reactants and products. The present data indirectly suggest that the maximum stability of the alumina-poor and alumina-rich chlorites is lower than that suggested by Nelson and Roy. The breakdown products of clinochlore and amesite obtained by Nelson and Roy (1958) do not appear to represent equilibrium assemblages. Runs of much greater duration than those carried out by Nelson and Roy on the chlorite breakdown may yield assemblages different from the forsterite, talc, and spinel obtained by those authors.

The information on the limits of stability of the quartz-chlorite mineral assemblage may be applied in a general way to the conditions of formation of potassium-deficient low-grade metamorphic rocks. As the chlorite minerals in the rocks always contain iron, however, the data can only indicate maximum temperatures in the pressure range under consideration. Moreover, the effect of other phases such as muscovite, biotite, feldspar, or epidote on the quartz-chlorite stability field is unknown.

It is clear from these results that previous investigators had not made runs of sufficient duration to obtain the quartz-chlorite assemblage. It can now be concluded that the synthetic studies support the field observations that Mg-rich as well as Fe-rich chlorites can coexist with quartz over a large *P-T* range. Limitation of the coexistence of quartz and chlorite in the natural rocks is due to complex reactions involving chlorite, quartz, or both.

Alkali-Rich Igneous Rocks and Minerals

The System Na_2O-Al_2O_3-Fe_2O_3-SiO_2 and Its Bearing on the Alkaline Rocks

J. F. Schairer and D. K. Bailey

The alkaline rocks, by virtue of their uncommon chemistry and mineralogy—with essential amounts of feldspathoids and alkali pyriboles in a wide range of proportions, and often with minor amounts of rare minerals—have engaged the attention of petrographers and analysts to an extent that belies their quantitative importance; consequently, a large body of information is available on them, and the problem of their origin has provoked much speculation and argument. It does not follow, however, that the considerable attention devoted to these rocks has been misdirected, for the typical alkaline centers, in common with kimberlites and carbonatites (with which alkaline rocks are frequently associated), are restricted to the stable continental areas of the earth's crust, and the rocks are probably the surface expression of deep-crustal and subcrustal activity in epeirogenic zones. The natural compositional ranges in the alkaline rocks, and the awesome range of rock names, make generalizations about their composition difficult, but a large proportion of the rocks are essentially assemblages of nepheline (or related feldspathoids such as sodalite and cancrinite), sodic pyroxene (or amphibole), and alkali feldspar, thus falling into two broad groups: ijolites (nepheline-pyroxene rocks) and foyaites (nepheline-feldspar-pyroxene rocks). Both types bulk largely in alkaline complexes, are commonly associates of carbonatite, and hence figure prominently in theories of the origin and the differentiation of these rocks. The study of the system Na_2O-Al_2O_3-Fe_2O_3-SiO_2 offers an opportunity to observe the essential rock-

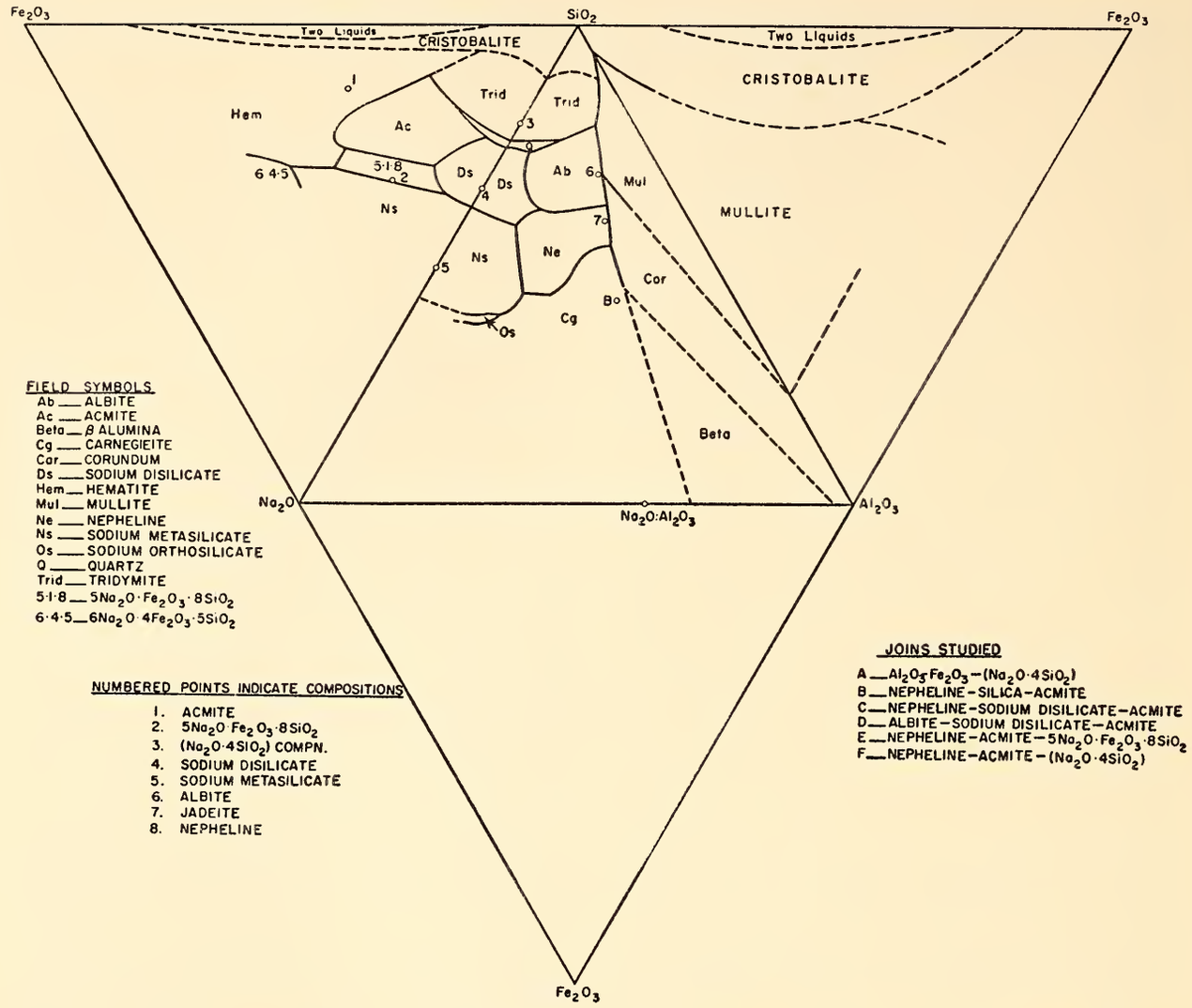

Fig. 27. The system Na_2O-Al_2O_3-Fe_2O_3-SiO_2 to show relations of the compounds and joins studied. Three faces of the tetrahedron have been laid flat in the plane of the base.

forming minerals nepheline, acmite, and albite in equilibrium with liquids the compositions of which are analogous to those of natural alkaline rocks.

Study of equilibrium within the quaternary system Na_2O-Al_2O_3-Fe_2O_3-SiO_2 at 1 atmosphere pressure began with an examination of the join jadeite-acmite in 1948–1949 (*Year Book 48*, p. 32) as part of a more general study of the stability relations of jadeite. This join is not binary, and it was found that the primary phase for compositions $acmite_{100-20}$ was hematite or hematite-corundum solid solution, and that compositions $jadeite_{100-80}$ gave nepheline-albite solid solution as the primary phase with hematite-corundum solid solution as the second phase. This meant that relations in the join could be described correctly only in terms of the quaternary system, and work was started in five joins in this system the following year.

Phase-equilibrium data for the bounding ternary system Na_2O-Fe_2O_3-SiO_2, establishing the incongruent melting of acmite, have been published by Bowen, Schairer, and Willems (1930) and for the system Na_2O-Al_2O_3-SiO_2 by Schairer and Bowen (1956). Because reduction of Fe_2O_3 to FeO increases with temperature, only the parts of the system Na_2O-Al_2O_3-iron oxide-SiO_2 with low liquidus temperatures can be treated as essentially quaternary and in the system Na_2O-Al_2O_3-Fe_2O_3-SiO_2. Fortunately this low-

temperature region embraces the compositions of greatest geological interest, approximately within the volume sodium metasilicate-acmite-nepheline-silica. The position of this volume within the tetrahedron Na_2O-Al_2O_3-Fe_2O_3-SiO_2 can be seen from figure 27. The positions of the five joins first studied can also be seen from this figure; they are $Na_2O \cdot 4SiO_2$-Al_2O_3-Fe_2O_3 (which includes the acmite and jadeite compositions), nepheline-silica-acmite, nepheline-sodium disilicate-acmite, albite-sodium disilicate-acmite, and nepheline-$5Na_2O \cdot Fe_2O_3 \cdot 8SiO_2$-acmite. Brief progress reports on these joins were given in *Year Books 49*, pp. 46–47, *50*, pp. 53–54, and *51*, pp. 52–53, noting the existence of various eutectics and piercing points without further details. Clearly, however, the system has a bearing on problems other than jadeite stability, which was the initial stimulus for its study. One such is its application to studies of alkaline rocks, and it was during a joint visit to the alkaline rock/carbonatite complex of the Fen area in southern Norway that we decided to extend the work on this system.

From the data on the first five joins in the system it is possible to deduce a crystallization flow diagram, but there were no observational data on the important univariant line nepheline-acmite-albite-liquid. Its existence and extent have to be inferred from other data and the geometry of the system. Such a univariant line should link the quaternary reaction point hematite-nepheline-acmite-albite-liquid and the quaternary eutectic point nepheline-acmite-albite-sodium disilicate-liquid, between which there is a considerable composition interval and a temperature interval of 200°C (from 915° ± 5°C to below 727°C). From the point of view of the alkaline rocks it was important to have an intersection of this univariant line to confirm these deductions. That such an intersection had not been found in any of the previous five joins—notably the composition plane nepheline-acmite-silica—is due to the incongruent melting relationship of acmite, the primary phase volume of hematite thus extending through this join.

It seemed most likely, from geometric considerations, that the join nepheline-acmite-$Na_2O \cdot 4SiO_2$ would intercept the univariant line nepheline-acmite-albite-liquid, and that in addition this plane should contain the ternary reaction point nepheline-acmite-hematite, which is also of particular interest in alkaline rock problems. During the past few months thirty-three compositions have been prepared, and work on this join is nearing completion; the preliminary equilibrium diagram is given here as figure 28. The piercing points of two univariant lines, acmite-albite-quartz-liquid and nepheline-acmite-albite-liquid, and the ternary reaction point nepheline-acmite-hematite have been located, at temperatures of 758°, 845°, and 905°C, respectively.

We intend to publish the complete diagrams for the six joins and the crystallization flow diagram in the near future, when we hope that the bearing of the results on the crystallization history of related alkaline rocks and other rocks such as peralkaline granites and rhyolites can be discussed. Some of the petrological implications are already apparent and will be of interest to geologists working on alkaline rock problems; for this reason a few of them are stated briefly here.

The univariant line along which the three important rock-forming minerals nepheline, acmite, and albite crystallize in equilibrium with liquid spans a relatively large composition and temperature interval with the composition of the liquid moving toward a quaternary eutectic where the fourth solid phase, sodium disilicate, begins to form. Liquid compositions along this line range from those analogous to ijolite to a foyaitic or nepheline syenitic composition, approximately from liquids containing 40 to 10 per cent potential acmite: the temperature interval is from 915° ± 5°C to below 727°C. This means that the three min-

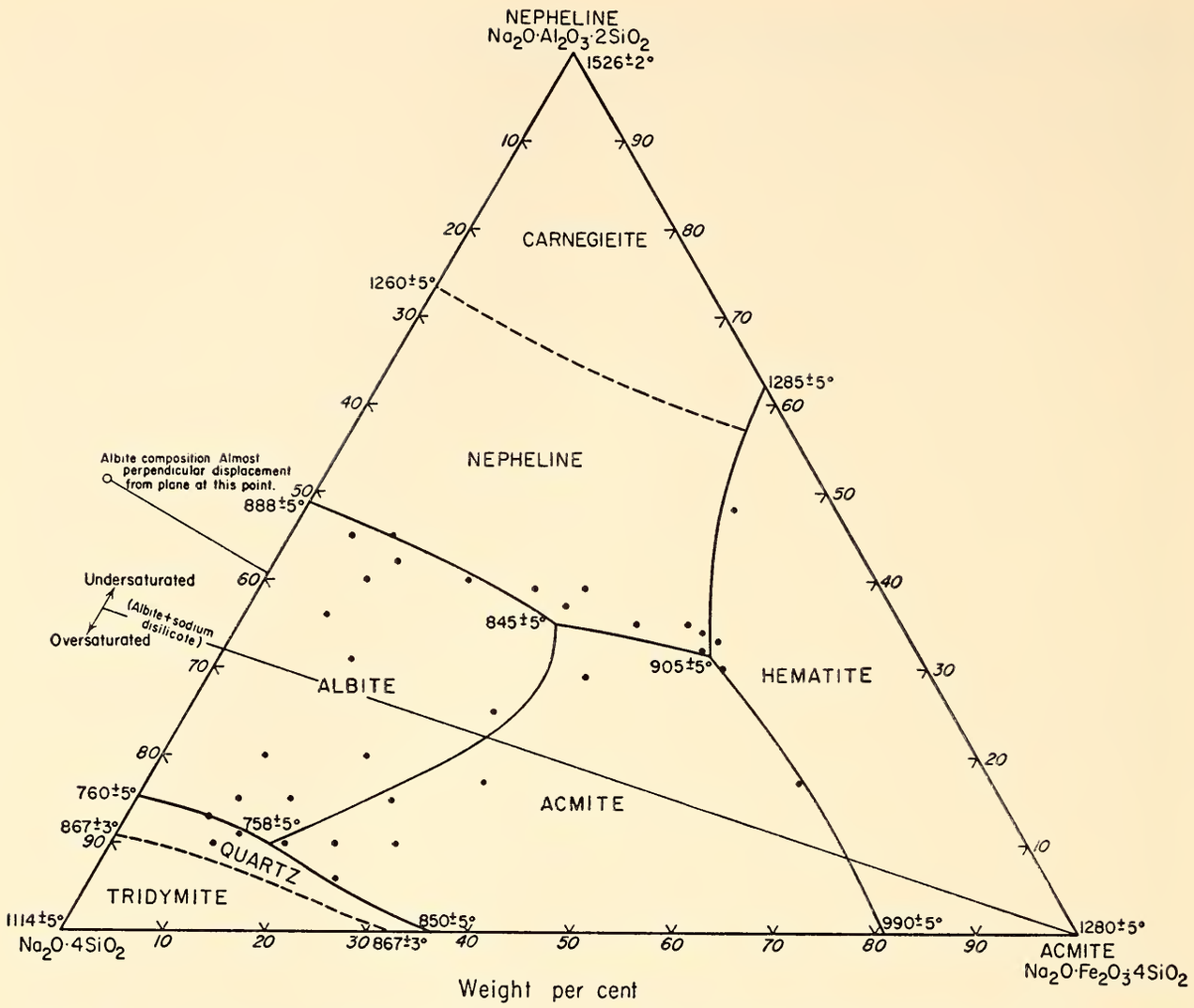

Fig. 28. Preliminary equilibrium diagram of the join nepheline-acmite-($Na_2O \cdot 4SiO_2$). This is a portion of the join nepheline-hematite-($Na_2O \cdot 4SiO_2$).

erals, in various proportions, can exist in equilibrium with a wide range of liquid compositions over a long temperature interval. If similar conditions pertain to magmas of analogous composition there would be ample scope for differentiation and separation of residual liquids by any of the normally invoked physical processes. Such a condition is entirely in keeping with the wide compositional ranges seen in alkaline complexes and even, in some hand specimens and outcrops, by segregation of early-formed minerals. Equilibrium between nepheline-acmite-albite and a liquid containing potential sodium disilicate is consistent with the observation that, of the analyses in Washington's (1917) tables that give sodium metasilicate in the norm, about half are of unsaturated alkaline rocks, but probably more important is the fact that the residual liquid is becoming progressively enriched in sodium disilicate with increasing crystallization. In nature the residual liquid would also become increasingly enriched in volatiles, and production of such residual fluids would explain the almost invariable alkali metasomatism of country rocks around alkaline intrusions.

The liquid phase at the ternary reaction point nepheline-acmite-hematite-liquid has the composition of a simplified ijolite, figure 28. This point should be a temperature maximum on the corresponding univariant line, such that liquids with an

initial composition on the more siliceous side of the composition plane move toward the quaternary reaction point nepheline-acmite-albite-hematite, i.e. have a foyaitic trend, whereas those on the opposite side move toward a quaternary eutectic at which $5Na_2O \cdot Fe_2O_3 \cdot 8SiO_2$ crystallizes with the other three phases. This increasingly basic liquid trend, with 60 per cent or more acmite in some compositions, may be compared with the ijolite-melteigite trend seen in natural rocks. This indication of the possibility of an ijolitic liquid's having two possible divergent differentiation trends depending on small compositional variations is interesting, but an even more interesting feature is that there appears to be very little temperature difference between the ternary reaction point nepheline-acmite-hematite and the quaternary reaction point nepheline-albite-acmite-hematite. This means that only slight fluctuations in physical conditions or composition will determine the differentiation trend of the liquid toward either foyaitic residual liquids or mafic melteigitic liquids.

The critical consideration in regard to all the above suggestions is whether the equilibrium relations at 1 atmosphere are seriously changed under pressure and in the presence of volatiles, and it is planned in the coming year to investigate the effects of water pressure on some of the critical joins in the system.

Peralkaline Residual Liquids: Some Petrogenetic Considerations

D. K. Bailey and J. F. Schairer

It was noted in the above discussion of the anhydrous system Na_2O-Al_2O_3-Fe_2O_3-SiO_2 that the residual liquids resulting from crystallization of nepheline, albite, and acmite became increasingly enriched in sodium disilicate. In nature this enrichment would be expected to be concomitant with enrichment in volatiles, and fluids of this type would be expected to react with wall rocks containing Al_2O_3 and SiO_2 to form nepheline, nepheline-albite, or albite, depending on the proportions of Al_2O_3 and SiO_2 present. For convenience of discussion the simplest expressions of the reactions are given in the equations

$$Na_2O \cdot 2SiO_2 + Al_2O_3 \rightarrow \text{nepheline} \quad (1)$$

$$Na_2O \cdot 2SiO_2 + 2SiO_2 + Al_2O_3 \rightarrow (\text{nepheline} + \text{albite}) \quad (2)$$

$$Na_2O \cdot 2SiO_2 + 4SiO_2 + Al_2O_3 \rightarrow \text{albite} \quad (3)$$

In pelitic wall rocks reaction 2 would have the most general application, the silica and alumina balance corresponding to that of the clay minerals. Calcareous and ferruginous pelites might be expected to push the reaction even further in the direction of greater production of nepheline. It is not inconceivable, for instance, that nephelinization such as that demonstrated at Bancroft, Ontario (Tilley, 1958), could result from reaction of sodium silicate-bearing fluids and impure aluminous members of the limestone series; such a reaction would require far less transfer of material than that needed for metasomatic replacement of pure limestone. With higher SiO_2-Al_2O_3 ratios in the country rocks the reactions would trend toward equation 3, giving eventually only feldspathization of country rocks. The generation of residual fluids rich in alkali silicate may thus be seen to offer a simple explanation of the metasomatism around alkaline intrusions.

The parent peralkaline undersaturated liquid giving rise to these fluids might arise by partial melting of alkali basalt. Bowen (1945, p. 88) pointed out that residual liquids from fractionation of basalt might become enriched in sodium silicate by operation of the "plagioclase effect," and he was well aware that reaction of such liquids with aluminous wall rocks would tend to form nepheline and albite. (Certainly such liquids would account for adinole formation at dolerite-shale contacts and might play a part in the formation of some spilites.) The

converse of residual liquid origin—partial melting of alkali basalt—could yield the same reactive undersaturated liquids and perhaps give rise to nepheline-syenite complexes with no associated basalt.

It is less obvious perhaps that oversaturated compositions could yield residual liquids capable of producing metasomatic effects similar to those derived from unsaturated liquids. In the system Na_2O-Al_2O_3-Fe_2O_3-SiO_2, quartz, acmite, and albite also crystallize in equilibrium with a liquid becoming enriched in sodium disilicate, the quaternary eutectic lying to the silica-poor side of the join acmite-nepheline-$Na_2O \cdot 4SiO_2$ (fig. 28), which means that liquid compositions near this point can be expressed in terms of the molecules acmite, albite, sodium disilicate, and silica, the amount of silica being less than that required for a composition $Na_2O \cdot 4SiO_2$. If residual fluids of this nature were to react with sediments in which the SiO_2/Al_2O_3 ratio was 2 or less, such as bauxitic, calcareous, or ferruginous pelites, conditions intermediate between equations 2 and 3 would obtain, and it would be possible to have nephelinization of certain rocks around a peralkaline granite intrusion.

Peralkaline residual liquids might result from a variety of originally oversaturated compositions, for, as Tuttle and Bowen (1958, pp. 84–87) have indicated, fractionation of liquids on the alkali side of the albite-orthoclase-quartz section in the system Na_2O-K_2O-Al_2O_3-SiO_2 would be expected to yield residuals rich in alkali silicates and volatiles. They point out that such liquids escaping from a cooling granite "may affect granitization of the adjacent rocks, providing the rocks have appropriate composition," but their data indicate that the content of alkali silicates in such liquids is such that reaction with aluminous country rocks could yield undersaturated mineral assemblages in the manner described above. These considerations only serve to underline a point that perhaps receives too little emphasis, namely that the system $NaAlSiO_4$-$KAlSiO_4$-SiO_2 ("petrogeny's residua system") is a residua system only for compositions that are subaluminous, in the sense used by Shand; compositions off this plane, on the alkaline side, would be expected to fractionate to liquids rich in alkali silicates. It follows that partial melting of basement or buried sediments, with a bulk composition slightly less than subaluminous, should first yield such a liquid fraction. Usually this liquid, during its uprise, would be expected to react with country rocks to produce granite, either magmatically or metasomatically, or it might produce a more diffuse regional metasomatism; but in an aluminous environment it should also be possible for undersaturated assemblages to result.

The System Nepheline-Diopside

J. F. Schairer, Kenzo Yagi,[1] and H. S. Yoder, Jr.

In view of the significance of the system nepheline-diopside as a principal join of the petrologically important tetrahedron diopside-nepheline-forsterite-quartz, further study was initiated in 1950–1951 (*Year Book 50*, p. 54), and it has been continued intermittently in subsequent years. The system was found to be of such complexity that over five hundred runs have been made in an effort to determine the stability regions of the various mineral solid solutions. Some forty years ago the system nepheline-diopside was investigated by Bowen (1922) in connection with the genesis of alnoitic rocks of the Province of Quebec, Canada. The subsolidus relations were not established at the time because of the difficulties in the determination of minute crystalline phases under the microscope without the aid of X-ray techniques.

As is evident from an inspection of figure 29, the system is not binary, and should be considered a join in the quinary system Na_2O-CaO-MgO-Al_2O_3-SiO_2. All the crystalline phases obtained are solid

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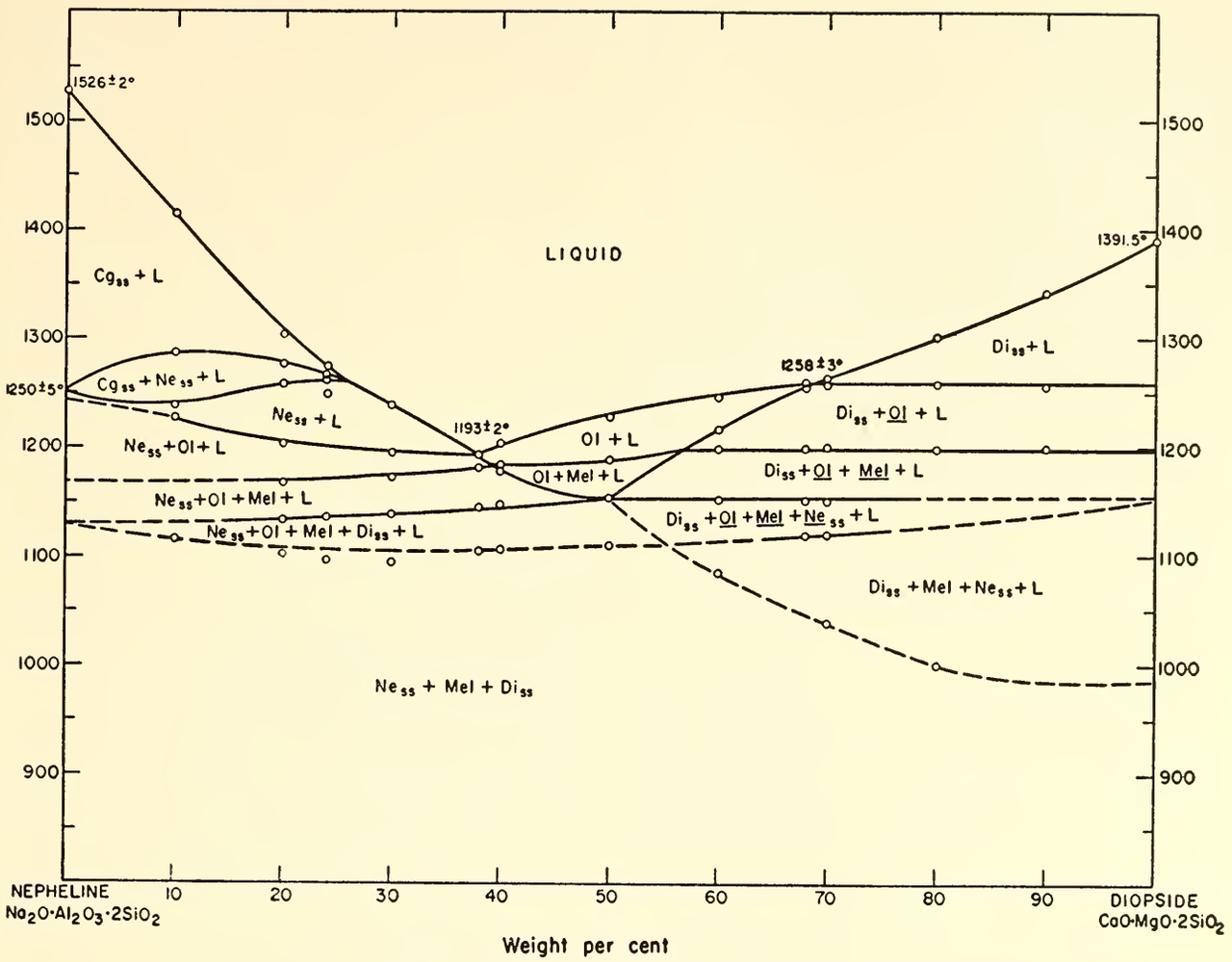

Fig. 29. Pseudobinary diagram of equilibrium in mixtures of nepheline and diopside.

solutions, and their precise compositions have not been determined; however, they may be designated by the principal end member present. The phases are diopside solid solution (Di_{ss}), nepheline solid solution (Ne_{ss}), carnegieite solid solution (Cg_{ss}), melilite (Mel), olivine (Ol), and liquid (L). In some regions of temperature and composition a specific solid solution may have a fixed composition. This conclusion is based on the principle that a solid solution of fixed composition will appear or disappear during cooling at the same temperature from a range of compositions. For example, in compositions rich in the diopside component, it is seen that Ol, Mel, and Ne_{ss} appear successively at specific temperatures. It may be concluded that in the designated ranges of bulk composition the solid solutions involved here were of fixed, but unknown, composition. Such solid solutions, believed to be of fixed composition, are underlined in the figure.

It was not possible to fix the solidus of the system with assurance because of the difficulty of recognizing small amounts of glass in the quench products or, for some compositions, the presence or absence of small amounts of olivine. In addition, crystal growth was sluggish and equilibrium could not be established with certainty. All runs having more than 5 per cent crystals were examined with powder X-ray diffraction techniques.

The new results reaffirm Bowen's observations that melilite and olivine separate from liquids whose total composition can be expressed as a mixture of nepheline and diopside. Olivine appears to react with liquid until consumed, producing diopside solid solution (see also

Schairer and Yoder, 1960, on the system nepheline-diopside-silica) and melilite. The intimate association of nepheline, clinopyroxene, and melilite in lavas suggests that melilite may indeed be a differentiation product of an alkali basalt magma. Further information is needed on the composition of the melilites that crystallize in the join nepheline-diopside and on the crystallization relations in the portion nepheline-diopside-albite-forsterite of the simplified basalt tetrahedron nepheline - diopside - forsterite - quartz of Yoder and Tilley (*Year Book 59*, p. 67).

A Reconnaissance of the Systems Acmite-Diopside and Acmite-Nepheline

Kenzo Yagi

The main constituent molecules of the pyroxenes in the alkaline rocks are diopside, hedenbergite, acmite, and jadeite. Jadeite is present only in minor amounts in most of these pyroxenes. When the compositions of these pyroxenes are plotted in the triangular diagram diopside-hedenbergite-acmite (+ jadeite) the points line in a zone extending from the diopside corner through the center of the triangle to the acmite corner. This suggests complete solid solution between diopside and acmite and extensive solid solution with hedenbergite (see Yagi, 1953). Nepheline syenites and related rocks have nepheline and acmitic pyroxene as the most important constituent minerals in addition to alkali feldspars. To investigate the relations in alkaline rocks a study of the joins diopside-hedenbergite-acmite and nepheline-diopside-acmite is necessary.

First the join nepheline-diopside was studied; the results are given elsewhere in this report (pp. 96–98). The results on the join acmite-diopside are given here as figure 30. There is a complete series of solid solutions between acmite and diopside. Bowen, Schairer, and Willems (1930) showed the incongruent nature of the melting of acmite at 990°C to hematite

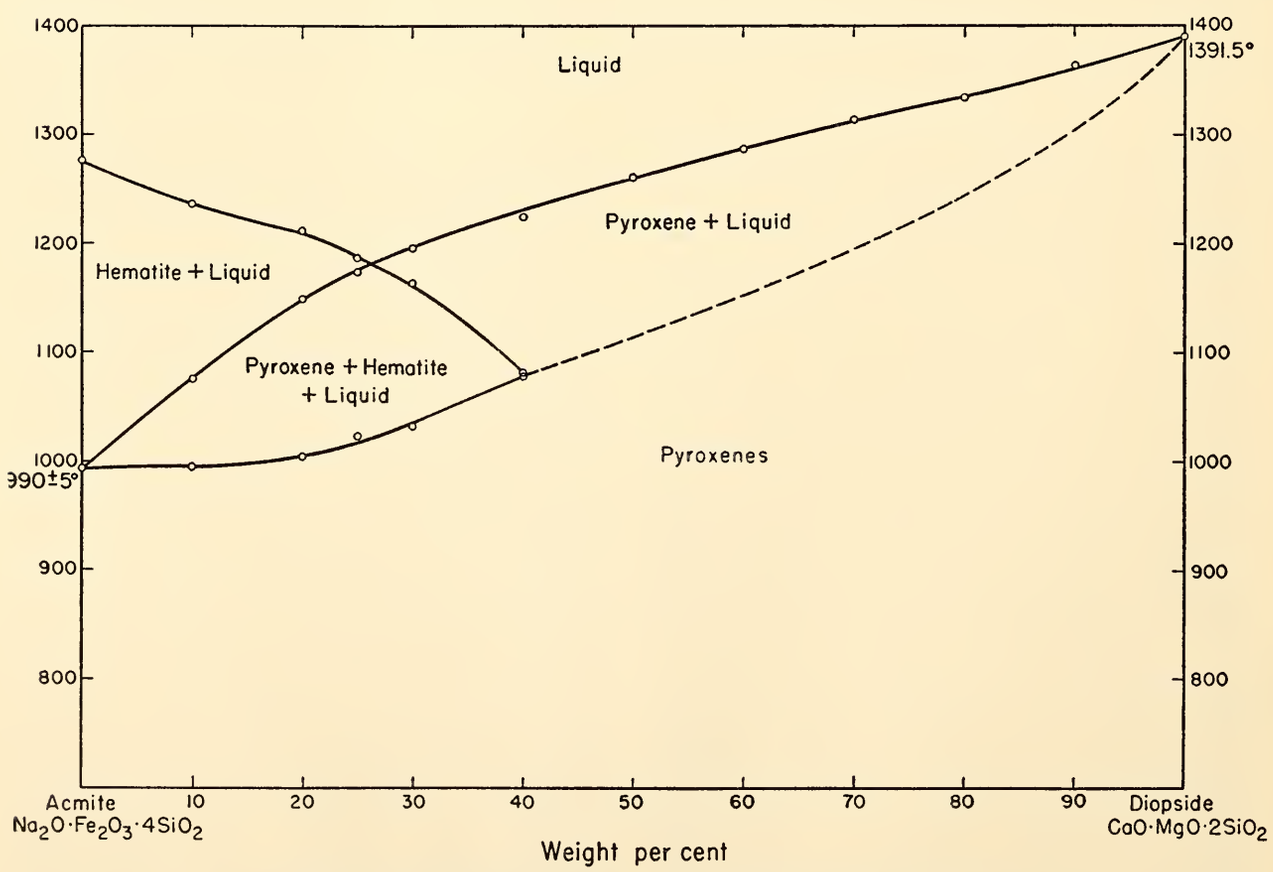

Fig. 30. Equilibrium diagram for the join acmite-diopside.

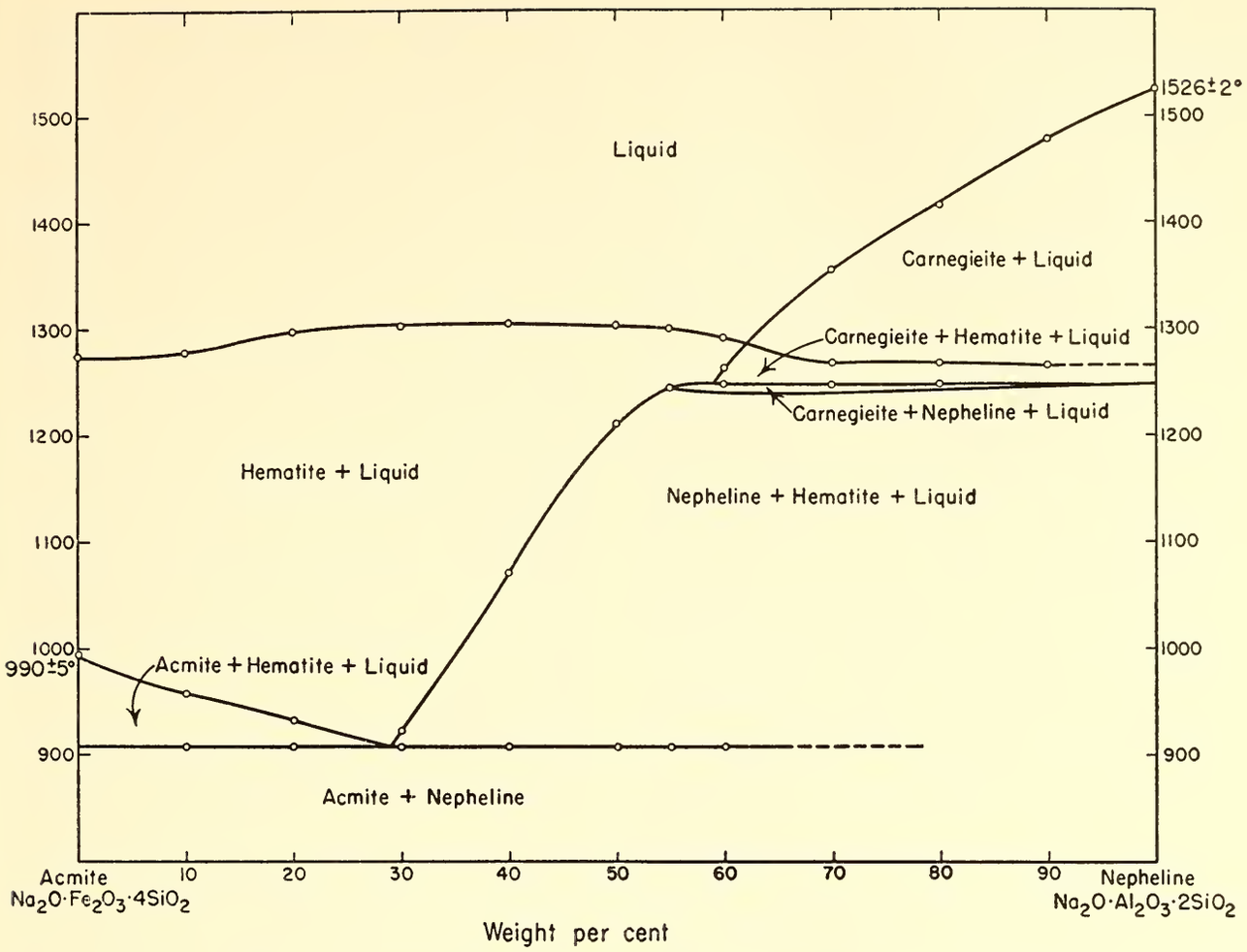

Fig. 31. Equilibrium diagram for the join acmite-nepheline.

and liquid. In the join acmite-diopside all compositions with 40 per cent or more acmite melt incongruently, and the join is not binary. Attention is called to the fact that some of the iron is always present as ferrous iron, although most of the iron in these melts is ferric iron. Therefore, the system is never truly binary even in the portion richer in diopside than 40 per cent, and there is always a small amount of liquid (glass) present in the region labeled pyroxenes.

The results of a study of the join acmite-nepheline are given here in figure 31. A very wide primary field of hematite appears on the liquidus surface as a result of the incongruent melting of acmite. The system is not binary. The phases present are acmite, hematite, carnegieite, nepheline, and liquid. There is a narrow region of coexistence of nepheline and carnegieite, suggesting that they are solid solutions with a narrow range of compositions. Hematite present in the melts varies in color from deep reddish brown to pale brown, suggesting differences in composition perhaps due to the presence of Al_2O_3 in solid solution in the hematite. The precise compositions of solid solutions have not been determined. Mixtures of acmite and nepheline begin to melt at about 908°C.

Studies of the join acmite-diopside-nepheline in progress at Tohoku University are nearly completed. They will be presented during the next year. Most of the studies on acmite-diopside and acmite-nepheline were made at Tohoku University, but some of the quenching experiments were run at the Geophysical Laboratory in December 1960 and January 1961.

Accessory Minerals

Investigations in the System FeO-Fe_2O_3-TiO_2

D. H. Lindsley

The system FeO-Fe_2O_3-TiO_2 contains several phases of geologic interest (fig. 32). In addition to the TiO_2 minerals rutile, anatase, and brookite, the important phases are:

1. The rhombohedral hematite-ilmenite (αFe_2O_3-$FeTiO_3$) series, with complete solid solution above ~950°C (Carmichael, 1961), referred to as the α series by Verhoogen (1962).

2. The cubic magnetite-ulvöspinel series, lying on the binary Fe_3O_4-Fe_2TiO_4 join, with complete solid solution above ~600°C (Vincent, Wright, Chevallier, and Mathieu, 1957), called β series or β spinels for convenience.

3. The cation-deficient spinels that lie on the Fe_2O_3 and TiO_2 side of the Fe_3O_4-Fe_2TiO_4 join, called γ spinels by analogy with maghemite (γFe_2O_3).

4. The pseudobrookite series, between Fe_2TiO_5 (pseudobrookite proper) and $FeTi_2O_5$, an unnamed end member not known to occur in nature. Complete solid solution in this series is found above 1150°C (Akimoto, Nagata, and Katsura, 1957). The term γ spinel is used only for convenience in reference, and does not imply existence of the hypothetical $\gamma FeTiO_3$ end member that has been postulated for these spinels (e.g., Nicholls, 1955). Inasmuch as these spinels apparently form by oxidation of β spinels, the concept of cation deficiency is more useful and more valid than that of $\gamma FeTiO_3$ solid solution.

Magnetite-ilmenite relations. Magnetite grains containing lamellae of ilmenite

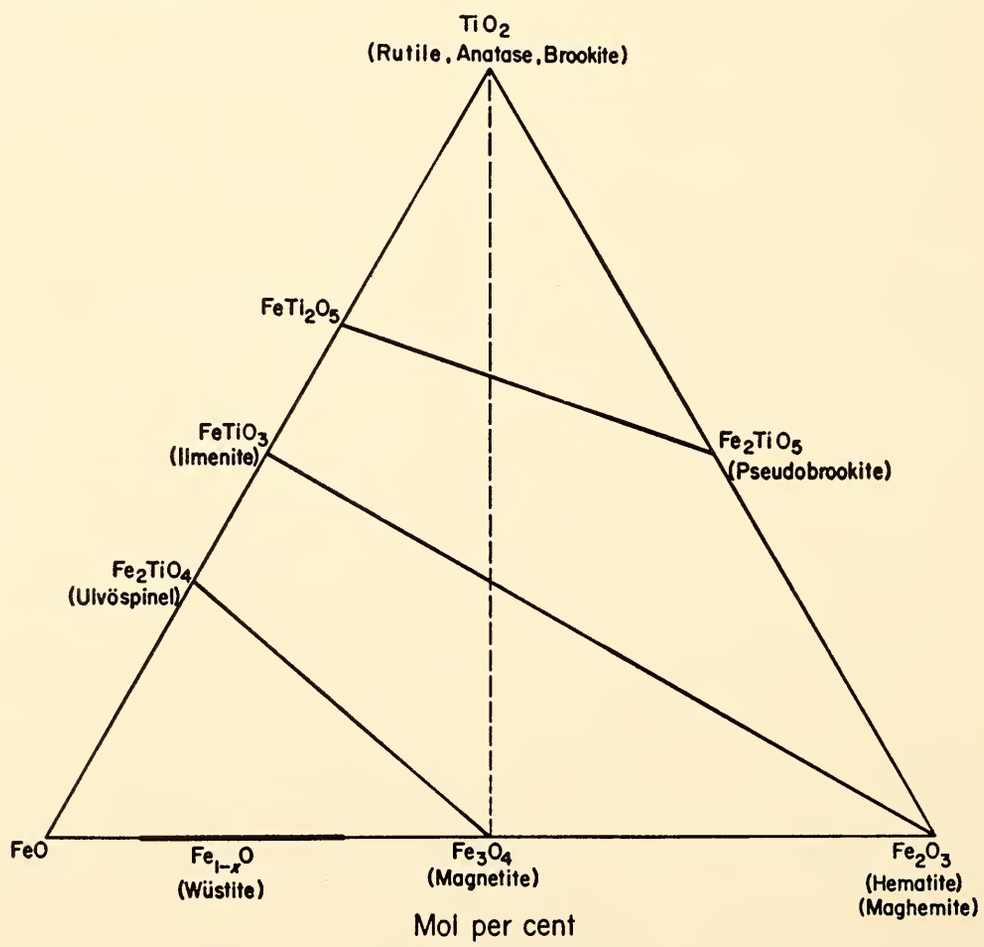

Fig. 32. Phases in the system FeO-Fe_2O_3-TiO_2. Temperatures of complete solid solution (heavy lines) in the β series, α series, and pseudobrookite series are approximately 600°, 950°, and 1150°C, respectively. The join magnetite-rutile (dashed line) is found at low temperatures.

oriented in the (111) planes of the host are found in a variety of rocks and ores. The ilmenite lamellae have been widely interpreted as due to exsolution from original ilmenite-magnetite solid solutions. From a study of natural specimens Ramdohr (1955) concluded that the parental phase could contain up to 50 mole per cent ilmenite. Several workers have noted, however, that ilmenite-magnetite intergrowths cannot be homogenized by heating to 1000° to 1200°C if bulk composition is maintained; it has therefore been suggested that the original phase contained ulvöspinel rather than ilmenite in solid solution and that ilmenite is formed by oxidation of ulvöspinel. Ramdohr (1953) pointed out that ulvöspinel exsolves in the (100) planes of magnetite and that ilmenite formed by oxidation of such ulvöspinel lamellae has oblique extinction—a mode of occurrence very different from that of most ilmenite-magnetite intergrowths. Several workers have also suggested that primary magnetite-ulvöspinel solid solutions may be oxidized to γ spinels, which then break down to ilmenite-magnetite intergrowths. The oxidation hypothesis has gained added support from recent experimental data (Webster and Bright, 1961; R. Taylor, unpublished Ph.D. thesis at Pennsylvania State University) and from theoretical considerations (Verhoogen, 1962), which indicate that the stable solubility of ilmenite in magnetite even at 1200° to 1300°C is much too small to explain observed amounts of ilmenite in natural ilmenite-magnetite intergrowths.[2] Results of the current investigation support a variant of the oxidation hypothesis and show that natural textures as well as assemblages can be explained by that hypothesis.

Reagents used were Fisher "certified" Fe_2O_3 and TiO_2, and United Mineral and Chemical Corporation 99.999 per cent Fe sponge. Before weighing, the Fe sponge was analyzed for O_2, and appropriate corrections were made in the proportions of Fe and Fe_2O_3. Material was mixed by grinding under acetone or toluene to inhibit further oxidation. Single-phase starting materials for hydrothermal experiments were synthesized from the mixes by heating at 1000°C in evacuated silica glass tubes or at 1200°C in Alundum crucibles in a controlled atmosphere of $N_2 + H_2$. Homogeneity was checked by optical and X-ray examination.

Stability relations were determined by the hydrothermal buffer technique of Eugster, using the buffers wüstite-magnetite (WM), fayalite-magnetite-quartz (FMQ), nickel-nickel oxide (NNO), and magnetite-hematite (MH) to control oxygen fugacity (f_{O_2}). Oxygen fugacities of these buffers as functions of temperature and total pressure can be derived from the expressions given in table 3. Alloying of Fe from the charge with Pt containers at low oxygen fugacities was a problem in early hydrothermal buffer experiments. Wrapping the charge in Ag foil or using Ag instead of Pt containers prevents Fe loss but introduces additional disadvantages. A. Muan (unpublished data presented in a Petrologists' Club lecture) has shown that Ag-rich Ag-Pd alloys have melting points higher than that of pure Ag, but are still almost immiscible with Fe. The high permeability of Ag-Pd alloys to hydrogen makes them ideal as charge containers for buffered hydrothermal experiments. Most of the data here presented were obtained from runs made in $Ag_{70}Pd_{30}$ (weight per cent) containers. Temperatures were measured to ± 2°C and regulated to ± 2°C. At and below 800°C most runs were made at 2 kb total pressure; at

[2] The extensive solubility of ilmenite in magnetite reported by Schmahl, Frisch, and Hartgartner (1960) at 1000°C is questionable because their experimental method could not distinguish between mixtures and solid solutions of ilmenite and magnetite, and no X-ray or optical observations were reported. The limits of solid solution shown in their phase diagram are based on Ramdohr's estimates from natural occurrences and hence cannot be used as independent evidence on the extent of natural solid solutions.

TABLE 3. Calculations of Oxygen Fugacities of Buffers as Functions of Temperature and Total Pressure

T in °K; f_{O_2} and P_{tot} in bars. From Eugster and Wones, 1962.

$$\text{Log } f_{O_2} = -\frac{A}{T} + B + C\frac{(P_{tot} - 1).}{T}$$

Buffer	A	B	C
Wüstite-magnetite (WM)	32,730	13.12	0.083
Fayalite-magnetite-quartz (FMQ)	27,619	10.55	0.092
Nickel-nickel oxide (NNO)	24,709	8.94	0.046
Magnetite-hematite (MH)	24,912	14.41	0.019

higher temperatures, lower pressures (usually 1 kb) were used to protect the pressure vessels. All compositions are given in mole per cent unless otherwise noted.

A series of standard β spinels ranging from Mt_{100} to $Mt_{20}Usp_{80}$ were synthesized hydrothermally at 800°C using the WM buffer, each composition being made in duplicate or triplicate. Ten measurements of the (333, 511) peak, using internal standards of CaF_2 or Si with Fe radiation on a Phillips powder X-ray diffractometer, were averaged for each sample. Pure ulvöspinel, which is not stable at the lowest f_{O_2} attainable with available buffers, was synthesized at 1200°C in a controlled atmosphere of N_2 and H_2. Because the (311) peaks of CaF_2 and Si interfere with the (333, 511) peak of ulvöspinel, quartz was used as an internal standard. Comparison of results obtained with samples of intermediate compositions using CaF_2, Si, and quartz standards showed no detectable differences in 2θ values. The 2θ (Fe $K\alpha_1$) versus composition data were plotted for use as a determinative curve. Corresponding unit-cell edges are given in figure 33, with the data of Akimoto, Katsura, and Yoshida (1957) for comparison. Repeated measurements of both standards and unknown specimens showed a reproducibility of $\pm 0.01°$ 2θ; this internal consistency permits determination of compositions to at least ± 2 mole per cent despite any systematic errors that might affect the absolute accuracy of the X-ray data. Unit-cell edges are believed to be accurate to ± 0.001 Å.

Data are now available on the compositions of titaniferous magnetite in equilibrium with ilmenite for the buffers NNO, FMQ, and WM. Starting materials were either β spinels alone or β spinels + ilmenite. The following reactions take place during buffered runs:

$$\text{Ulvöspinel-rich } \beta \text{ spinel} + O_2 = \text{Fe-Ti spinel} + ilm_{ss} \quad (1)$$

$$\text{Magnetite-rich } \beta \text{ spinel} + \text{ilm} = \text{Fe-Ti spinel} + ilm_{ss} + O_2 \quad (2)$$

(Ilm_{ss} means ilmenite with Fe_2O_3 in solid solution; for the buffers NNO, FMQ, and WM this Fe_2O_3 content is less than 15 per cent. Fe-Ti spinel is used as a general term to indicate either β or γ spinels.) For each temperature and oxygen fugacity the unit-cell edges of the Fe-Ti spinels formed in reactions 1 and 2 are nearly identical and are always intermediate between those of the initial β spinels, suggesting that the Fe-Ti spinels have approached the composition of the spinel that is in equilibrium with $ilmenite_{ss}$. However, the unit-cell edges alone cannot yield unique compositions if the product spinels are γ phases, which they must be if there is solid solution of ilmenite in the spinel. Akimoto, Katsura, and Yoshida (1957) have determined cell edges of β and γ spinels; their data permit determination of composition

from the cell edge if the Fe/Ti ratio of the spinel is known. The presence of ilmenite in a run, however, prevents identification of the Fe/Ti ratio of the product spinel with the known ratio of the entire charge. Chemical analysis of the spinel is likewise not feasible when ilmenite is present. The amount of ilmenite in solid solution with the Fe-Ti spinels of reactions 1 and 2 must therefore be determined indirectly.

The equilibrium compositions of the products of reactions 1 and 2 are uniquely fixed at constant pressure by the temperature and oxygen fugacity; the relative amounts of the products are determined by the bulk composition (or by the Fe/Ti ratio in f_{O_2} buffered systems) of the starting materials. For each fixed P, T, and f_{O_2} there must exist a bulk composition for which the Fe-Ti spinel formed will coexist with an infinitesimal amount of ilmenite$_{ss}$. That critical composition can be estimated for reaction 1 by making a series of runs in which the Fe_2TiO_4 content of the starting β spinel is successively lowered until no ilmenite is found in the products. The composition of the spinel thus formed can be determined from the unit-cell edge and by chemical analysis. No differences in unit-cell edge were detected before and after runs at several critical compositions, indicating within the accuracy of the X-ray data that the product spinel is essentially a binary β spinel of the starting composition.

One composition indicated by the

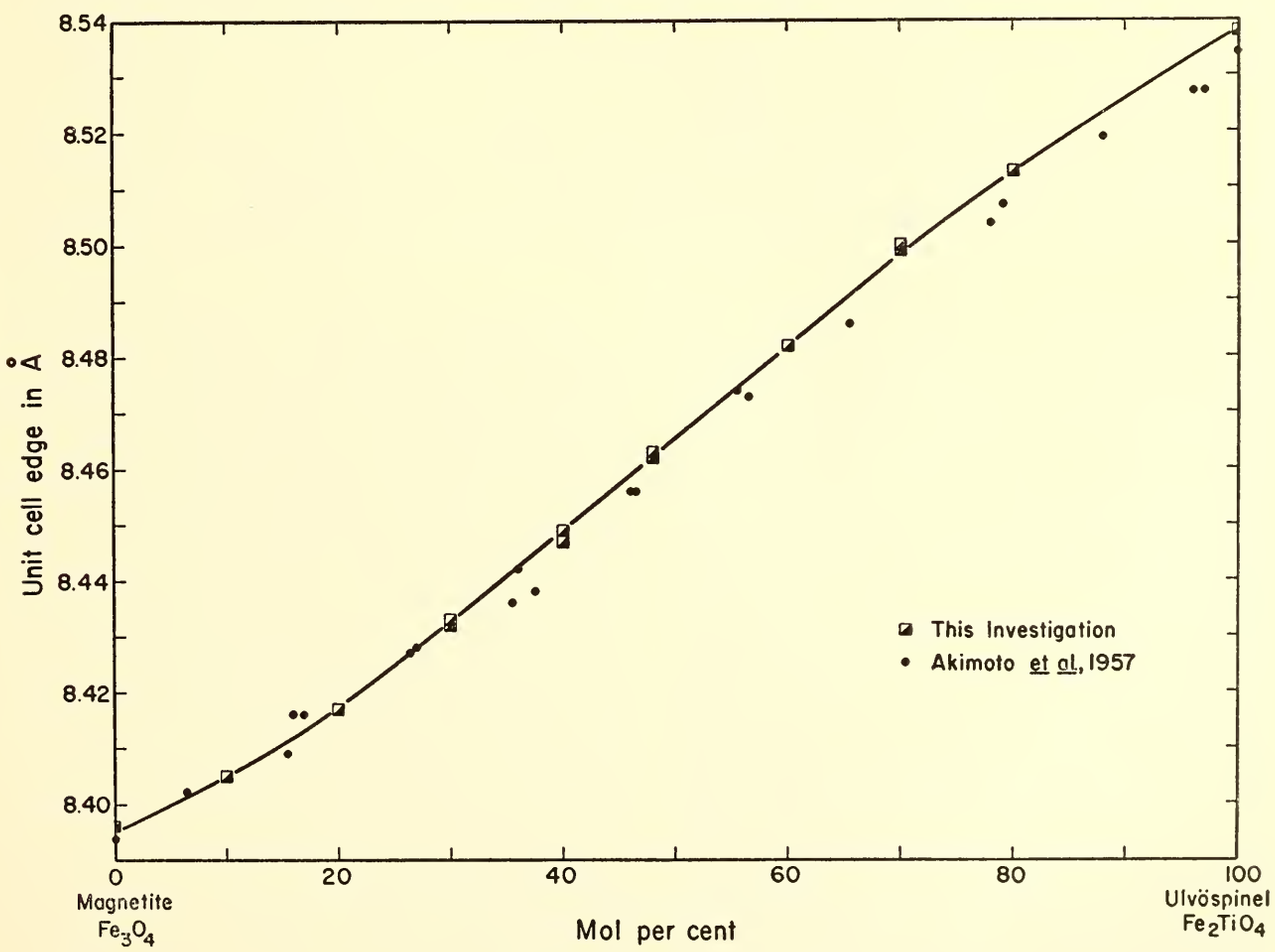

Fig. 33. Plot of composition versus unit-cell edge for magnetite-ulvöspinel solid solutions (β spinels). Vertical extent of data points shows uncertainty in cell-edge determinations; horizontal extent not significant. Compositions Mt_{100} to $Mt_{20}Usp_{80}$ made hydrothermally at 800°C; pure ulvöspinel made at 1200°C in $N_2 + H_2$ mixture. Data of Akimoto, Katsura, and Yoshida (1957) from samples made in evacuated silica glass tubes are shown for comparison.

X-ray data was checked as follows. At 960°C and the f_{O_2} of the NNO buffer, the spinel in equilibrium with ilmenite$_{ss}$ is $Mt_{52}Usp_{48}$ by X-ray determination. Runs were made on spinels of that composition at 960°C using the NNO, FMQ, and WM buffers, and at 800°C using the WM buffer. Part of each sample was removed for X-ray and optical examination, and the remainder, about 100 mg, was dried for 16 hours at 200°C in N_2 for chemical analysis. No ilmenite was detected optically or by X ray. Ferrous iron was determined by the modified Pratt method (table 4). The determinations of FeO are

Fe-Ti spinels in equilibrium with ilmenite can be determined from the unit-cell edges.

The available data indicate negligible solid solution of ilmenite in magnetite. That ilmenite-magnetite *intergrowths* (as well as ilmenite-magnetite *assemblages*) can be formed by reaction 1 is shown in figure 34, plate 1. Pure ulvöspinel was held at 1000°C at the f_{O_2} of the Stellite bomb (roughly equal to that of the NNO buffer) for 3 hours. The resulting texture—lamellae of ilmenite$_{ss}$ in the (111) planes of a $Mt_{50}Usp_{50}$ solid solution—closely resembles natural textures.

TABLE 4. FeO Contents and Unit-Cell Edges of Some Fe-Ti Spinels in Equilibrium with Ilmenite$_{ss}$

Run No.	T, °C	Buffer	Unit-Cell Edge, Å	Weight Per Cent FeO	Mole Per Cent FeO
L333	800	WM	8.462	46.2	59.3
L334	960	FMQ	8.462	45.6	58.8
L335	960	WM	8.462	45.0	58.3
L336	960	NNO	8.462	45.3	58.5
Theoretical FeO for $Mt_{52}Usp_{48}$				46.96	59.68

minimum values, as any alloying of Fe with the charge container, incomplete drying of the sample before weighing, or oxidation of the solution before titration would tend to reduce the value for FeO. Evidently the spinel in equilibrium with ilmenite$_{ss}$ at 960°C and the f_{O_2} of the NNO buffer deviates from a binary β spinel by approximately 1 mole per cent FeO, a deviation that is not detected by the X-ray method used. The maximum deviation of the $Mt_{52}Usp_{48}$ composition from the binary join is less than 0.5 mole per cent FeO at 800°C with the WM buffer and should be further lowered with decreasing temperature. The apparently stoichiometric binary compositions indicated by X-ray data for other spinels in equilibrium with ilmenite will be checked by similar analysis. It seems justified to conclude that any deviation is small and that the approximate composition of

Reactions 1 and 2, carried out at a series of temperatures for each of several buffers, permit us to bracket the compositions of β spinels that are in equilibrium with α phases. Data obtained using the buffers NNO, FMQ, and WM are presented in figure 35; in all runs with these buffers the coexisting α phases are ilmenite rich. Exact compositions of the α phases are now being determined. Runs are also being made using the buffers MH and MnO-Mn_3O_4. Figure 35 is simply a graphical representation of the compositions of β spinels that coexist with ilmenite$_{ss}$ for given temperatures and buffers; it is not a phase diagram, inasmuch as the compositions of the ilmenites are not represented. For a given temperature and buffer f_{O_2}, a spinel of a composition to the right of the appropriate curve will break down by reaction 1 to yield two phases: ilmenite$_{ss}$ plus a

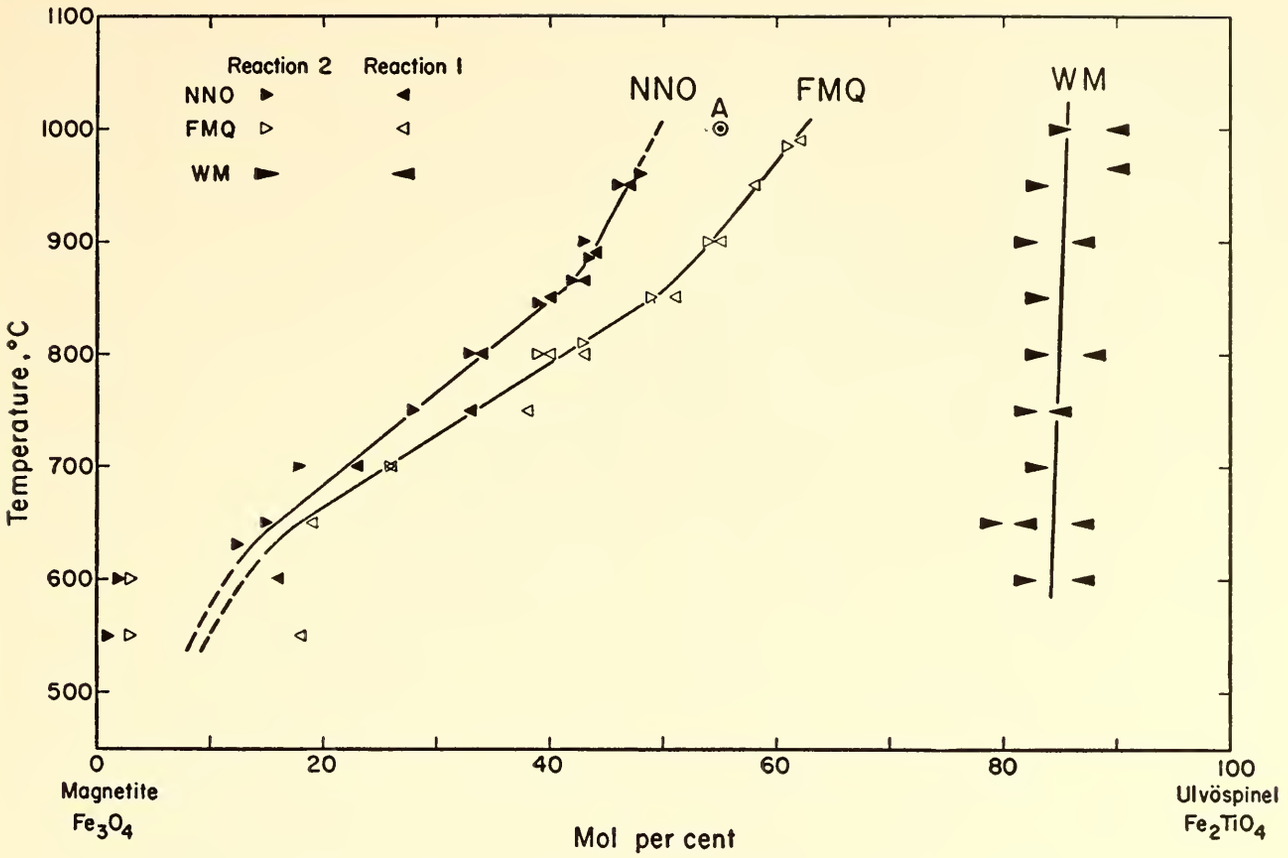

Fig. 35. Composition of β spinel in equilibrium with ilmenite$_{ss}$ as a function of temperature and the oxygen fugacities of three buffers. NNO, nickel-nickel oxide buffer; FMQ, fayalite-magnetite-quartz buffer; WM, wüstite-magnetite buffer. Reaction 1, ulvöspinel-rich β spinel + O_2 = Fe-Ti spinel + ilmenite$_{ss}$. Reaction 2, magnetite-rich β spinel + ilmenite = Fe-Ti spinel + ilmenite$_{ss}$ + O_2. Point A is discussed in the text.

spinel whose composition is indicated by the curve. Any spinel to the left of the curve is stable by itself but in the presence of ilmenite will form by reaction 2 the spinel indicated by the curve. For example, at 1000°C, the spinel Usp_{55} (point A in fig. 35) is unstable at the f_{O_2} of the NNO buffer and will oxidize to Usp_{51} + ilmenite$_{ss}$. The same spinel (A) with the FMQ buffer is stable down to 910°C; at successively lower temperatures spinel A is unstable and breaks down to spinels successively richer in magnetite, plus ilmenite$_{ss}$. At the lower oxygen fugacities of the WM buffer, spinel A would remain stable upon cooling until the magnetite-ulvöspinel solvus is reached (~500° to 550°C according to the data of Vincent, Wright, Chevallier, and Mathieu, 1957).

It is well to recall that the curves in figure 35 are drawn not at constant oxygen fugacity but at the fugacity of each buffer, which varies with temperature (see, for example, Eugster and Wones, 1962). The data are nevertheless sufficient to establish the principle that the composition of β spinel in equilibrium with ilmenite$_{ss}$ is strongly dependent on oxygen fugacity as well as on temperature.

Application of experimental data to natural minerals. Available experimental data in the ternary system FeO-Fe_2O_3-TiO_2 indicate that the stable solubility of ilmenite (= cation deficiency) in Fe-Ti spinels is small at and below magmatic temperatures, although metastable cation-deficient Fe-Ti spinels are easily made by oxidation of β spinels in air at 400° to 550°C. It thus seems likely that natural cation-deficient Fe-Ti spinels ("titano-

maghemites") form metastably by oxidation at moderate temperatures, say below 600°C. (However, many natural γ spinels contain minor amounts of Mg, Mn, Al, V, and Cr, and the experimental data from the pure synthetic system cannot rule out the possibility that the presence of these elements might stabilize the cation-deficient structure.) As cation-deficient spinels are less dense than the equivalent assemblages β spinel + α phase, high pressure should inhibit their formation in plutonic rocks.

The experimental data presented here do not disprove the theory that ilmenite-magnetite intergrowths form by exsolution from a primary ilmenite-magnetite solid solution, but they strongly support the alternative hypothesis that such intergrowths result from the oxidation of magnetite-ulvöspinel solid solutions. An intermediate γ phase may form in some volcanic and hypabyssal rocks, but direct oxidation to magnetite-rich β spinel + ilmenite_{ss} seems likely in plutonic rocks. In rocks where oxygen fugacity remains sufficiently low upon cooling, little or no ilmenite will form and ulvöspinel-magnetite intergrowths may result.

Relations between ilmenite, hematite, magnetite, and rutile. Attempts to determine the hematite-ilmenite solvus hydrothermally have been unsuccessful because no buffer is available with an oxygen fugacity at which hematite_{ss} and ilmenite_{ss} can coexist. Initial compositions of $\text{Hem}_{50}\text{Ilm}_{50}$ are oxidized to hematite_{ss} + $\text{pseudobrookite}_{ss}$ (or hematite_{ss} + rutile) by the MH buffer, and are reduced to magnetite_{ss} + ilmenite_{ss} by the NNO buffer. If both hematite_{ss} and ilmenite_{ss} can coexist at equilibrium for a given temperature, the f_{O_2} ranges at which each is stable must overlap; the zone of overlap must lie between the oxygen fugacities of the MH and NNO buffers. As the compositions of the coexisting hematite_{ss} and ilmenite_{ss} move farther apart upon cooling, the corresponding f_{O_2} range of mutual stability probably decreases. It is possible that at low temperatures (say below 200° to 400°C) there is no f_{O_2} at which both hematite_{ss} and ilmenite_{ss} can coexist at equilibrium. Under this hypothesis hematite-ilmenite intergrowths exsolved at low temperatures are metastable. They may form because less energy is required for migration of Fe and Ti in the inherited oxygen framework of the original phase than for complete reorganization into new phases such as magnetite plus rutile. In this regard it is significant that many low-grade metamorphic rocks contain the assemblage magnetite + rutile, which is chemically equivalent to hematite + ilmenite. The reaction

$$Fe_3O_4 + TiO_2 = Fe_2O_3 + FeTiO_3 \quad (3)$$

has a small positive free energy, $\Delta G(3) = +1$ to $+2$ kcal, over the temperature range 100° to 1200°C, according to the best available data. $\Delta G(3)$ is probably smaller than the uncertainty involved in its derivation; if, however, its sign is correct it accounts for the assemblage magnetite + rutile in low-grade metamorphic rocks. At higher temperatures the free energy of mixing of Fe_2O_3 becomes sufficient to favor ilmenite-hematite solid solutions over the magnetite + rutile assemblage.

Stability Relations of Dravite, a Tourmaline

C. R. Robbins[3] *and H. S. Yoder, Jr.*

The most abundant and geochemically most important of the boron minerals are the tourmalines. They are found in a variety of igneous, metamorphic, and sedimentary rocks of all ages. Their authigenic formation at low temperatures in some limestones and sandstones is of particular interest.

Chemically, tourmalines are complex borosilicates of variable composition, the variation resulting from the large number of substitutions permitted by the structure. Preliminary calculations suggest

[3] U. S. National Bureau of Standards.

that tourmalines may be described as isomorphous mixtures of several end members.

A number of the tourmalines have been synthesized from rather complex systems, but their stability relations were not determined. The objective of the present study is the determination of the pressure-temperature stability range of a tourmaline of specific composition, the iron-free end member dravite, $NaMg_3Al_6B_3Si_6O_{27}(OH)_4$. This composition was chosen because the crystal structure studies of Hamburger and Buerger (1948) had established the ideal formula. It was also of interest to relate this composition to the petrologically important system MgO-Al_2O_3-SiO_2-H_2O.

For this work a glass of the requisite anhydrous composition was carefully prepared in several steps to avoid loss of Na_2O and B_2O_3. In addition, a natural tourmaline from Dobruva, Carinthia, Austria (U. S. N. M. no. 103.791), was selected for comparative studies. A chemical analysis of the material by H. B. Wiik showed it to be exceptionally close to the ideal dravite composition. Indices of refraction and unit-cell dimensions of synthetic dravite and the Dobruva tourmaline are given in table 5.

TABLE 5. Comparison of Indices of Refraction and Unit-Cell Dimensions of Synthetic Dravite and Dobruva, Austria, Tourmaline

	a, Å	c, Å	ω	ϵ
Synthetic dravite	15.93	7.18	1.632	1.610
Dobruva tourmaline	15.931	7.197	1.634	1.613

Preliminary results of this study at various temperatures, and water pressures up to 5000 bars, are summarized in figure 36. The part of the curve below 500 bars was calculated from the integrated Clausius-Clapeyron equation. It is evident that dravite is stable over a wide range of temperatures and pressures.

At temperatures above 865°C and pressures up to 2000 bars the main decomposition products are cordierite, liquid, gas, and a crystalline phase that has not yet been identified. Sporadic occurrences of trace amounts of spinel, mullite (?), and, once, of sapphirine have been observed microscopically in the dissociation products of both the natural tourmaline and the glass. These dissociation products are fine grained and frequently occur as inclusions in the cordierite or glass. They may well be the result of leaching.

Above 895°C and 5000 bars both glass and natural tourmaline form the assemblage kornerupine + sapphirine + liquid + gas. Previous synthesis of kornerupine is unknown to the writers. The phase is well crystallized, and its X-ray pattern agrees well with that of a natural mineral from Kazebanza, Quebec (U. S. N. M. no. 106.774).

At 925°C and 5000 bars kornerupine is no longer stable, and the phases coexisting in equilibrium are sapphirine + liquid + gas. Inclusions observed in the sapphirine appear to be minute spinel octahedra and probably result from leaching.

Although these results are only preliminary, it would appear that they will have application to natural occurrences, since the associations tourmaline + cordierite, tourmaline + kornerupine, and cordierite + kornerupine + sapphirine (Ussing, 1889) are known.

Mantle Minerals

F. R. Boyd, Jr., and J. L. England

The discovery that pressures as low as 5 kb cause enstatite to melt congruently (Boyd and England, *Year Book 60*) raises

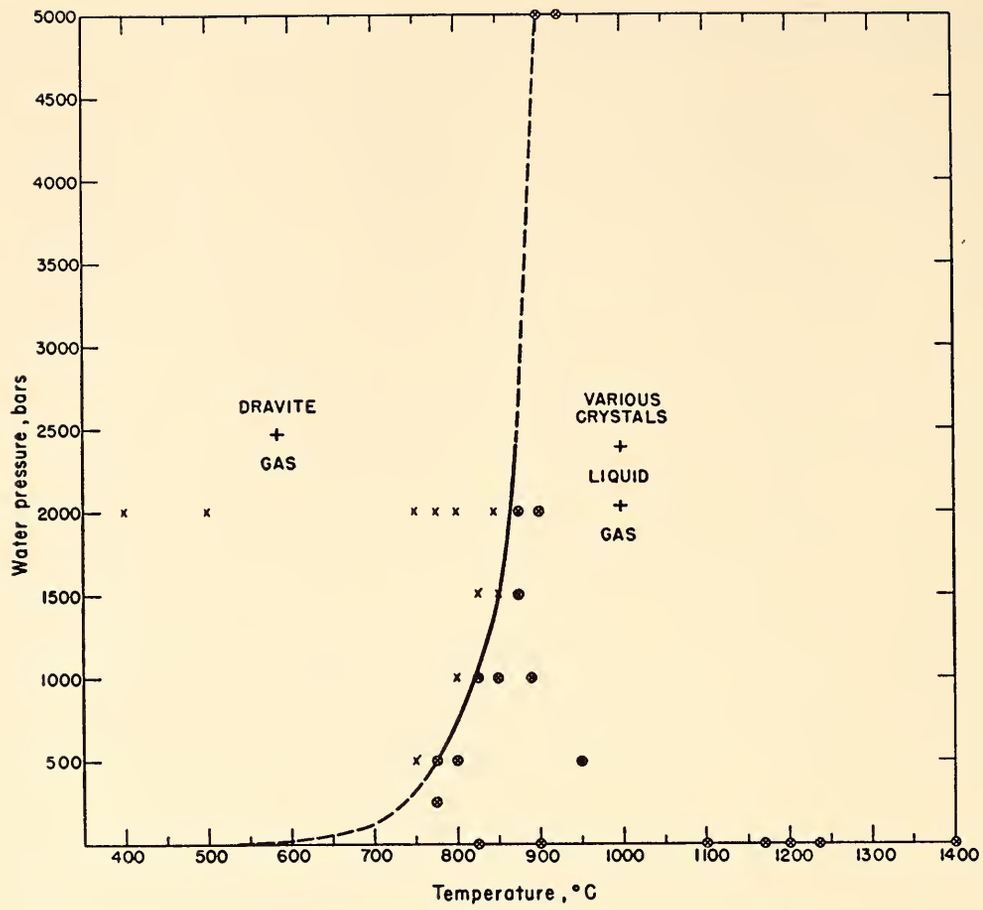

Fig. 36. Preliminary *P-T* diagram of the system dravite-water.

a problem in accounting for the formation of basaltic magma that is oversaturated in silica. Basalts in the Pacific Ocean basin are sometimes considerably oversaturated in silica. For example, the degree of oversaturation of the primitive shield basalts of Hawaii ranges up to about 6 weight per cent SiO_2 (Powers, 1955, p. 81). Nevertheless, there is considerable evidence to indicate that the mantle rocks from which these basalts were derived contain olivine. The principal minerals in rocks in the upper mantle are probably enstatite, diopsidic pyroxene, olivine, and pyrope-rich garnet.

It is impossible to derive a liquid oversaturated in silica by partial fusion of a mixture of pyroxene, olivine, and garnet in the absence of an incongruent melting reaction. For many years it was thought that the incongruent melting of enstatite found by Bowen and Andersen (1914) at atmospheric pressure provided a mechanism for generating oversaturated liquids from olivine-bearing parent rocks at depth. High-pressure data, however, indicate that this reaction must be restricted to relatively shallow depths in the crust. Estimates of the depth of formation of basaltic lava in the ocean basins range from 50 to 100 km where the pressure is in the range 15 to 30 kb. Since experimental data have shown that enstatite melts congruently in this range, an alternative explanation for the composition of these oversaturated basalts must be sought.

A possible alternative is that an incongruent melting reaction involving garnet is effective at depth in the mantle. Boyd and England suggested in *Year Book 60* that pure pyrope must melt incongruently and that this reaction might also be present in more complex natural melts. The melting relations of pyrope have been restudied with im-

proved techniques, and it has been found that pyrope does melt incongruently over a wide *P-T* range. As is described hereafter there are at least three incongruent melting reactions for pyrope in the pressure range 25 to 36 kb. Above 36 kb pyrope melts congruently.

The principal incongruent melting reaction of pyrope is to spinel + liquid. Since spinel contains no silica, the liquid that forms in the incongruent interval contains more silica than pyrope composition does. In the melting of pure pyrope the composition of the liquid lies in the three-phase field pyrope + Al-enstatite + quartz. If this incongruent melting reaction were present in more complex natural systems, oversaturated basalts could theoretically be generated by fractionation of liquid from a partly melted garnet peridotite. By analogy with the melting relations for pure pyrope it would be expected that the incongruent melting would be present over a restricted depth range. Pyrope-rich garnet would not be stable in the melting interval at lower pressures and would melt congruently at higher pressures. For pure pyrope the depth range would be from about 75 to 105 km, but it would probably be shallower in a natural system.

The phase relations determined in the pyrope study are a further demonstration of the importance of high pressure in modifying crystal-liquid equilibria in silicate systems. In the pressure range 20 to 40 kb a liquid of pyrope composition can be crystallized by at least five different paths, depending on the pressure. It is not certain that the incongruent melting of pyrope is a significant feature of magma differentiation at depth in the mantle, but there is little question that pressure will prove to have a major influence on such differentiation.

Effect of Pressure on the Melting of Pyrope

Phase relations for pyrope composition in the pressure range 15 to 50 kb are shown in figure 37. The subsolidus boundary (*A* in fig. 37) is essentially the same as that given in our preliminary diagram in *Year Book 58* except that no friction correction has been made for the present results. Experience has shown that the friction in single-stage runs at high temperature is less than was initially estimated. The pressure on the run is believed to be within ±5 per cent of the load pressure. The results shown for temperatures above 1500°C are new and were obtained by techniques developed in a study of the melting curves of albite and diopside. Details of these techniques have been published recently (Boyd and England, 1962).

Liquid of pyrope composition cannot be quenched to a glass over most of the investigated *P-T* range. Recognition of the various melting reactions, therefore, depends on textural differences in the runs. Fortunately, pyrope itself will not form in the quench. Runs quenched from the fields above curves *B*, *C*, and *D* crystallize in the quench to assemblages consisting wholly or largely of aluminous enstatite.

At temperatures below curve *E* and at pressures below curve *A* pyrope composition crystallizes to a fine-grained mixture of aluminous enstatite, sapphirine, and sillimanite. Enstatite and sapphirine can be distinguished in X-ray diffractometer patterns of such runs, and they can be recognized under the microscope. Aluminous enstatite forms about 80 per cent of the products. The presence of sillimanite is known from the study of other compositions in the system MgO-Al_2O_3-SiO_2 in this *P-T* range, but the amount that forms in a run on pyrope composition is too small to detect by optical or X-ray methods.

At 1615°C and 24.5 kb there is a pronounced break in the boundary of the pyrope stability field. This break is believed to be due to the intersection of a melting curve in the breakdown products field with the pyrope subsolidus boundary. Most likely, though not certainly, the melting curve (*E* in fig. 37) is the solidus

curve for the breakdown products. It is known that aluminous enstatite is a stable phase in the *P-T* field between curves *E* and *F*, but it is not known whether *E* marks the disappearance of sapphirine or sillimanite. Runs quenched from pressures and temperatures immediately above or below curve *E* have essentially identical X-ray patterns and are indistinguishable under the microscope.

Curve *F* in figure 37 is the liquidus curve for aluminous enstatite. In runs quenched from below curve *F* the enstatite forms a fine-grained mosaic of crystals. In runs quenched from above curve *F* the enstatite crystallizes as coarse blades with undulate extinction

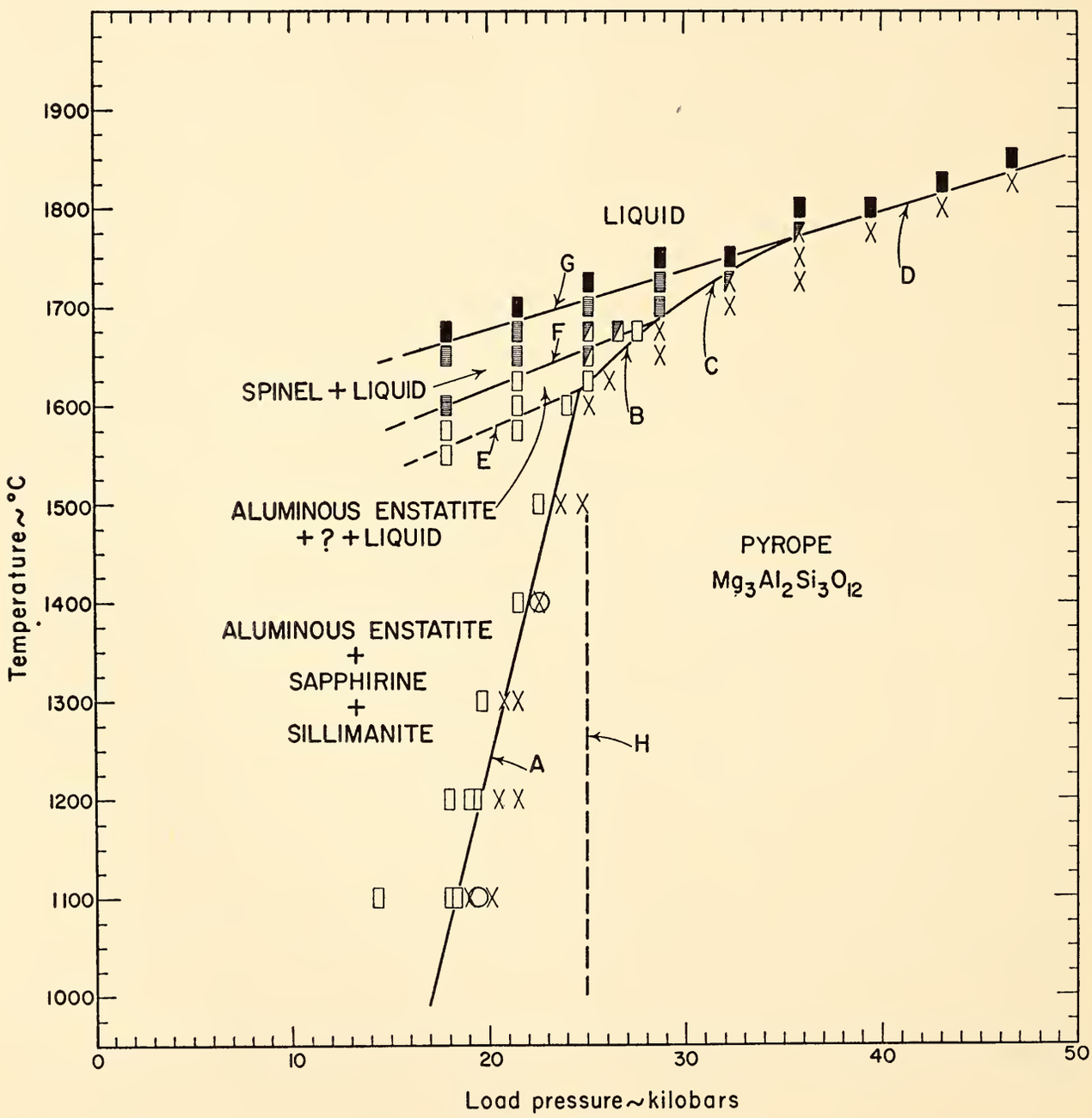

Fig. 37. The stability field of pyrope garnet. Synthetic pyrope was used as starting material for all runs shown except for the two runs indicated by open circles on curve *A*. In these two runs pyrope formed from seeded, crystalline breakdown products. The dashed curve, *H*, is a nucleation boundary; pyrope forms readily from glass or crystalline starting materials at pressures higher than curve *H*, but it will not nucleate in the *P-T* field between curves *A* and *H*. For a further discussion of the nucleation problem, see Boyd and England, *Year Book 58*.

under crossed nicols. This texture is characteristic of enstatite crystals that have formed in the quench in high-pressure runs (Boyd and England, *Year Book 60*).

In the *P-T* field bounded by curves *F*, *C*, and *G* the quench crystals of enstatite contain scattered, subhedral grains of an isotropic phase with a refractive index appreciably higher than that of the enstatite in which they are poikilitically enclosed. These isotropic grains have rounded to rectangular shapes when seen under the microscope and are 1 to 5 microns in diameter. The texture of the runs quenched from this field is strikingly similar to that of the granules of primary forsterite embedded in quench enstatite found in runs on $MgSiO_3$ composition cooled from a temperature within the incongruent melting interval at atmospheric pressure. The isotropic granules decrease in abundance as the temperature is raised in the interval between curves *F* and *G*. Runs quenched from above curve *G* contain only glass and/or quench crystals of enstatite metastably rich in Al_2O_3.

The quantity of the isotropic primary phase in the field bounded by curves *F*, *C*, and *G* is insufficient to show on an X-ray diffractometer pattern. Optical properties obtained indicate that it must be either spinel or sapphirine. The refractive indices of spinel and sapphirine are similar. Sapphirine has a low birefringence, but it appears isotropic in grains only a few microns in diameter. A test was devised, however, that showed the primary phase to be spinel.

At constant temperature and pressure, changing the proportions of phases in equilibrium will not change the kinds of phases present or their compositions. Hence, if the primary isotropic phase was spinel, it would be possible to add spinel to the mixture of spinel and liquid on pyrope composition without changing the phase relations. If the primary phase was spinel and sapphirine was added, the sapphirine would not be stable and should react to form some other phase.

A mixture of 20 per cent crystalline sapphirine + 80 per cent pyrope glass and a mixture of 24 per cent crystalline spinel + 76 per cent crystalline pyrope were run at temperatures midway between curves *F* and *G* at the pressures 21.5 kb and 28.7 kb. The products of these runs looked identical with those of runs made in this *P-T* field on pyrope composition except that the concentration of the primary, isotropic phase poikilitically enclosed in enstatite quench crystals was greatly increased. X-ray patterns of these runs showed that the products obtained with both mixes were spinel + enstatite. Since sapphirine was converted to spinel in these experiments, the primary phase in the field bounded by curves *F*, *C*, and *G* is proved to be spinel.

The melting relations shown in figure 37 only partly define the melting of pyrope composition in the pressure range 15 to 29 kb. There must be at least one more curve than is shown in the *P-T* range below curve *F*. Quenching difficulties and the small amounts of phases other than aluminous enstatite that are present on pyrope composition prevented the identification and location of this curve. Study of the melting of a variety of compositions in MgO-Al_2O_3-SiO_2 in this *P-T* range would undoubtedly clarify the picture, but the tendency of these compositions to crystallize in the quench remains a formidable problem.

The melting curves of the breakdown products (*E*, *F*, and *G*) intersect the pyrope stability field and give it a faceted boundary. The principal incongruent melting reaction is to spinel + liquid, but at least two other reactions in which pyrope melts to Al-enstatite + liquid + other crystalline phases must be present in the pressure range 25 to 29 kb. Above 36 kb the melting is congruent. The maximum incongruent melting interval at constant pressure is about 90° at 25 kb and diminishes as the pressure is raised until the melting becomes congruent. The pressure interval over which the

melting is incongruent is 11 kb, corresponding to a depth interval in the upper mantle of about 30 km.

The average slope of the pyrope solidus curves (*B* and *C*) in the incongruent melting interval between 25 and 36 kb is about 16.5°/kb. Above 36 kb, where the melting is congruent, the slope decreases to 5.5°/kb. The only other silicate melting curve thus far determined in the pressure range above 30 kb is diopside. The slope of the diopside curve in the range 35 to 50 kb is 6.9°/kb. These slopes are substantially less than was earlier estimated for most silicates on the basis of data obtained in a lower pressure range.

STATISTICAL PETROGRAPHY

Sanidine Phenocrysts in Some Peralkaline Volcanic Rocks

F. Chayes and E. G. Zies with X-ray data by Y. Suzuki

The petrologist often uses bulk chemical analysis as in some sense a substitute for modal analysis, and recent improvements in modal analysis have prompted a revival of Rosiwal's countersuggestion that chemical composition be inferred from modes. There are circumstances in which the first procedure is unavoidable, and there are also circumstances in which the second seems very convenient. We hope the work reported here, part of a long-range and rather general study of rhyolites and trachytes, persuades the reader that much may also be gained by using analytical chemistry and petrography as supplements to rather than substitutes for each other.

In the current report year we have completed examination of four peralkaline specimens and the feldspar concentrates prepared from them. Siliceous lavas of this type provide an excellent—perhaps the best—opportunity to study the relation between crystal composition and bulk composition in a natural "system" closely resembling the experimentalists' version of "petrogeny's residua." The specimens include a trachyte from Paris de Besa, Sardinia, a porphyritic pantellerite from Pantelleria, and two comendites from the type localities Le Commende and Le Fontane, Isola San Pietro, Sardinia. The pantellerite, collected by H. S. Washington, was kindly released to us by the U. S. National Museum. The other three specimens were collected by Chayes, in company with Professor S. Vardbasso and Dr. A. Atzeni, of the University of Cagliari.

The Paris de Besa trachyte flow, situated about 5 km west of the town of Ales and described briefly by Atzeni (1959), is exposed by a small window through the post-Miocene basalts on the southeastern flank of Monte Arci. It is a fine-grained blue-gray rock studded with numerous blocky phenocrysts of glass-clear sanidine. As Atzeni remarks, these sometimes contain cores of oligoclase. The transition from oligoclase core to sanidine mantle may be either blurred and gradual or sharp and abrupt. In the former case the crystals usually show highly undulant extinction, and the "core" is likely to have a jagged outline marked by many reentrants; in the latter there is no suggestion of strain or replacement, and the sharply euhedral core usually shows polysynthetic twinning. There are also occasional phenocrysts of acmitic diopside, sublenticular clots of tridymite, and irregular inclusions of other, possibly cognate, volcanic rocks. As in most Sardinian volcanics so far collected in this project, the feldspar phenocrysts show little indication of alteration, although joint surfaces throughout the rock are usually stained yellowish brown and in thin section similar staining sometimes occurs in phenocrysts. Carlsbad twins are common,

but in our specimens no other variety of twinning has been observed in the sanidine, which is also free of micro- and cryptoperthitic intergrowth. Its optic angle is variable but always very small. (Unless our material is entirely atypical, Atzeni's identification of the alkali feldspar of this rock as microcline is erroneous.) The mode of our specimen (no. 25B10) is shown in column 1 of table 6.

trifling amount. Despite much effort we were unable to isolate enough of either cossyrite or acmite for analysis. The mode of Washington's porphyritic pantellerite from Gelkhamar (U. S. N. M. no. PRC 2000) is shown in column 2 of table 6.

The comendites of Isola San Pietro were discovered by Bertolio and described in considerable detail by Johnsen (1912); since Johnsen's work no new information

TABLE 6. Modes of the Analyzed Specimens

Phenocrysts	25B10	PRC 2000	39B2	40B5
Quartz	0.1	0.8	8.8	6.0
Sanidine	19.1	10.7	16.8	9.3
Plagioclase	0.7			
Acmite	0.5	0.2		
Cossyrite		0.8		
Opaque	0.7			
Tridymite and others	0.4			0.3
Groundmass	78.5	87.5	74.4	84.3

25B10, trachyte, Paris de Besa, Sardinia.
PRC 2000, pantellerite, Gelkhamar, Pantelleria.
39B2, comendite, "Commende type," Capo Sandolo, Isola San Pietro, Sardinia.
40B5, comendite, "Fontane type," Le Fontane, Isola San Pietro, Sardinia.

The porphyritic pantellerite is H. S. Washington's specimen from Gelkhamar, Pantelleria, described and analyzed by him (Washington, 1914). A molar excess of alkalies over R_2O_3, signified in the CIPW system by the appearance of Ns (sodium metasilicate) in the norm, has long attracted attention to the Pantellerian lavas. This specimen was one of two recently reanalyzed; for a comparison of the new and old analyses see Zies (1960). The feldspar analysis, made at the same time as the bulk analysis, appears here for the first time. The principal phenocryst of this specimen is sanidine, called soda microcline by Washington. In a careful examination of two thin sections and of many granular products obtained at various stages of the sample preparation no second feldspar was observed. Phenocrysts of bipyramidal quartz are abundant; phenocrysts other than quartz and sanidine, chiefly cossyrite and acmite, are present only in

on these interesting rocks appears to have been published. Our specimen 39B2 is from a roadside exposure about 500 meters east of the lighthouse at Capo Sandalo and about 1 km west southwest of Le Commende, from which the rock type takes its name. It is a completely devitrified blue-gray glass containing numerous phenocrysts of bipyramidal quartz and blocky, water-clear sanidine, the sanidine usually showing a pronounced schiller. The matrix consists of spherulitic masses of extremely fine-grained quartz, feldspar, and an acicular green mineral, which may be either acmite or arfvedsonite. Specimen 40B5 is from the quarry at Le Fontane, about 500 miles southwest of the town of Carloforte. It is a bluish gray glass, closely matching the description of Johnsen's "Fontane type" comendite. Both quartz and feldspar phenocrysts are similar in appearance to those already described from Le Commende, though much less abundant

than in our particular specimens from that locality. Prominent in 40B5 are stringers of a dense black glass distributed through the rock in conspicuously laminar fashion. The groundmass is glass showing little evidence of devitrification. The modes of specimens 39B2 and 40B5 are shown in columns 3 and 4 of table 6.

Bulk analyses and norms. Analyses and CIPW norms of the four specimens, as well as of the material forming one of the dark stringers in 40B5, are shown in table 7. It will be noted that although all the rocks are peralkaline Ns appears in only one of the four norms. This is, of course, the pantellerite from Gelkhamar, Pantelleria; the large amount of normative Ns shown in the original analysis of this specimen was the principal occasion for its reanalysis. Ns is recorded in seven of the ten available peralkaline norms of the Pantellerian lavas (Washington, 1914), the first and still the most extreme example of molar excess of alkalies over ferric oxide and alumina.

Ns is not present in either of our comendite norms, occurs in only one of the norms of the seven comendite analyses given by Johnsen (1912), and is evidently both uncommon and quantitatively insignificant in the type locality of comendite. This contrast between comendite and pantellerite is perhaps particularly striking because in the petrographic literature the names are often used interchangeably. The differences between our two comendites and the Gelkhamar pantellerite are about what would be anticipated from inspection of earlier analyses. In connection with these particular rocks, however, such an inspection raises more problems than it solves. Even if we agree to ignore sampling difficulties, which are unusually acute, and the total amount of information, which is, as usual, rather small, Johnsen

TABLE 7. Bulk Analyses and Norms
(Specimens as identified in table 6; 40B5 inc. is fragment of a stringer in 40B5.)

	Analyses						Norms			
	25B10	PRC 2000	39B2	40B5	40B5 inc.		25B10	PRC 2000	39B2	40B5
SiO_2	67.12	69.81	75.36	75.31	75.01	Q	13.22	28.07	33.67	35.33
Al_2O_3	15.66	8.59	11.44	10.43	10.53	Or	35.91	26.56	28.06	27.50
Fe_2O_3	2.58	2.28	2.30	3.22	3.06	Ab	41.70	19.20	32.41	27.75
FeO	0.69	5.76	0.76	0.80	1.14	An	2.67			
MgO	0.36	0.10	0.13	0.10	0.10	Ac		6.61	1.80	5.31
CaO	1.01	0.42	0.07	0.13	0.10	Ns		5.14		
BaO	0.01	----*	----	tr.	tr.	Di	1.04	1.11	0.15	0.35
Na_2O	4.93	6.46	4.07	3.99	4.05	Hy		10.04		
K_2O	6.08	4.49	4.75	4.65	4.68	En	0.41		0.25	0.09
H_2O^+	0.30	0.14	0.75	0.51	0.44	Il	1.26	0.85	0.30	0.39
H_2O^-	0.06	0.05	0.38	0.38	0.27	Mt	0.44		2.22	2.32
TiO_2	0.66	0.45	0.16	0.21	0.21	Hm	2.27		0.14	
ZrO_2	0.01	0.25	0.10	0.18	0.17	Hl		1.30		
P_2O_5	0.16	0.13	0.02	0.03	0.03	Ap	0.37	0.30	0.05	0.07
SO_3	0.01	0.06	----	tr.	tr.	Z		0.25	0.15	0.27
Cl-H_2O sol.	----	0.03	----	0.05	----	Rest	0.41	0.25	1.15	0.89
Cl-H_2O insol.	0.02	0.76	0.02	0.05	0.08		99.76	99.68	100.35	100.27
MnO	0.05	0.28	0.07	0.09	0.09					
		100.06								
O for Cl		0.18								
Sum	99.71	99.88	100.38	100.13	99.96					

* Sought but not found.

points out that his analyses of comendite differ markedly from the earlier, incomplete analyses of Bertolio, Washington points out that his analyses of pantellerite differ markedly from the earlier, incomplete analyses of Förstner, and there is now reason to suspect that Washington's TiO_2 estimates were systematically high (Zies, 1960, p. 306), an analytical bias that would necessarily generate overestimates both of the amount of Ns and of the frequency of its occurrence in any set of norms. Like so many of the problems of modern descriptive petrography, satisfactory comparison of these two rock types will require a manifold increase in the number of rock analyses with no sacrifice, and preferably with some improvement, in their quality.

Feldspar phenocrysts of the analyzed rocks. In all four specimens the principal phenocryst is sanidine, characterized by very small optic angle, apparent monoclinic symmetry of X-ray powder spectra, absence of multiple twins, and lack of cryptoperthic structure or anorthoclase type grill. The geological occurrence is of course the classic one for sanidine; the blocky, sharply euhedral habit and the glassy, often transparent character of the crystals are equally appropriate. Despite careful search, no other feldspar has been identified in three of the specimens; in the Paris de Besa trachyte, as already noted, sanidine crystals sometimes contain cores of oligoclase. The X-ray spectra of the Paris de Besa and Gelkhamar feldspars show very little submicroscopic unmixing, whereas those from the Capo Sandalo and Le Fontane comendites seem to be almost entirely unmixed, the powder patterns being interpretable as mixtures of nearly pure Or and Ab. This unmixing can be detected only by X ray. It is therefore rather startling to discover that in the very year in which Laue first predicted that crystals ought to diffract X rays Johnsen (1912, p. 6) unhesitatingly attributed the schiller of the comendite sanidine to incipient unmixing, which, carried to completion, would finally transform an "unstable monoclinic" crystal into a "stable triclinic" one. The line between science and prescience is sometimes very hard to draw!

Analyses and norms of the best feldspar concentrates that could be obtained from each specimen by magnetic and heavy-liquid separation are shown in table 8.

TABLE 8. Analyses and Norms of Four Alkali Feldspars
(Specimens as identified in table 6.)

	25B10	PRC 2000	39B2	40B5
SiO_2	65.56	67.82	67.30	67.12
Al_2O_3	19.68	18.06	18.42	18.32
Fe_2O_3	0.40	1.11	0.59	0.83
FeO	0.05	----	0.02	0.04
MgO	----*	----	0.02	----
CaO	1.14	----	0.06	----
BaO	0.03	0.07	----	----
Na_2O	6.08	7.17	6.95	5.89
K_2O	6.78	5.99	6.60	7.92
TiO_2	0.06	0.02	0.01	----
MnO	----	----	0.02	----
H_2O†	0.02	----	0.01	----
Sum	99.80	100.24	100.00	100.12
		Norms		
Q	1.74	3.44	1.63	2.53
Or	40.08	35.41	39.02	46.82
Ab	51.45	59.27	58.01	49.83
An	5.65		0.31	
Cs	0.08	0.19		
Ac		1.25	0.69	
En			0.05	
Mt			0.09	0.12
Il	0.12		0.02	
Hm	0.40	0.69	0.35	0.75
C	0.22			0.05
Rest	0.02		0.01	

* Sought but not found.
† Samples dried at 110°C before analysis.

Results of fragment counts made on three of the analyzed specimens are recorded in table 9; in the feldspar from 40B5 no quartz or plagioclase was found, and the amount of groundmass adhering to sanidine grains was too small to estimate by this technique. In the counts, incidentally, the precision of ratios of

TABLE 9. Fragment Counts of Analyzed Feldspar Concentrates
(Specimens as identified in table 6.)

	25B10	PRC 2000	39B2
Alkali feldspar	92.4	95.4	98.4
Plagioclase	3.3		
Quartz	3.0	3.9	1.0
Others		0.2	0.7
Groundmass	1.3	0.4	
Count length	1347	1446	1827

minerals to each other is about that appropriate to the count length, but, since "groundmass" occurs almost entirely as thin discontinuous margins about sanidine in all four concentrates, estimation of the amount of groundmass from the number of grains in which it is observed requires an "adjustment" or "correction" factor that is not much better than a shrewd guess. The values given are probably overestimates.

Although FeO appears in barely more than trace amounts in the analyses of table 8, Fe_2O_3 is present in quantities far greater than can reasonably be attributed to visible impurities or analytical error. In all four feldspars a strong buff tint can be produced by heating for 10 minutes or less at ~850°C in air, and discharged by heating over a Meker blast for a few minutes at ~1100°C. In our view most of the Fe_2O_3 in these analyses must be regarded as part of the feldspar, probably proxying for Al_2O_3, as already suggested by Johnsen (1912, p. 6).

It will be noted that quartz and hematite are present in all four and a little corundum in two of the feldspar norms. In three of the specimens the content of normative quartz is roughly comparable to the modal amounts shown in table 9. The agreement is far from exact, but the presence of measurable amounts of modal quartz sharply limits the use of these analyses as a basis for speculation about the nature of the silica-cation balance in alkali feldspars. We may point out, however, that despite diligent search no quartz at all was noted in feldspar 40B5, yet the norm shows 2.53 per cent. Most of the norms of literature analyses of alkali feldspar so far examined show normative quartz in comparable or greater amounts, amounts large enough so that they could scarcely be overlooked by the petrographer or fabricated by the chemist. Many also show appreciable amounts of normative hematite and corundum. Despite serious analytical and sampling uncertainties in available data it seems to us that the possibility of systematic departure from the assumed 1:1:6 ratio of $RO:R_2O_3:SiO_2$ in alkali feldspar deserves more than casual consideration.

Projection of results into "petrogeny's residua system." Ternary coordinates of the four rock-feldspar phenocryst pairs

TABLE 10. Rock and Feldspar Compositions Projected into the Ternary System Q-Or-Ab
(Numbered specimens as identified in table 6; Commende and Fontane from Johnsen, 1912.)

	25B10	PRC 2000	39B2	40B5	Johnsen	
					Commende	Fontane
			Rock			
Q	14.6	38.0	35.8	39.0	33.8	40.1
Or	39.5	36.0	34.4	30.4	31.4	30.2
Ab	45.9	26.0	29.8	30.6	34.9	29.8
			Feldspar			
Or (analytical)	44.0	37.4	39.9	48.4	40.8	48.6
Or (X-ray)	40	35	37	44		

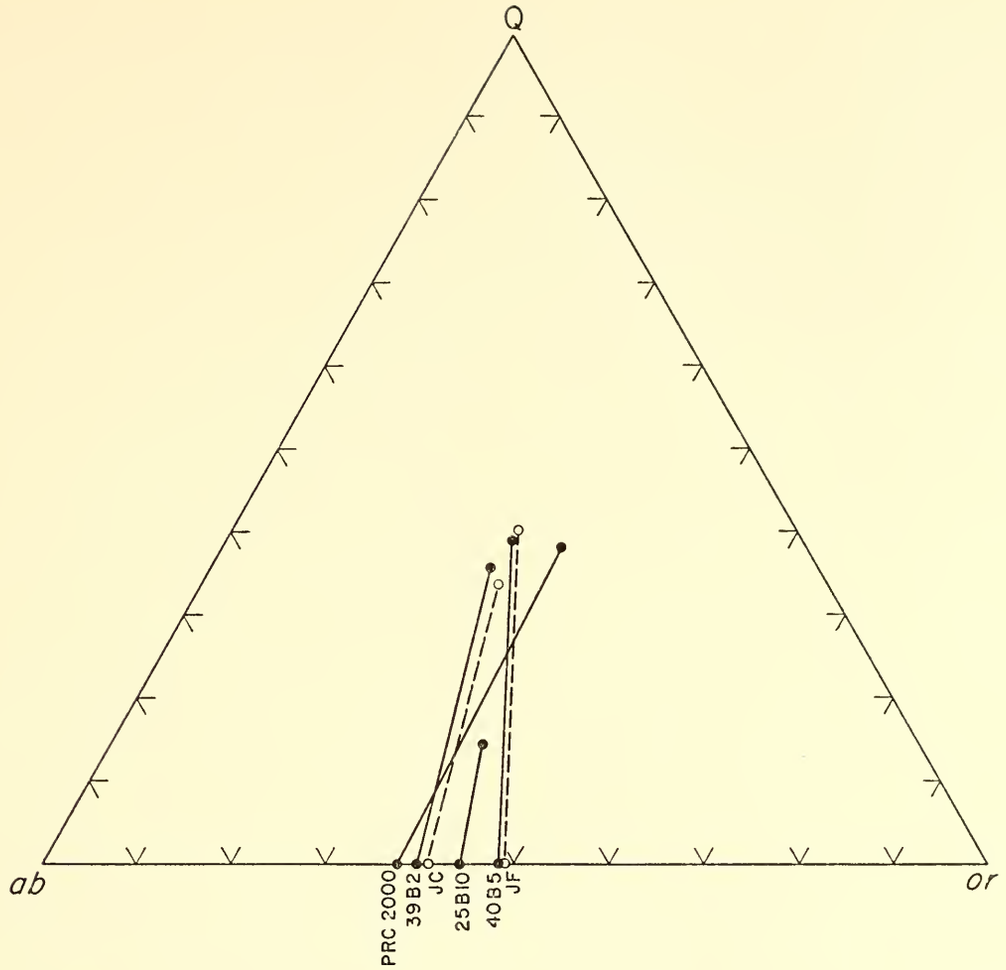

Fig. 38. Data of table 10 plotted in Q-Or-Ab diagram. JC, rock anal. p. 11, no. 2, feldspar anal. p. 5, no. 1; JF, rock anal. p. 22, no. 4, feldspar anal. p. 19, no. 1, in Johnsen (1912). Other specimens as identified in table 6.

described in this note together with two evidently similar pairs taken from Johnsen (1912) are listed numerically in table 10 and shown graphically in figure 38. The last line of the table gives compositions of alkali feldspar determined in the way described by Tuttle and Bowen (1958, pp. 11–13) on specimens homogenized at 850°C and room pressure for 24 hours. As far as could be determined from the X-ray powder spectra the materials, which were initially monoclinic, were completely homogenized by this treatment. We could find no significant differences between Or content estimated on specimens treated in this way and that obtained from specimens heated hydrothermally for extended periods of time. It will be noted that all four estimates of Or by X ray are lower than the relevant Or/(Or + Ab) ratios calculated from the analytical data. We suspect that the high Fe_2O_3 and excess SiO_2 already noted may be responsible for this discrepancy, since the determinative curves are developed for synthetic materials as free of Fe and as close to the 1:1:6 ratio as possible.

In figure 38 a line connects each projected bulk composition with its appropriate feldspar, our data being shown with solid dots and lines, the two pairs taken from Johnsen by open circles and dashed lines. Our Fontane specimen obviously checks Johnsen's very closely, and the two Commende pairs are also in good agreement. The differences between the slopes of these lines may appear small in ternary projection but are in fact very large. In compositions distributed along the line connecting the Pantellerian

feldspar and its host rock the slope of the regression of Or as a function of Ab or Q would be zero and the slope of the regression of Ab as a function of Q would be -1. In compositions distributed along the line connecting our Fontane feldspar with its host rock, on the other hand, the slope of the regression of Or as a function of Ab would be $+1$ and the slope of the regression of either Ab or Or as a function of Q would be $-\frac{1}{2}$.

The relations between phenocryst and host implied by these two pairs are thus very different. In the first the normative groundmass feldspar must be much richer in Or than the phenocryst feldspar; in the second the two must be nearly identical in composition. From data already given we may estimate that the normative Or content of groundmass feldspar in the Gelkhamar pantellerite is 61.2 compared with 37.4 in the phenocrysts. In the Capo Sandolo comendite, on the other hand, the Or content of the normative groundmass feldspar is 47.7 as compared with 39.9 in the phenocrysts, and in the Fontane comendite the comparable values are 50.7 and 48.4, this last pair probably differing by less than the total experimental error. The possible importance of this distinction between pantellerite and comendite is obvious; fractionation of the Pantellerian type would involve extensive end-stage enrichment in potassium, whereas fractionation of the comenditic type would proceed with no notable shift in the Na/K ratio. It is equally obvious that a discussion of this problem based on data from three or four specimens is scarcely more than idle speculation. Once more the desirability of a drastic increase in analytical potential becomes apparent.

Variance Relations in Some Published Harker Diagrams

F. Chayes

Much of the interest in petrographic closed arrays centers on relations between silica and other essential oxides in suites of rock analyses, as portrayed graphically in the Harker variation diagram.[4] Because of algebraic restrictions arising from the cloture property, covariances and correlations are not independent of variances, as is normally assumed in statistical (or other) testing. The larger the variance of a particular variable, the more strongly negative are its expected correlations with other variables, and as long as no variance is greater than the sum of the other variances all expected correlations are negative. The particular effect of variance or covariance of most importance for an appreciation of the Harker diagram may be illustrated by a very simple model.

Let us suppose an M-variable closed array in which the parent variances, σ_j^2, of variables X_j are equal for $2 \leqslant j \leqslant M$, but in which $\sigma_1^2 \neq \sigma_j^2$. Further, we specify that, although σ_1^2 is potentially variable, the system remains stable during any particular sampling or set of samplings. Under these conditions the expected correlation between X_1 and X_j in such a sampling is

$$\rho_{1j} = \sigma_1/\sigma_j(1 - M) \qquad (1)$$

and that between any pair of variables not including X_1 is

$$\rho_{jk} = \frac{1}{2 - M}\left[1 - \frac{\sigma_1^2}{(M - 1)\sigma_j^2}\right] \qquad (2)$$

Now σ_1 is by definition nonnegative, and it can be shown (see, for instance, Chayes, 1960) that $\sigma_1 < (M - 1)\sigma_j$. We are thus concerned with variations in ρ_{1j} and ρ_{jk} that may arise as a consequence of permitting σ_1 to vary in the range $0 \leqslant \sigma_1 \leqslant (M - 1)\sigma_j$.

By substitution of these values in equations 1 and 2 we have

$$\left.\begin{aligned} 0 \geqslant \rho_{1j} &> -1 \\ -1/(M - 2) \leqslant \rho_{jk} &< +1 \end{aligned}\right\} \qquad (3)$$

As σ_1 moves from its lower to its upper limit, the negative correlation to be

[4] The diagram known by Harker's name seems to have been invented by Iddings (1892).

expected between X_1 and X_j becomes progressively stronger while the initially strong negative correlation between X_j and X_k becomes progressively weaker; if $\sigma_1^2 > (M-1)\sigma_j^2$ the expected value of the latter correlation is positive. For the present we specify no mechanism by which to manipulate the variances, arguing only that, *if* variance relations of the specified sort did in fact occur, the correlations to be expected in the absence of any other relation between the variables would be dictated by them.

That the principal negative correlations of the Harker diagram seemed intimately related to the variances in the fashion suggested had already been noted (Chayes, 1962), but in fact this inference was largely based on examinations of graphs. During the report year calculations were carried through on twenty-five suites of analyses of volcanic rocks that had served as the basis of published Harker diagrams. Satisfactory description of the results calls for bibliographical and other detail not appropriate in a report of this sort. Certain of the findings are so extreme, however, that further work is hardly likely to lead to substantial modification.

In every suite, for instance, the variance of silica was considerably larger than that of any other oxide. In twenty-three of the twenty-five suites the variance of silica was larger than the *sum* of all other variances, both exceptions being suites of oceanic lavas, assemblages to which the Harker diagram is rarely applied. The ratio of silica variance to the sum of other variances is so far never larger than 3.38; its average value, 1.95, taken as an estimate of the ratio $\sigma_1^2/\sigma_j^2(M-1)$ would lead to $\rho_{1j} \sim -0.63$ in equation 1, if we count the variables in the way proposed in last year's report. Although this is hardly more than a very crude approximation, the variance relations are clearly such as to require very strong negative correlation between silica and all other oxides that contribute materially to the total variance of a Harker array. These oxides are, in order of increasing average variance, Fe_2O_3, Al_2O_3, FeO, MgO, CaO; curiously, and perhaps significantly, this is also in order of increasingly strong negative correlation with SiO_2. Since TiO_2, Na_2O, and K_2O do not contribute materially to the total variance, the effect of cloture on their correlations with SiO_2 should be negligible. Although TiO_2 is usually negatively correlated with SiO_2, it is well known that the correlations of Na_2O and K_2O with SiO_2 are nearly always strongly positive. Systematically strong positive correlations involving SiO_2 thus emerge only where the variance relations permit.

A considerable excess of silica variance over the sum of other variances seems to be characteristic of the continental basalt-andesite-dacite-rhyolite association; if the basic and acid parts of such suites are considered separately, the excess variance of silica usually persists in the rhyolite or dacite-rhyolite and, to date, always persists in the basalt-andesite parts of the assemblages. An apparently similar excess is not uncommon in suites of the oceanic-basalt-trachyte association but seems to be generated here by the grouping of analyses that do not belong together, as suggested in a later section of this report. At any rate, in the basaltic portions of oceanic basalt-trachyte suites silica variance never exceeds the sum of the other variances and, indeed, is rarely the largest variance. (Unfortunately, there are very few oceanic suites in which nonbasalts are sufficiently numerous to make separate computation worth while.) A full account of variance-covariance computations in volcanic suites is now in preparation.

The Treatment of FeO and Fe_2O_3 in Harker Diagrams

F. Chayes

In most Harker diagrams only one Fe oxide is shown; customarily, a new variable is formed by adding the adjusted weight of one of the oxides to the posted

amount of the other in each analysis, viz.,

$$(Fe_2O_3)_T = Fe_2O_3 + 1.11FeO$$

or

$$(FeO)_T = FeO + 0.901Fe_2O_3$$

Although modern justifications of it are rarely explicit, the practice itself was proposed by Iddings (1892) in the first publication containing what we would now call Harker diagrams. In this pioneering discussion Iddings often shows total Fe as FeO, but *always* shows FeO and Fe_2O_3 separately as well. (In the first text treatment of the subject, however, Harker [1909] shows the iron oxides separately in only six of the twelve diagrams he presents.)

From close examination of the "very carefully executed analyses of the rocks from the region of the Yellowstone Park," Iddings argues (1892, p. 153) that "In this group of rocks the reciprocal behavior of the ferrous and ferric oxides is one of the most marked chemical features" and concludes "it seems highly probable that during the differentiation of the magma all of the iron existed in the ferrous condition . . . and that subsequently it was in part more highly oxidized, so that the more ferric oxide was produced the less ferrous remained." Before hastily concluding that this dictum implies negative correlation between FeO and Fe_2O_3, the reader is urged to examine figure 39, a display of the data upon which it is based. The correlation between FeO and Fe_2O_3 is actually positive, though very weak. Denoting SiO_2 by x, Fe_2O_3 by y, and FeO by z, the Iddings' data give $r_{yz} = +0.246$. The partial correlation is very different, viz., $r_{yz \cdot x} = -0.690$. For a fixed silica content there is indeed some tendency for FeO and Fe_2O_3 to vary inversely in the sample, but the tendency hardly seems strong enough to warrant either Iddings' detailed speculations about a pooled Fe variable or the tacit conviction of modern petrographers that no other Fe variable is desirable in Harker diagrams.

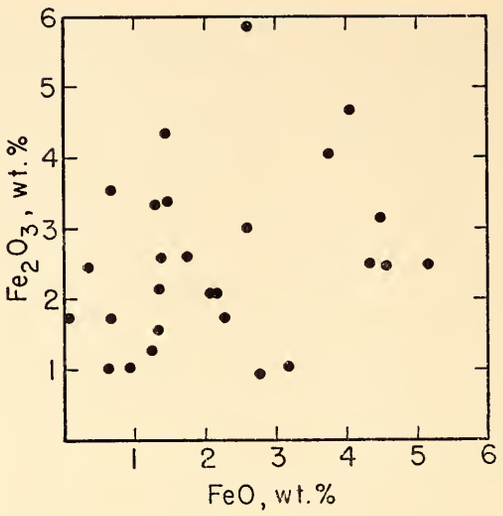

Fig. 39. FeO and Fe_2O_3 in volcanic rocks of Yellowstone Park (data from table 1 of Iddings, 1892).

There is, nevertheless, strong negative correlation between both iron oxides and silica in this earliest "Harker array," as in so many of its successors. In fact, for the Iddings data $r_{xy} = 0.643$, $r_{xz} = -0.834$, and $r_{x(y+z)} = -0.925$. This last correlation is, I believe, the common though rarely stated occasion for pooling the Fe oxides into a single variable. We

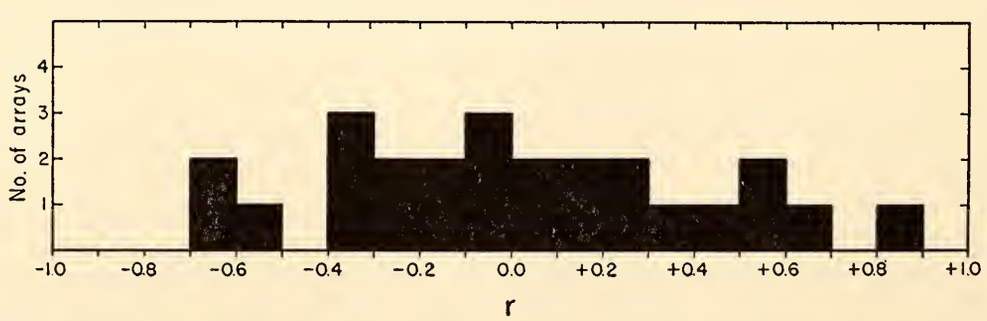

Fig. 40. Histogram of product moment correlations between FeO and Fe_2O_3 in the raw data of twenty-five published Harker diagrams.

all like points that lie fairly close to fairly simple curves, and in most Harker arrays the linear correlation between silica and some form of the sum of the iron oxides will be stronger than that between silica and either of the iron oxides separately. The explanation offered by Iddings is only one of a large class of hypotheses compatible with this relationship; not all members of this class require inverse or "reciprocal" variation of the iron oxides, whether the correlation implied is total or partial.

The correlation between FeO and Fe_2O_3 in Harker arrays is in fact extraordinarily variable. Figure 40 is a histogram showing the distribution of r_{yz} in the twenty-five arrays for which computations have so far been completed. Observed relations between FeO and Fe_2O_3 run the gamut from strong negative correlation to virtually perfect positive correlation. To the extent that the Harker diagram is intended to provide a condensed description of the data the use of a single Fe variable will often be either misleading or uninformative. It would be preferable to return to the practice of Iddings; the separate oxides of iron should be retained as Harker variables whether or not some form of pooled Fe variable is also constructed.

On the Relative Scarcity of Intermediate Members in the Oceanic Basalt-Trachyte Association

F. Chayes

There seems to be no question that basalt is by an enormous margin the principal oceanic lava and that trachyte —often moderately feldspathoidal in either norm or mode—is a poor but uncontested second. The next most important oceanic lava is probably phonolite, and where phonolite is abundant, as in Tahiti or Réunion, for instance, hypabyssal or even plutonic feldspathoidal rocks may occur. Rhyolite is on the whole very uncommon, though there are indeed a few occurrences whose authenticity is beyond any reasonable doubt. (For a review of the oceanic lava associations, see, for instance, Tilley [1950], or Turner and Verhoogen [1951, pp. 124–155].)

We are equipped with a full complement of names for lavas intermediate in composition between basalt and trachyte —trachybasalt, trachydolerite, trachyandesite, kohalaite, mugearite—but the rocks themselves seem not at all common on oceanic islands. This apparent scarcity of intermediate members, noted by a number of petrologists, has been perhaps most dramatically presented by Barth (Barth, Correns, and Eskola, 1939, p. 65). Citing Hawaii as an example, Barth remarks that in the intrapacific province rocks containing between 53 and 58 per cent of silica seem to be completely lacking. Since Barth wrote, however, three Hawaiian specimens with silica in the forbidden range have been reported, two from Maui (Macdonald and Powers, 1946, pp. 119 and 122) and one from Oahu (Tilley, 1950, p. 41). Although the search for them was perhaps in part stimulated by Barth's remark, these new finds do render his example strictly incorrect. And whether the precept it illustrated was ever correct will depend on whose analyses are to be discarded. From elsewhere in the intrapacific province there are—and were at the time of Barth's writing—at least seven other analyses of lavas falling in the forbidden silica range, one from Easter Island and two each from Samoa, the Marquesas, and the Society group. But there are also at least twenty analyses of Pacific lavas in which $58 < SiO_2 < 63$, so that, although lavas with silica in the range 53–58 are perhaps not so rare in this region as Barth suggested, analyses of them are nevertheless considerably less common than those of true trachytes. In other oceans this central minimum is not quite so clear cut; in the Indian Ocean, for instance, available data suggest no shortage of intermediate silica values for the Kerguelen archipelago but at Réunion

and Mauritius the situation is about like that in the Pacific. In the Atlantic a "Barth gap" seems to occur, though very weakly, at St. Helena, but on Ascension and in the Canaries the shortage is most evident in the 58–63 per cent SiO_2 range, while there are many analyses in the range 63–68 per cent.

Table 11 shows the incidence of silica values in classes whose width is $\frac{1}{6}$ of the range observed in each island or island group. It will be noted that zeros occur only in classes 4 and 5, and that with two exceptions 1's are confined to classes 3, 4, and 5. The column totals of the table leave little doubt that, for the array as a whole, the inference of some kind of hiatus—either a minimum or an outright discontinuity—in the parent distribution(s) is almost unavoidable.

Is it possible that this preponderance of trachyte over trachyandesite is merely a consequence of traditional cabinet-specimen sampling aimed at collecting rare and unusual material? On the assumption that the various lavas can be adequately identified in the field, it does not seem at all likely that collectors would have ignored material as rare and interesting as trachyandesite. If, as is suggested in many of the source papers, the hand-specimen distinction between trachyte and basalt is in fact difficult and unsatisfactory, there is even less likelihood of a serious sampling bias. There seems no reason to doubt that in this respect the distribution of analyses reflects, approximately at least, the distribution of rocks, and that lavas intermediate in composition between basalt

TABLE 11. Frequency of Silica Values in Sixths of Group Ranges for Larger Groups of Oceanic Basalt-Trachyte Suites

	Range	Class						Σ
		1	2	3	4	5	6	
Pacific Ocean								
Kohala-Hulalai[1]	41–62	9	6	2	0	1	3	21
Georgian and Society Islands[2]	41–63	10	10	2	1	3	8	34
Marquesas[3]	42–66	14	3	1	1	7	2	28
Samoa[4]	43–72	5	1	1	0	2	5	14
Easter[5]	42–74	4	4	1	2	2	1	14
		42	24	7	4	15	19	111
Atlantic Ocean								
Ascension[6]	47–73	2	4	1	2	4	3	16
St. Helena[7]	43–63	3	2	1	0	0	5	11
Canary Islands[8]								
Feldspathoidal	39–64	9	10	3	4	18	7	51
Nonfeldspathoidal	38–71	12	15	3	1	5	7	43
Azores[9]	37–68	3	10	2	1	3	6	25
		29	41	10	8	30	28	146
Indian Ocean								
Réunion[10]	44–64	4	6	2	1	0	3	16
Mauritius[11]	43–63	9	3	1	1	0	6	20
Kerguelen[12]	43–69	8	9	3	3	2	2	27
		21	18	6	5	2	11	63
All		92	83	23	17	47	58	320

Sources: 1, Washington (1923); 2, Iddings and Morley (1918) and Lacroix (1923, pp. 279–289); 3, Chubb (1930); 4, Daly (1924); 5, Bandy (1937); 6, Daly (1925); 7, Daly (1927); 8, Fuster, Ibarrola, and Lobato (1954); 9, Berthois (1953); 10, Lacroix (1923, pp. 227–237); 11, Walker and Nicolaysen (1954); 12, Edwards (1938).

and trachyte appear to be less abundant than trachytes because they are in fact less abundant. One is tempted to point out imposing continental analogies: the lavas of eastern Otago, East Africa, the Iki Islands of Japan, etc. It is important to realize, however, that, although the data as a whole appear to indicate a minimum in the frequency distribution of silica in oceanic lavas, *from only one island group do we have enough analyses to provide a reliable test for the significance of observed departures from uniform density in classes 3, 4, 5, and 6 of table 11!*

This is a regrettable and highly unsatisfactory state of affairs, one that should be remedied at the earliest opportunity. With the increasing funds available for oceanographic research it is to be hoped that the lavas of the oceanic islands will soon receive from oceanographers the same kind of attention space scientists are devoting to meteorites.

Granite in Port Clyde Peninsula

Y. Suzuki and F. Chayes

It is commonly supposed that there is a gradual transition from true granites to gabbros, with parallel tendencies toward increase of plagioclase over potash feldspar, increase of An over Ab in plagioclase, increase of color index, and decrease of quartz content. Our area, however, provides no support for this classical notion of a compositional continuum. Rather, there is a very strong suggestion that the two principal facies, biotite-muscovite granite and biotite-hornblende granite, are quite distinct and readily distinguishable.

In the peninsula stretching from Rockland southwestward to Port Clyde, Maine, there are many excellent exposures of granitic and dioritic rocks. These rock types outcrop within the outlined areas of figure 41, and sample localities are marked. They are intrusive into Paleozoic sediments. The unmarked area is underlain by Paleozoic rocks or glacial drift. Along the shoreline outcrops are abundant, although often deeply weathered. The area was once the center of a large quarrying industry.

Following up some earlier studies of the quarries by Chayes, Suzuki spent about a month during the summer of 1960 attempting to sample the intrusive complex systematically on a 1-km grid. The final distribution of specimens is shown in figure 41. Despite the enormous amount of natural and artificial outcrop in the area, the sample density varies greatly over the grid. By conventional standards, however, we have an unusually large sample of the complex upon which to base our report.

Most of the outcrop area is underlain by fine-grained two-mica granite. The northeast part of the complex consists of coarse-grained hornblende-biotite granite. Trondhjemite and biotite-quartz diorites are mostly confined to the western border.

The average values for the two main granite facies are shown in table 12. The first column is based on modal analyses of thirty-nine specimens, the second on modes of twelve specimens. By conventional variance analyses all differences are highly significant. Although the differences in the two average modes are in the expected directions, their numerical values hardly suggest the expected continuity. The color index of the hornblende facies is only 4.1 per cent greater than that of the muscovite facies, but its quartz content is 8.0 per cent less. Differences between the other major constituents are, similarly, very much larger than the color-index difference. The real clue to the situation seems to be the presence or absence of hornblende—sometimes of very little hornblende.

Although plagioclase is dominant over potash feldspar in all the hornblende-bearing granites, the most calcic plagioclase so far encountered is An 35, and the quartz content of these rocks is usually well within the granite range (fig. 42).

Rocks with no potash feldspar at all

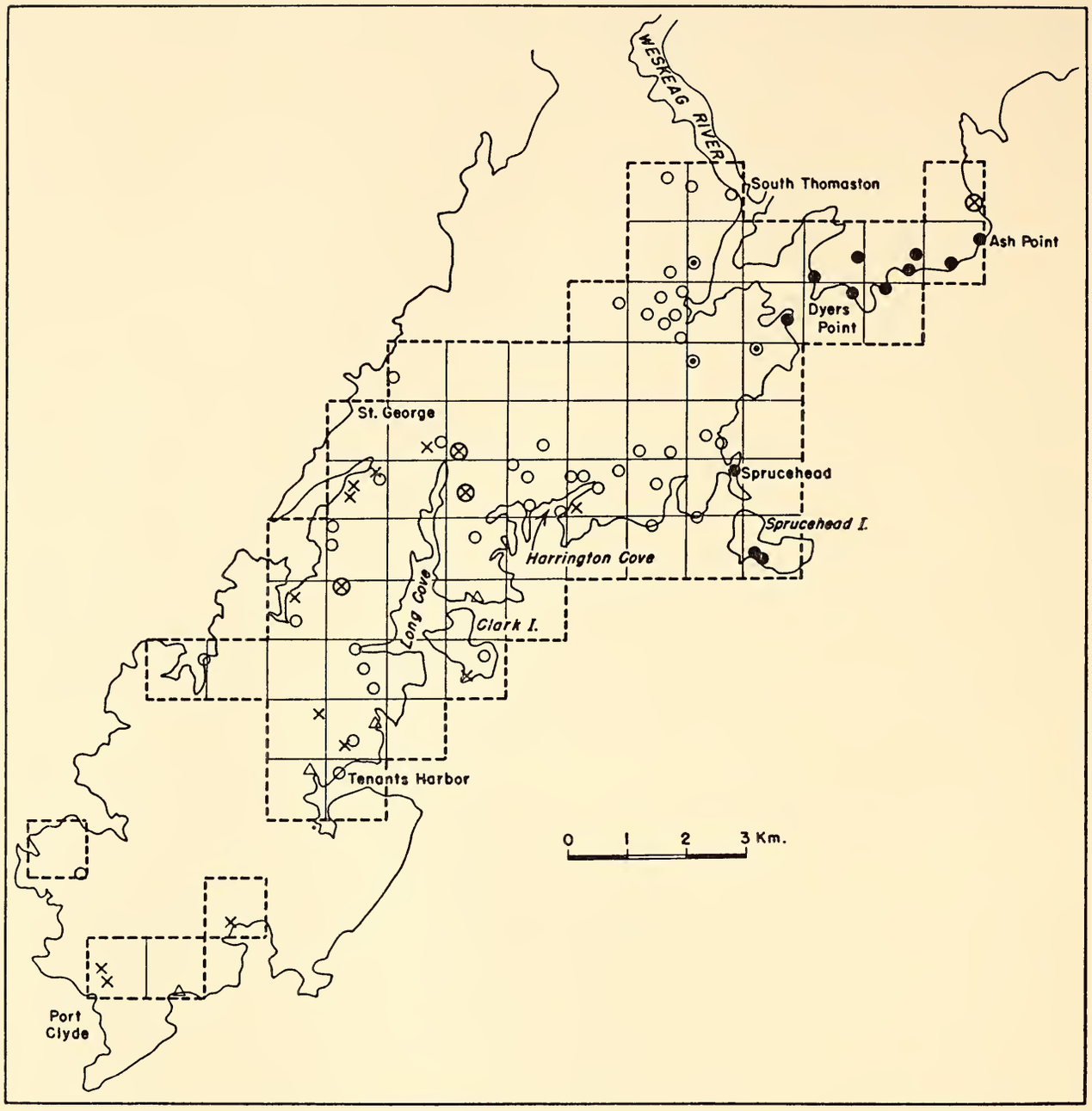

Fig. 41. Sample localities in the Port Clyde peninsula complex. Double circle, muscovite granite; open circle, muscovite-biotite granite; cross circle, biotite granite; solid circle, hornblende-biotite granite; triangle, trondhjemite; diagonal cross, quartz-biotite diorite.

may or may not be conspicuously intermediate between granite and gabbro in quartz content. But rocks containing even a little potash feldspar are, in this respect, not transitional at all. Rather, they are simply granitic.

The plagioclase of all facies of the complex exhibits mild but persistent zoning, so that reliable estimation of its An content is difficult. The work on this problem has already been described (*Year Book 60*, p. 169). Here it is only necessary to point out that, although in broad outline the results are compatible with the proposed transitional relation, there are striking exceptions, and the total range of An content is rather small. The plagioclase of the two-mica granites is oligoclase, or occasionally andesine. In the hornblende-biotite granite it is sodic andesine, rarely oligoclase.

Sharp contacts between sizable masses

TABLE 12. Modal Compositions of Muscovite-Biotite Granite and Hornblende-Biotite Granite of Port Clyde Peninsula, Maine

	Muscovite-Biotite Granite		Hornblende-Biotite Granite	
	Mean	Standard Deviation	Mean	Standard Deviation
Quartz	31.91	3.55	23.90	3.30
Potash feldspar	27.79	6.64	15.17	7.78
Plagioclase	29.95	6.27	46.52	5.74
Muscovite	2.52	1.45		
Biotite	6.81	4.23	12.18	2.98
Hornblende			1.40	1.63
Color index	10.35	4.57	14.42	3.95
Sample size	39		12	

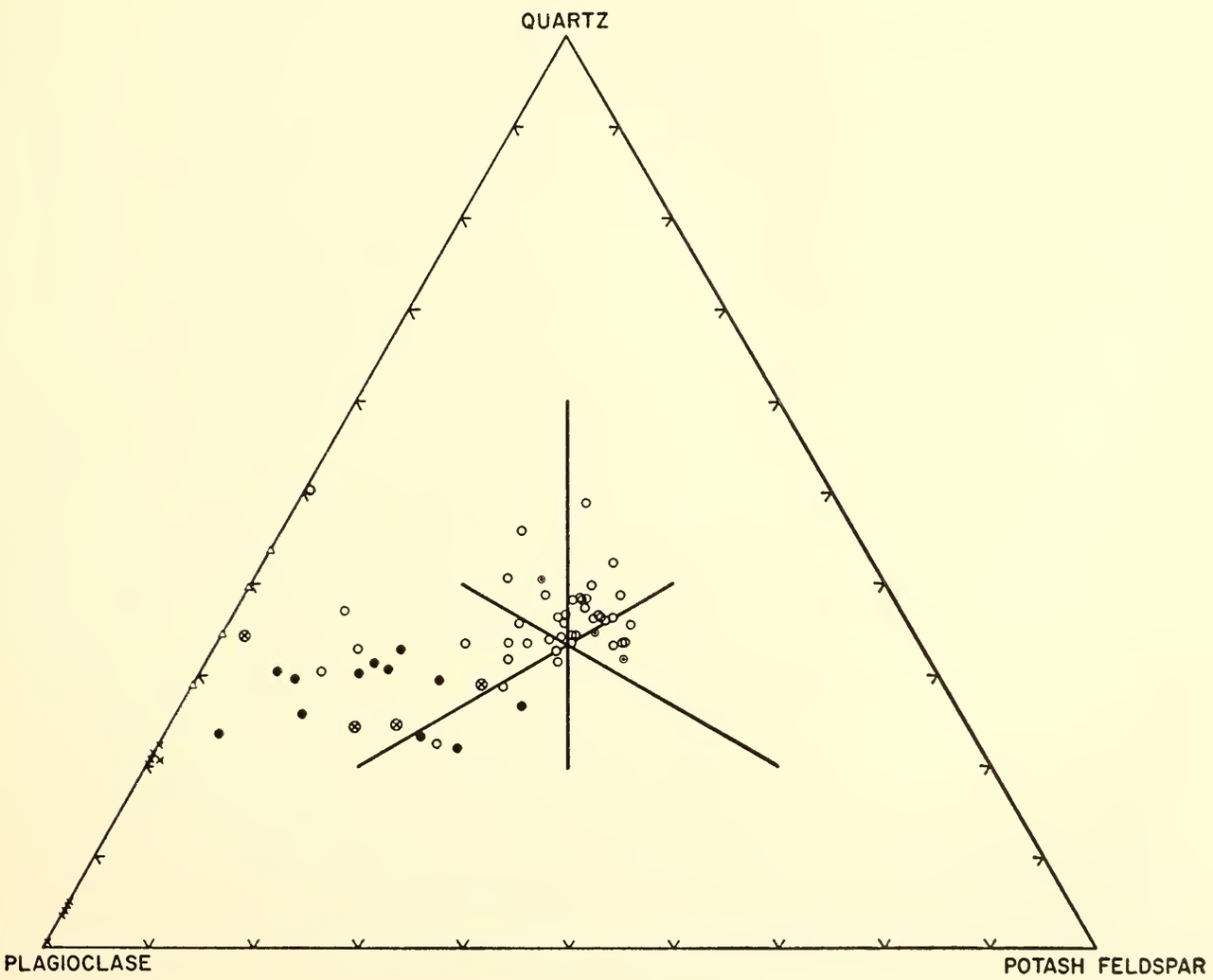

Fig. 42. Modal ternary ratios of Port Clyde peninsula specimens. Symbols as defined in figure 41.

of the two principal types of granite are nowhere exposed, but we have so far found no evidence for compositional gradation between the two. Although reliable identification cannot always be made in hand specimen, microscopic examination nearly always permits ready assignment of an outcrop to one or the other of the two main classes. Difficulties arise only when both muscovite and hornblende are absent, and such rocks are rare.

The exact relation between hornblende-biotite and muscovite-biotite granite in the Port Clyde area is still not known, but it seems quite clear that some type of geochemical or stratigraphic discontinuity separates them. Further, there is field evidence—inclusions, schlieren, brecciation, etc.—that the relation of the granites to the diorites and gabbro-diorites of the complex involves extensive hybridization.

It is to be noted, too, that it is the two-mica granite, not the hornblende-biotite granite, that is usually involved in this migmatization. Indeed, the large mass of hornblende-biotite granite in the northeastern part of the outcrop area is separated by the two-mica granite from the principal outcrop area of the dioritic facies, which lies to the southwest.

Thus, although the notion of a complete compositional continuum might provide here, as elsewhere, a convenient nomenclature and classification for the various facies of the complex, genetic inferences drawn from or based upon such a concept would be misleading.

Feldspar in the Granite of the Port Clyde Peninsula

Y. Suzuki

This section describes variations in Ab content of potash feldspar in the granites of the Port Clyde peninsula and reports attempts to determine whether such fluctuations appear to be systematically related to variations in other measurable modal or mineralogical properties of these rocks.

The Ab content of a grain of potash feldspar may receive contributions from: (1) plagioclase not removed by the separatory procedure, (2) perthitic intergrowths, (3) Ab in solid solution. Source 1, contamination, can be held suitably low in most of the Port Clyde peninsula rocks if only small amounts of concentrate are required. Optical properties measured on individual fragments or parts of fragments of perthitic intergrowths are concerned only with source 3, but in estimates of composition by X-ray powder techniques preliminary heat treatment[5] converts contribution from source 2 into contribution from source 3. If the perthite appears to be of replacement origin, the composition determined by X ray after heat treatment may be mineralogically interesting but petrographically uninterpretable. If, as in the Port Clyde area rocks, there seems no reason to suppose other than an exsolution origin for the perthites, the composition determined by X ray after homogenization (and inversion) is properly regarded as an estimate of the composition of the alkali feldspar before exsolution, and this is a valuable datum.

We should also like to know the Ab content of the K-feldspar phase now visible in the rock. At present the only methods purporting to give this information are optical, and they are exceedingly rough. The biggest index difference between pure orthoclase and pure albite, for instance, is 0.017 (Tuttle, 1952). With measurements subject to an uncertainty of, say, 5 in the fourth place, it is obvious that if the apparent difference between two observations is not zero it cannot be less than 3 per cent Ab. Whereas the X-ray procedure gives an average value for the homogenized specimen, the index measurements give a minimum value for Or from the maximum gamma found in a particular sample.

[5] At 800°C, 1 kb, 1 week, water saturated, after grinding.

In table 13 the average Or per cent as determined by X ray is shown in column 2, and in column 3 the maximum observed gamma index is recorded, followed by an estimate of the equivalent Or content from Tuttle's diagram (1952, p. 559). The fourth column gives the angle from which the "triclinicity" of Goldsmith and Laves (1954) is computed, and the fifth the average An content of accompanying plagioclase, determined by measurement of X-ray powder diagrams. The An content of accompanying plagioclase is clearly smaller in two-mica granites than in hornblende granites. The Goldsmith-Laves angle is somewhat larger in the two-mica granites, the largest value in the hornblende-biotite granites being less than the smallest in the two-mica granite. The difference between minimum and average Or per cent in alkali feldspar is large enough to suggest that the K_2O content of this mineral varies from grain to grain. In several specimens its heterogeneity seems beyond reasonable doubt, and it is curious that three of the five hornblende-biotite granites fall in this category. It is also curious that, although

TABLE 13. Composition of Alkali Feldspar and Accompanying Plagioclase in Granite of the Port Clyde Peninsula

No.	Average Or Per Cent	Or Per Cent from Minimum γ		Angle between 2θ 131 and $1\bar{3}1$	Average An Per Cent in Associated Plagioclase
		γ	Or%		
		Two-Mica Granite			
21	83	1.5260	77	0.79°	16
31	89	1.5265	75	0.78	30
49B	88	1.5245	86	0.80	12
51	88	1.5260	77	0.78	18
58	83	1.5255	80	0.80	13
62	84	1.5260	77	0.78	19
65	90	1.5275	69	0.79	27
67	82	1.5270	72	0.77	25
68	81	1.5260	77	0.78	16
80	86	1.5265	75	0.78	18
89	87	1.5260	77	0.78	18
93	86	1.5270	72	0.78	20
109	88	1.5260	77	0.77	30
		Hornblende-Biotite Granite			
25	90	1.5275	69	0.74	32
27	93	1.5265	75	0.71	29
28	91	1.5280	66	0.71	30
41	87	1.5245	86	0.64	25
42	86	1.5255	80	0.70	29
		Muscovite Granite			
26	94	1.5260	77	0.79	3
55	91	1.5240	89	0.80	5
		Biotite Granite			
71	80	1.5275	69	0.76	33
44	92	1.5250	83	0.61	18
		Two-Mica Granite (minor dike)			
77	83	1.5265	75	0.78	30

TABLE 14. Or in K Feldspar and An in Plagioclase by Rock Types
(Data of table 13)

	No. Samples	Average Or Per Cent in Potash Feldspar	Minimum Or Per Cent in Potash Feldspar	Average An Per Cent in Plagioclase
		All Data		
Average	23	87.04	76.09	21.30
Standard deviation		3.91	5.61	8.69
		Two-Mica Granite		
Average	13	85.77	76.23	20.03
Standard deviation		2.89	4.10	6.06
		Hornblende-Biotite Granite		
Average	5	89.40	74.20	29.02
Standard deviation		2.88	7.59	2.54

in the two-mica granites both the modal content of K feldspar and the minimum Or content of K feldspar show strong negative correlation with An in accompanying plagioclase, the average Or per cent in K feldspar does not. Table 14 shows, by rock type, the average values of columns 2, 4, and 6 of table 13.

Certain of the classical "gradations" appear to be present within the mica granites. There is, for instance, a markedly inverse variation between the amount of K feldspar in the mode and the average An content of the plagioclase in the rock; since the total feldspar content is relatively stable it is not surprising then to find rather strong positive correlation between An content of plagioclase and plagioclase content of rock.

Although the Goldsmith-Laves angle of microcline increases almost linearly with increase of An content in plagioclase of the hornblende-biotite granite, there is a strong suggestion of an opposite trend in the two-mica granites; at present we have no explanation to offer for either of these effects. Although average Or in K feldspar does not appear to be significantly correlated with any other sample statistic, there is a fairly strong inverse variation between minimum Or in K feldspar and average An content of plagioclase.

Two-Mica Granite and Hornblende-Biotite Granite

Y. Suzuki

The marked modal differences between the two-mica and hornblende-biotite granites of the Port Clyde peninsula prompted a literature search for quantitative modal data about other closely associated granites of these two types. As might have been expected, this search was unsuccessful; the only detailed comparison between these rock types possible at present is one that utilizes chemical analyses, and such a comparison is now in progress.

To qualify for inclusion, an analysis (1) must be of a rock called granite in the source publication, (2) must list determinations of the nine essential oxides, (3) must be accompanied in the source publication by a "qualitative" mode or, at least, a list of essential minerals. (This list must of course show that it belongs in one of the two groups under discussion.)

The need for the third requirement is obvious. The second was adopted largely as a means of eliminating partial analyses, on the perhaps questionable assumption that if a rock is not sufficiently interesting to warrant a full analysis it may also fail

TABLE 15. Averages of Muscovite-Biotite and Hornblende-Biotite Granites

	Muscovite-Biotite Granite		Hornblende-Biotite Granite	
	Mean	Standard Deviation	Mean	Standard Deviation
SiO_2	72.20	2.22	70.70	2.67
Al_2O_3	14.54	1.28	14.02	1.31
Fe_2O_3	0.69	0.41	1.03	0.50
FeO	1.46	0.88	2.43	0.96
MgO	0.58	0.58	0.69	0.58
CaO	1.82	0.87	2.20	1.07
Na_2O	3.24	0.70	3.36	0.68
K_2O	4.39	1.40	4.31	1.26
TiO_2	0.25	0.17	0.39	0.22
Sample size	30		48	

to rate a good partial analysis. In fact, however, FeO and Fe_2O_3 turn out to be of major importance.

The desirability of the first requirement will be immediately apparent only to readers who have attempted to make use of any of the standard petrographic classifications in a study of granitic rocks. Although the classifications without exception assign very broad compositional limits to "granite"—up to 80 per cent SiO_2 in CIPW, or as little as 5 per cent quartz in Johannsen, for instance—petrologists have customarily used the term in a much more restrictive sense. Our interest here is with real rocks that have actually been described as granite.

To date, 78 analyses satisfying all three requirements have been found: 32 from North America, 16 from Finland, and 30 from Japan. Means and standard deviations of the nine essential oxides are shown in table 15. The difference between silica averages is suggestive, whereas that between the ferrous oxide averages is decisive. The range of SiO_2 in the hornblende-biotite granites is 64.47–76.68, and that of the muscovite-biotite granites is 67.20–75.86. There is thus a suggestion that FeO and SiO_2 may be merely compensating for each other. This, however, is by no means the whole story. In subsets containing, respectively, all analyses with (*a*) more than 69 per cent SiO_2,

TABLE 16. Effect of Various Silica Restrictions on Average Compositions of Muscovite-Biotite (A) and Hornblende-Biotite (B) Granites

Restriction of SiO_2 Per Cent	More than 69.00 Per Cent		More than 72.00 Per Cent		Between 71.00 and 74.00 Per Cent	
	A	B	A	B	A	B
SiO_2	72.86	71.87	73.51	73.92	72.74	72.64
Al_2O_3	14.32	13.72	13.90	13.07	13.91	13.41
Fe_2O_3	0.66	0.93	0.64	0.84	0.72	0.76
FeO	1.26	2.15	1.09	1.62	1.22	2.17
MgO	0.43	0.50	0.37	0.24	0.51	0.37
CaO	1.67	1.83	1.56	1.22	1.57	1.57
Na_2O	3.14	3.37	3.15	3.35	3.35	3.44
K_2O	4.55	4.47	4.74	4.98	4.78	4.72
TiO_2	0.25	0.32	0.23	0.22	0.31	0.25
Sample size	26	35	20	15	15	15

(*b*) more than 72 per cent silica, and (*c*) between 71 and 74 per cent silica, the amount of FeO is significantly greater in the hornblende-biotite granites. These calculations are summarized in table 16.

One-third of the hornblende-biotite granites and two-thirds of the muscovite granites contain less than 2 per cent FeO, and no significant differences between these "less than 2 per cent FeO" subgroups were found. For these specimens the distinction between hornblende-biotite and biotite-muscovite granite is thus primarily physical rather than chemical. Work on this problem is continuing.

CRYSTALLOGRAPHY

Relationships between Crystal Structure and Crystal Morphology

J. D. H. Donnay[6] *and G. Donnay*

The second generalization of the law of Bravais, which was reported under this heading in last year's report (*Year Book 60*, pp. 208–214), has now been successfully applied to the unraveling of a particularly challenging morphology, that of the mineral barite. Ever since Mallard (1879, p. 318) and Friedel (1904, p. 339) applied the classical law of Bravais to barite, the morphological development of this species has remained an enigma to the present day, even though the crystal structure has been known for a long time (James and Wood, 1925). The first generalization (1937) of the law of Bravais is powerless, as was shown by Hartman and Perdok (1955) and by Seager (1959); the consideration of pseudoperiods (Hartman, 1961) was helpful but not entirely satisfactory.

Barite has an ionic crystal structure. It is well known that in certain simple ionic structures ions of equal charges but of opposite signs play the role of equivalent points when the law of Bravais is called upon to explain the morphology. The punctualization of the ionic charges is the basic postulate in this interpretation. Friedel made use of it in the classical case of NaCl, the crystal structure of which is governed by a face-centered cubic lattice, but where the morphology is controlled by the primitive cubic lattice, with half the cell edge, that is obtained when all the ions are replaced by unit charges concentrated in the nodes of this new lattice. The sign of the charge can be disregarded because the strength of the bond Na^{+}-Cl^{-} is equal to that of the bond Cl^{-}-Na^{+}. Friedel explains the morphology of calcite in the same way: the rhombohedral lattice whose nodes carry the double charges (either positive or negative) is the morphological lattice obtained by applying the law of Bravais of 1849; in this case, not only elementary ions (Ca^{++}) but complex ions $(SO_4)^{=}$ as well are punctualized. The application of the second generalization of the law of Bravais to the problem of barite has led us to a new type of punctualization, namely that of pairs of neighboring ions with the same sign. The reasoning proceeds as follows.

Let us start with the structural space group of James and Wood (1925), *Pnma*, with axial ratios $a:b:c = 1.6304:1:1.3136$. We first note that the dominant general form *z* receives the symbol (211) in this structural setting, whereas it should be symbolized (111) from the morphological point of view; it would then correctly define a primitive morphological lattice. The conclusion is that all (*hkl*) faces in the structural notation must obey the criterion "*h* even," which implies the halving of the structural *a* unit length in the three-dimensional bond assemblage. This agrees with a previous result of Hartman and Perdok (1955), that the energy period of the bond chain along the *x* axis is $a/2$.

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The zone of the (0*kl*) faces is a *simple zone* with (011) dominant, which requires the corresponding reciprocal-lattice net to be primitive from the point of view of morphology, that is to say, of *bonds*. But the structural net b^*c^* has its mesh centered, owing to the *n* glide plane. We must, therefore, postulate additional extinction criteria that will require both *k* and *l* to be even. The dominant face will have to be symbolized (022). To the reciprocal net ($2b^*$, $2c^*$) there should correspond a direct net ($b/2$, $c/2$) that will express the periodicity of the two-dimensional bond assemblage of the projection of the crystal structure onto the *yz* plane. Turning now to the known barite structure (fig. 1 of James and Wood, 1925), we actually observe the predicted net if we replace by equivalent points the pairs of neighboring projected ions with the same sign.

The zone of the (*h*0*l*) faces is also a *simple zone* with unit face dominant, which must obey the criterion "*h* even" (see above) and the additional criterion "*l* even" in order that (202) be the dominant face. As a group, the faces (*h*0*l*) do not occur frequently enough to have their indices co-prime: multiplying all the indices by 2 makes the faces in this zone recede to their correct ranks in the list of decreasing frequencies predicted by the generalized law of Bravais. Whereas the structure, projected onto the *zx* plane, has a primitive net with mesh *ca*, the two-dimensional bond assemblage should have a primitive mesh ($c/2$, $a/2$). This predicted mesh can indeed be recognized in figure 1 of James and Wood (1925) after the *ca* projection is suitably extended. The pairs of ions with the same sign that are to be punctualized are not as obvious on inspection as in the *bc* projection: there are two kinds of pairs of Ba ions and two kinds of pairs of SO_4 ions, according as the line segment that connects the two ions in a pair slopes to the right or to the left.

The zone of the (*kk*0) faces is a *simple zone* with (210) dominant. The condition "*h* even," which is imposed on all (*hkl*) faces, is also the criterion of the structural *a* glide plane, which requires the unit length *a* to be halved in the *xy* projection of the structure. This halving holds for morphology too: this zone does not yield any information other than the prediction that both the projected structure and the corresponding two-dimensional bond assemblage have the same periodicity. As shown in figure 1 of James and Wood (1925) no punctualization of ions or pairs of ions can be found to define a mesh other than ($a/2$, b).

In addition to planar projections, we must consider linear projections, on the coordinate axes. Such a projection of the crystal structure has a one-dimensional bond assemblage, whose period may be the same or smaller. The relative importances (frequencies of occurrence) of the pinacoids constitute the experimental data: we observe that *c* is the most frequent, and *a* the least frequent.

According to the structural space group *Pnma*, the linear projections onto the coordinate axes *x*, *y*, *z* have periods $a/2$, $b/2$, $c/2$, respectively. This would imply the sequence *a*(200), *c*(002), *b*(020) as the order of importance of the pinacoids, which is contrary to facts. To express the fact that *a* is the least frequent of the pinacoids, we must write it *a*(400), so that the predicted sequence becomes *c*(002), *b*(020), *a*(400). This, in turn, requires that the one-dimensional bond assemblage of the projection of the structure onto the *x* axis have period $a/4$. This prediction can be checked in figure 1 of James and Wood (1925): the projected charges along the *a* length look as follows:

$$+\ +\ -\ -\ +\ +\ -\ -\ +\ +$$

where the first and the last pair of positive signs are separated by translation *a*. If all pairs of equal signs are considered equivalent and replaced by points, the length *a* is divided by 4.

We must now check that the other two linear projections of the structure are *not* divided by 4. This is immediately

apparent for the b axis (fig. 1, James and Wood, 1925). Here, equal numbers of plus signs and minus signs are projected on the same points at $y = 0$ and $y = \frac{1}{2}$; the structure is composed of electrically neutral planes; b is halved, both for the projected (linear) structure and for its one-dimensional assemblage. The situation is not so clear for the a axis. Here the centers of the barium and sulfur atoms do not lie exactly in the same plane (parameters that should ideally be equal to $\frac{1}{3}$ are found to be 0.333 and 0.305 by James and Wood, who place their origin at a center of symmetry). Although the structure cannot be said to consist of electrically neutral *planes*, it nevertheless results from the stacking of neutral *layers*, with period $c/2$. This period controls the linear bond assemblage in keeping with the morphological symbol (002) of the basal pinacoid.

Finally we must justify the punctualization of pairs of ions. If we replace by a single central charge the two charges of a pair of ions with the same sign, we must introduce a compensating quadrupole that consists of the original two charges and two opposite charges placed in the center of the pair, next to the punctualized charge. Then we see that the bonds between successive equipoints of the bond assemblage are indeed rigorously equal in strength. Consider, for instance, along the a length, a first pair of Ba ions, followed by a pair of SO_4 ions, itself followed by a second pair of Ba ions. The interactions to be taken into account between two successive pairs of ions are of four kinds: charge-charge, charge-quadrupole, quadrupole-charge, and quadrupole-quadrupole. These interactions between the first Ba pair and the SO_4 pair are equal, each to each, by symmetry, to the interactions between the SO_4 pair and the second Ba pair. The centers of charge can thus be considered equivalent points from the point of view of bonding.

We are indebted to Dr. H. F. Hameka, Johns Hopkins University, for suggesting to us the consideration of the quadrupole. The above results were given in the special issue of *Kristallografiya* published in honor of Professor N. V. Belov.

Lattice Constant Refinement

Charles W. Burnham

Practically all phases of experimental mineralogy require knowledge of precise crystallographic lattice constants. Such values form the basis of detailed three-dimensional crystal structure refinements as well as studies of subsolidus phase-equilibrium relationships. To place precise lattice constant determination on a routine basis a least-squares technique for lattice constant refinement has been developed and programmed for the IBM 7090 digital computer.

The refinement procedure is completely general in the following respects:

1. It is applicable to crystals of any symmetry.
2. It will accept data, from cards or tape, either as angle measurements for any wavelength or in the form of calculated d values.
3. Observations may be suitably weighted according to any scheme.
4. Up to nine systematic correction terms may be included with each observation. Each term consists of an unknown refinable parameter and a coefficient whose form may be any one of five different types. The functional form of each type of coefficient is programmed in a separate subroutine to suit individual experimental conditions.

To allow for systematic errors, Bragg's law is modified to include an error in θ:

$$n\lambda/2d = \sin(\theta + \Delta\theta) \qquad (1)$$

In practice n is absorbed by the reflection indices and will not appear in subsequent equations. Following the method of Cohen (1935), equation 1 is squared and expanded in a Taylor series retaining terms not involving powers of the error, $\Delta\theta$:

$$\left[\frac{\lambda}{2d_{hkl}}\right]^2 = \sin^2\theta + \sin 2\theta\,\Delta\theta \quad (2)$$

The term $\Delta\theta$ contains all systematic errors; it can be expanded to separate n distinct types of errors:

$$\left[\frac{\lambda}{2d_{hkl}}\right]^2 = \sin^2\theta + \sum_{k=1}^{n} \sin 2\theta\,\Delta\theta_k \quad (3)$$

The error terms, $\Delta\theta_k$, are of the form $X_k f_k(\theta)$, where X_k is an experimental factor whose value is initially unknown and is to be refined, and $f_k(\theta)$ is a function of θ, which generally vanishes at $\theta = \pi/2$ (Buerger, 1942; Klug and Alexander, 1954).

Cohen's method may be generalized by introducing reciprocal lattice notation:

$$\left[\frac{\lambda}{2d_{hkl}}\right]^2 = \frac{\lambda^2}{4}(\mathbf{r}_{hkl}\cdot\mathbf{r}_{hkl}) \quad (4)$$

Here $\mathbf{r}_{hkl}$ is the reciprocal lattice vector for the reflection hkl. When the dot product is evaluated in terms of reciprocal lattice constants equation 3 is expanded and rearranged to give

$$h^2a^{*2} + k^2b^{*2} + l^2c^{*2} + 2hka^*b^*\cos\gamma^* + 2hla^*c^*\cos\beta^* + 2klb^*c^*\cos\alpha^* + \sum_{k=1}^{n} g_k(\theta)X_k = \frac{4\sin^2\theta}{\lambda^2} + \epsilon \quad (5)$$

where

$$g_k(\theta) = -f_k(\theta)\,(4/\lambda^2)\sin 2\theta \quad (6)$$

and ϵ represents random error in the observation.

Equation 5 can be transformed to a linear equation in terms of the variations of the parameters by expansion in a Taylor series about a set of trial reciprocal lattice constants and experimental unknowns. If only the first two terms of the expansion are retained, the transformation yields

$$Q_{\text{calc}} + \sum_{j=1}^{6}\frac{\partial Q}{\partial a_j}\delta a_j + \sum_{k=1}^{n}\frac{\partial Q}{\partial X_k}\delta X_k = Q_{\text{obs}} + \epsilon \quad (7)$$

where Q_{calc} represents the left side of equation 5 evaluated using the trial parameters, the a_j are the reciprocal lattice constants, and

$$Q_{\text{obs}} = (4\sin^2\theta_{\text{obs}})/\lambda^2$$

Since equation 7 is linear in terms of the parameter variations, δa_j and δX_k, a set of m of these equations, one for each observed θ, can be solved by standard least-squares techniques (Whittaker and Robinson, 1944) for the parameter variations, provided that $m \geqq n + 6$ (in the triclinic case). The refinement program generates and inverts the least-squares normal equations matrix according to a method developed by Busing and Levy (1962).

When each observation is weighted in proportion to its reliability, equation 7 is multiplied by $\sqrt{w_i}$, and the least-squares procedure minimizes $\sum_i w_i\epsilon_i^2$. The two-term Taylor expansion is exact for orthogonal unit cells, hence the least-squares parameter shifts, when algebraically added to the trial parameters, will yield a set of lattice constants for which the random errors are minimized. Two, or perhaps three, consecutive cycles in which the corrected parameters from the preceding cycle make up the new set of trial parameters may be required for complete convergence in the nonorthogonal crystal systems.

Since the residual, ϵ, represents the difference between an observed and a calculated Q, the standard deviation of a measurement of θ must be converted to the equivalent standard deviation of Q. The program automatically computes the proper least-squares weight for each Q according to

$$\sqrt{w_Q} = \frac{1}{\sigma_Q} = \frac{\lambda^2}{4\sigma_\theta \sin 2\theta} \quad (8)$$

The least-squares standard error of fit, corresponding to the standard error of an observation of Q of unit weight, is computed after each cycle of refinement according to

$$\sigma_0 = \left[\frac{\sum_{i=1}^{m} w_i\epsilon_i^2}{m-n}\right]^{1/2} \quad (9)$$

where m is the number of observations and n is the total number of varied parameters. The variance-covariance matrix, $|V_l|$, of the varied parameters is obtained from

$$|V_l| = \sigma_0 |B|^{-1} \qquad (10)$$

where $|B|$ is the $n \times n$ least-squares normal equations matrix. The standard errors of the varied parameters are, of course, the square roots of the diagonal terms of $|V_l|$.

Following each least-squares cycle the new values of the direct lattice constants and the unit-cell volume are evaluated using standard formulas (Buerger, 1942). The direct lattice constant variance-covariance matrix, $|V_d|$, is obtained from the reciprocal variance-covariance matrix according to (D. Handwerker, personal communication, 1962):

$$|V_d| = |D|\ |V_r|\ |D|^T \qquad (11)$$

where $|V_r|$ is the 6×6 reciprocal lattice constant variance-covariance matrix, containing terms from $|V_l|$ plus appropriate zeros for nontriclinic cases, and

$$|D| = \begin{vmatrix} \frac{\partial a}{\partial a^*} \frac{\partial a}{\partial b^*} \cdots \frac{\partial a}{\partial \gamma^*} \\ \frac{\partial b}{\partial a^*} \cdots \cdots \\ \cdot \\ \cdot \\ \cdot \\ \frac{\partial \gamma}{\partial a^*} \frac{\partial \gamma}{\partial b^*} \cdots \frac{\partial \gamma}{\partial \gamma^*} \end{vmatrix} \qquad (12)$$

The standard errors of the direct lattice constants correspond to the square roots of the diagonal terms of $|V_d|$. The standard error of the unit-cell volume is evaluated in an analogous manner:

$$\sigma^2_V = |E|\ |V_d|\ |E|^T \qquad (13)$$

where $|E|$ is the row vector containing the partial derivatives of V with respect to the direct lattice constants.

The printed results from each refinement cycle include a list of observed and calculated d values, the residuals $(d_{obs} - d_{calc})$ and $(Q_{obs} - Q_{calc})$, and the weighted residuals $(d_{obs} - d_{calc})/\sigma_d$ and $(Q_{obs} - Q_{calc})/\sigma_Q$ based on the trial parameters. The least-squares results contain the reciprocal lattice constant and systematic correction term experimental parameter shifts and standard errors in addition to the direct lattice constant shifts and standard errors. The asymmetric part of the direct lattice constant variance-covariance matrix is made available for subsequent inclusion in interatomic distance and angle error computations.

To illustrate the results obtained with this procedure, table 17 lists refined lattice constants for kyanite, Al_2SiO_5 (triclinic). A single crystal of kyanite from Burnsville, North Carolina used for intensity measurement for structure refinement (Burnham, 1962), was used to obtain precision Weissenberg (Buerger, 1937) photographs about the a, b, and c axes. Of the 79 film measurements employed in the least-squares analysis 23 were of $0kl$ reflections, 20 were of $h0l$ reflections, and 36 were of $hk0$ reflections. Since the precision Weissenberg film measurement, f, is linearly related to θ, and all measurements were considered to have equal precision, all θ_{obs} were weighted unity. Column 1 of table 17 lists the results obtained when systematic correction terms were included to compensate for film shrinkage, specimen absorption, and camera eccentricity. The coefficients, $g(\theta)$, of equation 5 were assigned the following forms (Buerger, 1942):

Film shrinkage:

$$g(\theta)_{shr} = \frac{4}{\lambda^2}\left(\frac{\pi}{2} - \theta\right) \sin 2\theta$$

Absorption:

$$g(\theta)_{abs} = \frac{4}{\lambda^2} \cos^2 \theta \sin 2\theta$$

Eccentricity:

$$g(\theta)_{ecc} = \frac{4}{\lambda^2} \sin^2 2\theta$$

Separate film shrinkage and absorption corrections were applied to data from

TABLE 17. Kyanite (Al_2SiO_5) Lattice Constants

	Seven Systematic Correction Terms	No Systematic Correction Terms	Precession (Skinner, Clark, and Appleman, 1961)
a, Å	7.1192 ± 0.0005	7.1197 ± 0.0004	7.121 ± 0.002
b, Å	7.8473 ± 0.0004	7.8479 ± 0.0003	7.846 ± 0.002
c, Å	5.5724 ± 0.0006	5.5736 ± 0.0004	5.577 ± 0.005
α, deg	89.977 ± 0.005	89.969 ± 0.006	89.97 ± 0.08
β, deg	101.121 ± 0.005	101.126 ± 0.006	101.15 ± 0.08
γ, deg	106.006 ± 0.003	106.001 ± 0.003	106.00 ± 0.08
Unit-cell volume, Å^3	293.16 ± 0.06	293.28 ± 0.03	292.74

different films. One eccentricity term was applied to all observations. Complete least-squares convergence was attained after two iterations.

Column 2 of table 17 contains the results obtained with the same data using no systematic correction terms. Column 3 lists the results obtained by Skinner, Clark, and Appleman (1961) with quartz-calibrated precession data from another specimen of Burnsville kyanite.

It must be emphasized that the least-squares standard errors represent the precision attainable with a specific set of data. The precision will, of course, vary with the ratio of observations to refinable variables. The values are, in general, conservative, since they implicitly involve all correlation, or parameter interaction, effects. They should not be construed, however, as the accuracy to be expected when several sets of parameters obtained by the same or different X-ray techniques on the different samples are compared.

The Crystal Structure of Sillimanite

Charles W. Burnham

Details of the crystal structure of sillimanite are essential to an understanding of the crystal chemical relationships between the Al_2SiO_5 polymorphs (andalusite, sillimanite, kyanite). Because of the extreme similarity of their X-ray diffraction patterns, a well determined sillimanite structure must, in addition, form the basis of detailed studies of the structures of mullites of various compositions. A three-dimensional refinement of the previously determined sillimanite structure (Taylor, 1928; Hey and Taylor, 1931) was undertaken with single-crystal counter diffractometer data measured on a small cleavage fragment of clear sillimanite from LaBelle County, Quebec. The unit-cell dimensions of this specimen were refined to the following values: $a = 7.4856 \pm 0.0006$, $b = 7.6738 \pm 0.0003$, $c = 5.7698 \pm 0.0008$ Å.

Preliminary least-squares refinement of the structure (Burnham, 1961) reduced the unweighted disagreement factor R to 10.3 per cent and the weighted (root-mean-square) R to 4.8 per cent. At that stage agreement between observed and calculated structure factors for the substructure reflections (l even) was excellent (table 18) whereas that for reflections with l odd indicated almost complete lack of complement structure convergence. Attempted refinement of disordered models and a noncentrosymmetric model failed to improve complement structure agreement but had little adverse effect on the substructure R value. This indicated that the substructure reflections are very insensitive to minor structural changes and that substantial convergence of the complement structure will be required before the details of the structure can be evaluated.

During the past year the sillimanite study has been continued. Analysis of the refinement procedure demonstrated that convergence had not been attained because of strong mathematical interactions

TABLE 18. Sillimanite Disagreement Factors, R

	Hey and Taylor (1931), %	Weighted Refinement, %	Unweighted Refinement, %
Unweighted R $\left[\frac{\Sigma\lvert\lvert F_o\rvert - \lvert F_c\rvert\rvert}{\Sigma F_o}\right]$	29.5	10.3	5.6
Weighted R $\left[\frac{\Sigma w(\lvert F_o\rvert - \lvert F_c\rvert)^2}{\Sigma w F_o^2}\right]^{1/2}$	26.2	4.8	4.7
Even-level unweighted R	23.5	5.0	3.6
Odd-level unweighted R	61.9	40.1	16.8

between structure parameters. Least-squares correlation coefficients between pairs of atomic coordinates whose differences determine the complement structure varied from −0.66 to −0.81.

Observed structure factors had been assigned least-squares weights in inverse proportion to their variances as determined by counting statistics. The distribution of sillimanite structure factor magnitudes in reciprocal space is not random; reflections on odd reciprocal lattice levels normal to the c axis receive intensity contributions from the complement structure alone. Because of the resulting unfavorable counting statistics the average weight assigned to these observations was 0.07, compared with the average weight of 0.20 assigned to the substructure observations on even reciprocal lattice levels.

Underweighting of the critical class of observations proved to be the primary cause of the strong parameter interactions. When all structure factors with measurable values were assigned weight 1.0 and those whose values were below the minimum observable value were assigned weight 0.01, all structure parameters were effectively uncoupled. Com-

TABLE 19. Sillimanite Atom Coordinates

Atom, coordinate	Hey and Taylor (1931)	Total Change	Final	Standard Error
O_a: x	0.35	+0.0099	0.3599	0.0006
y	0.43	−0.0222	0.4078	0.0006
z	¾		¾	
O_b: x	0.35	+0.0076	0.3576	0.0006
y	0.43	+0.0052	0.4352	0.0006
z	¼		¼	
O_c: x	0.47	+0.0053	0.4753	0.0007
y	0.03	−0.0292	0.0008	0.0007
z	¾		¾	
O_d: x	0.11	+0.0148	0.1248	0.0004
y	0.22	+0.0037	0.2237	0.0004
z	0.5	+0.0164	0.5164	0.0006
Si: x	0.14	+0.0135	0.1535	0.0003
y	0.35	−0.0096	0.3404	0.0003
z	¾		¾	
Al_1: x	0		0	
y	0		0	
z	0		0	
Al_2: x	0.14	+0.0019	0.1419	0.0003
y	0.35	−0.0053	0.3447	0.0003
z	¼		¼	

plete convergence was attained after nine additional least-squares cycles during which all atomic coordinates and anisotropic temperature factors were varied. The final R values, listed in table 18, confirm *Pbnm* as the correct sillimanite space group.

The refined atomic coordinates are compared with those of Hey and Taylor (1931) in table 19. Although refinement produced significant coordinate shifts, it did not alter the basic geometrical relationships between coordination polyhedra. Chains of slightly distorted aluminum octahedra run parallel to the c axis and are supported by double chains of aluminum and silicon tetrahedra. Differences in interatomic distances (table 20)

TABLE 20. Sillimanite Interatomic Distances*

Atom Pair	Multiplicity	Distance, Å	Standard Error
Si tetrahedron			
Si-O_a	1	1.629	0.007
Si-O_c	1	1.564	0.006
Si-O_d	2	1.633	0.004
O_a-O_d	2	2.628	0.005
O_a-O_c	1	2.608	0.007
O_c-O_d	2	2.627	0.006
O_d-$O_{d'}$	1	2.696	0.007
Al tetrahedron			
Al_2-O_b	1	1.758	0.005
Al_2-O_c	1	1.721	0.006
Al_2-O_d	2	1.800	0.004
O_b-O_d	2	2.834	0.005
O_b-O_c	1	2.903	0.007
O_c-O_d	2	2.843	0.006
O_d-$O_{d'}$	1	3.074	0.007
Al octahedron			
Al_1-O_d	2	1.957	0.003
Al_1-O_a	2	1.919	0.003
Al_1-O_b	2	1.861	0.003
O_a-O_b	2	2.893	0.001
O_a-$O_{b''}$ (shared)	2	2.434	0.006
O_a-O_d	2	2.776	0.005
O_b-O_d	2	2.698	0.005
$O_{d'''}$-O_a	2	2.705	0.005
$O_{d'''}$-O_b	2	2.703	0.005

* Atoms designated with a single prime represent transformation of the unprimed atom in the same coordination polyhedron according to $x' = x$, $y' = y$, $z' = \frac{1}{2} - z$. Double primes represent transformation according to $x'' = -x$, $y'' = -y$, $z'' = \frac{1}{2} + z$. Triple primes represent transformation to a centrosymmetric equivalent.

show that the distribution of aluminum and silicon in the tetrahedra is ordered. Figure 43 illustrates the bonding within and between coordination polyhedra.

The tetrahedral double chains are of particular crystal chemical interest. Each double chain may be thought of as a continuous series of four-membered rings, each ring containing two silicon and two aluminum tetrahedra in the sequence Si-Al-Si-Al. The Si-O_c-Al_2 bond angle of 171.6° and the Si-O_d-Al_2 bond angle of 114.4° control the basic configuration of the ring. Whereas the O-Si-O tetrahedral angles are close to ideal (107.4° to 111.3°), the Si-O bond distances show that the silicon atom is not at the center of its tetrahedron but is measurably displaced toward O_c. The aluminum tetrahedron is more irregular, but, again, the cation is closest to O_c.

The average Si-O distance is 1.615 Å, and the average tetrahedral Al-O distance is 1.770 Å. Smith and Bailey (1962) show that this average Si-O distance is close to that for other silicates in which three corners of each tetrahedron are shared with other tetrahedra. They also suggest that the average Al-O distance is close to the extrapolated value for layer silicates. If these are to be accepted as "expected" averages for sillimanite, the anomalous positions of the cations must be explained.

The anisotropic temperature factors for O_c indicate a vibrational configuration corresponding to an oblate spheroid whose circular equator lies in the plane normal to the mirror plane containing Si, Al_2, and O_c, and is essentially parallel to the bisector of the Si-O_c-Al_2 angle. The root-mean-square amplitude of vibration in the equatorial section is 0.12 ± 0.01 Å; that normal to this section and directed toward Si and Al_2 is 0.06 ± 0.02 Å. The equivalent isotropic temperature factor calculated for O_c is 0.86, as compared with values ranging from 0.35 to 0.50 for the other three oxygen atoms in the asymmetric unit, and 0.36 to 0.42 for the oxygen atoms in andalusite (Burnham and Buerger, 1961).

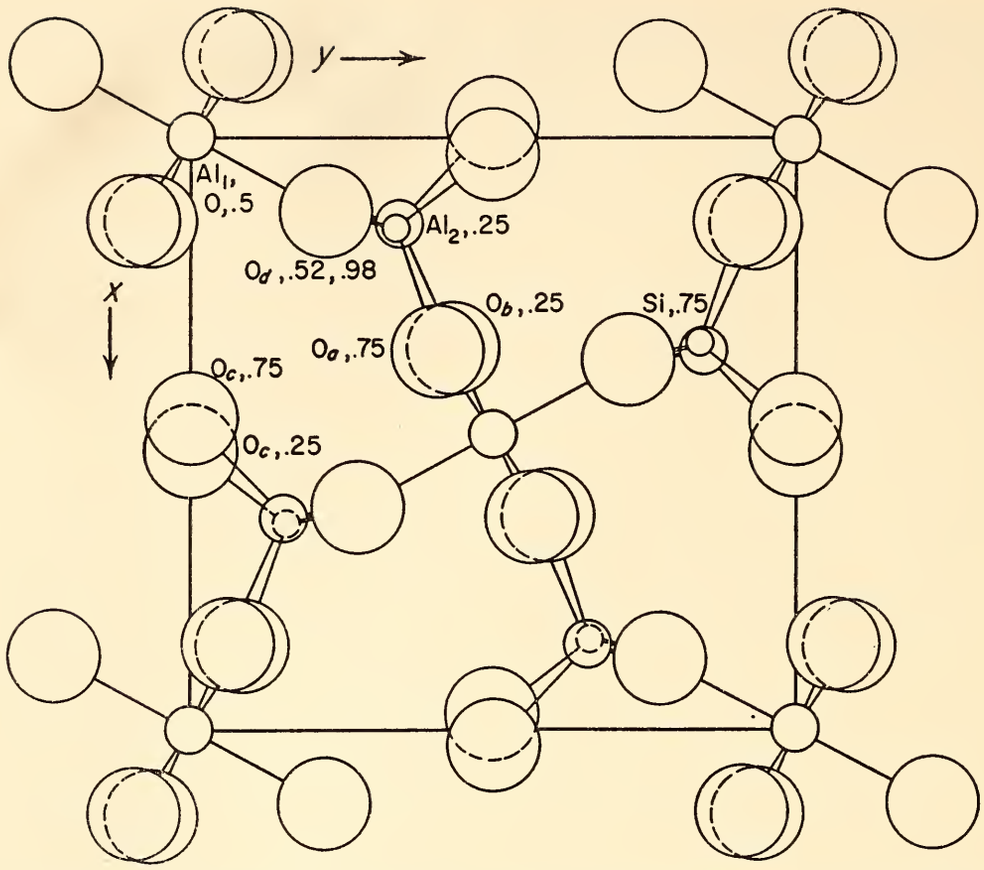

Fig. 43. Projection on (001) of the refined structure of sillimanite showing cation-anion bonds. The z coordinate of each atom is given beside its designation.

Since there is a local charge imbalance of -0.25 on O_c, the abnormally short bond distances could be attributed to excess Coulomb attraction between the cations and O_c. The indicated vibrational anisotropy may consequently arise as compensation for an electron-density distribution related to anomalous bond character not considered in the spherical scattering factor curves used in refinement. This explanation, however, does not appear to be consistent. The Al_2-O_b distance is 0.04 Å larger than the Al_2-O_c distance, yet O_b also bears a charge deficiency of -0.25. The shortest Si-O bond in andalusite is directed to an oxygen with a charge excess of 0.2, and the two shortest Al-O bonds in the five-coordinated aluminum group of andalusite involve oxygens with charge imbalances of -0.4 and $+0.1$.

Alternatively, the indicated thermal motion of O_c may represent an average electron-density distribution of atoms with normal vibration amplitudes but different time-average coordinates in different unit cells. Under this hypothesis the actual position of O_c from ring to ring is displaced from the coordinates listed in table 19 to positions within the equator of the oblate vibrational spheroid but not necessarily on the mirror plane. An approximation to the resulting effect on bond distances can be computed by averaging the distances over the indicated thermal motion assuming the cation and anion to vibrate independently. Averaging increases the Si-O_c distance to 1.576 Å and the Al_2-O_c distance to 1.732 Å. Both increases are significant relative to the standard errors of the bond distances, but are not sufficiently large to normalize the distances.

Further studies of this important crystal chemical problem are now under way. The positional variation hypothesis

will be tested by examining the structure at very low temperatures. If the oxygen atom, O_c, is, in fact, statistically distributed, the apparent thermal motion should not diminish with decreased temperature. If, however, the large temperature factor actually represents thermal vibration, it will be measurably reduced at low temperatures.

The Crystal Structure of Fe Mica

N. Morimoto, J. D. H. Donnay,[7] *and G. Donnay*

Work on synthetic iron mica (*Year Book 60*, p. 214) has been continued. The crystal structure was determined by the three-dimensional least-squares methods. The computations were carried out at the National Bureau of Standards, with the help of Dr. Helen Ondik, on the IBM 7090, using the modified Busing program. The intensity data, without absorption correction, gave $R = 0.23$, including nonobserved reflections, and $R = 0.13$, excluding nonobserved reflections, after four cycles of least-squares refinement. The absorption correction was then applied to the data by means of the program of C. W. Burnham. The corrected data gave $R = 0.21$ or 0.09, according as the nonobserved reflections were or were not included, after three cycles of refinement. These last computations were performed on the IBM 7090 of the Johns Hopkins Computing Center, using the Trueblood program as modified by Koenig with different isotropic temperature factors for the different atoms. The work is still in progress.

On the Transitions of Bornite

N. Morimoto

The transition mechanisms of the three polymorphic forms of bornite (Morimoto and Kullerud, 1961) were studied from the structural viewpoint.

The crystal structure of the high-temperature form is essentially the anti-fluorite structure, only slightly more complicated. The sulfur atoms occupy the nodes of the cubic face-centered lattice with $a = 5.50$ Å, being cubically close-packed. Each sulfur tetrahedron, on the average, contains ¾ of a metal atom. This fractional atom is itself statistically distributed over twenty-four equivalent sites inside the sulfur tetrahedron. Thus, in the whole unit cell, six metal atoms are statistically distributed over $24 \times 8 = 192$ sites.

The cubic edifice of the metastable form is a result of twinning of a large number of small domains in eight different orientations. Each such crystal has a rhombohedral cell with $a_{rh} = 6.70$ Å and $\alpha = 33°32'$.

The structure of this rhombohedral form can be derived from that of the high-temperature form considered along the body diagonal (111) of the cube (fig. 44). All the sulfur atoms stay in place, retaining the cubic close packing. Of the four sulfur tetrahedra sites, two do not change at all. One becomes vacant, and the metal atom that occupied it in the high-temperature form is redistributed among the other three sites. The corresponding three sulfur tetrahedra now contain one full atom apiece. To compensate for the vacant site, the last metal site is slightly displaced. The statistical distribution of ¾ of a metal atom among twenty-four possible sites inside each sulfur tetrahedron changes to the statistical distribution of one metal atom among four possible sites.

Figure 45 shows the structural relations between the high-temperature and the metastable forms, both of which consist of layers parallel to $(111)_{rh}$. Two structures are built on the basis of the cubic close packing of the sulfur atoms. The statistically distributed metal atoms are represented as bands.

The distance between the M_I layer and the sulfur layer becomes shorter in the metastable form, suggesting the possibility that the Fe atoms concentrate in M_I layers. Although the structure of the

[7] The Johns Hopkins University.

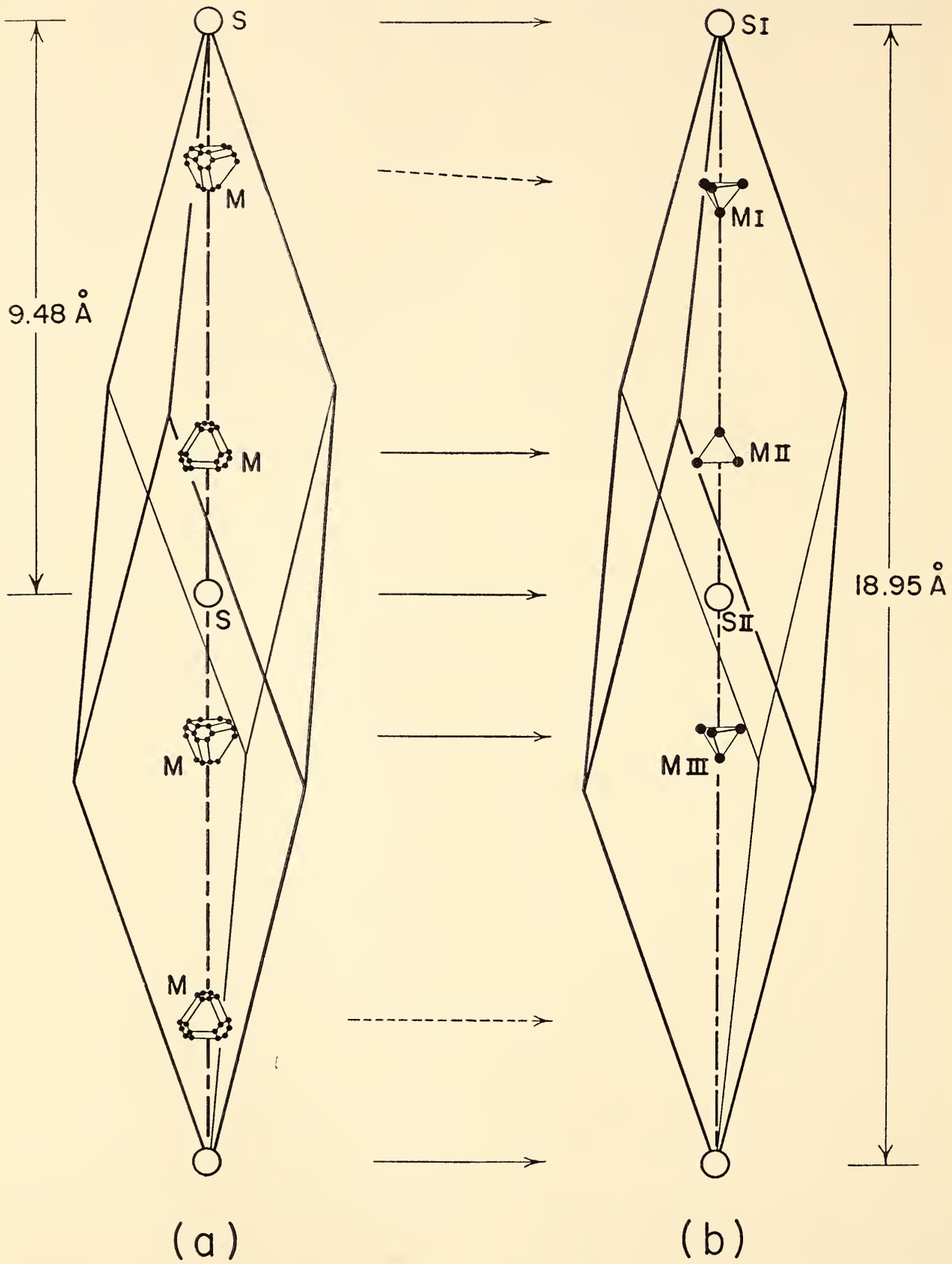

Fig. 44. Derivation of the structure of metastable form from that of high-temperature form.

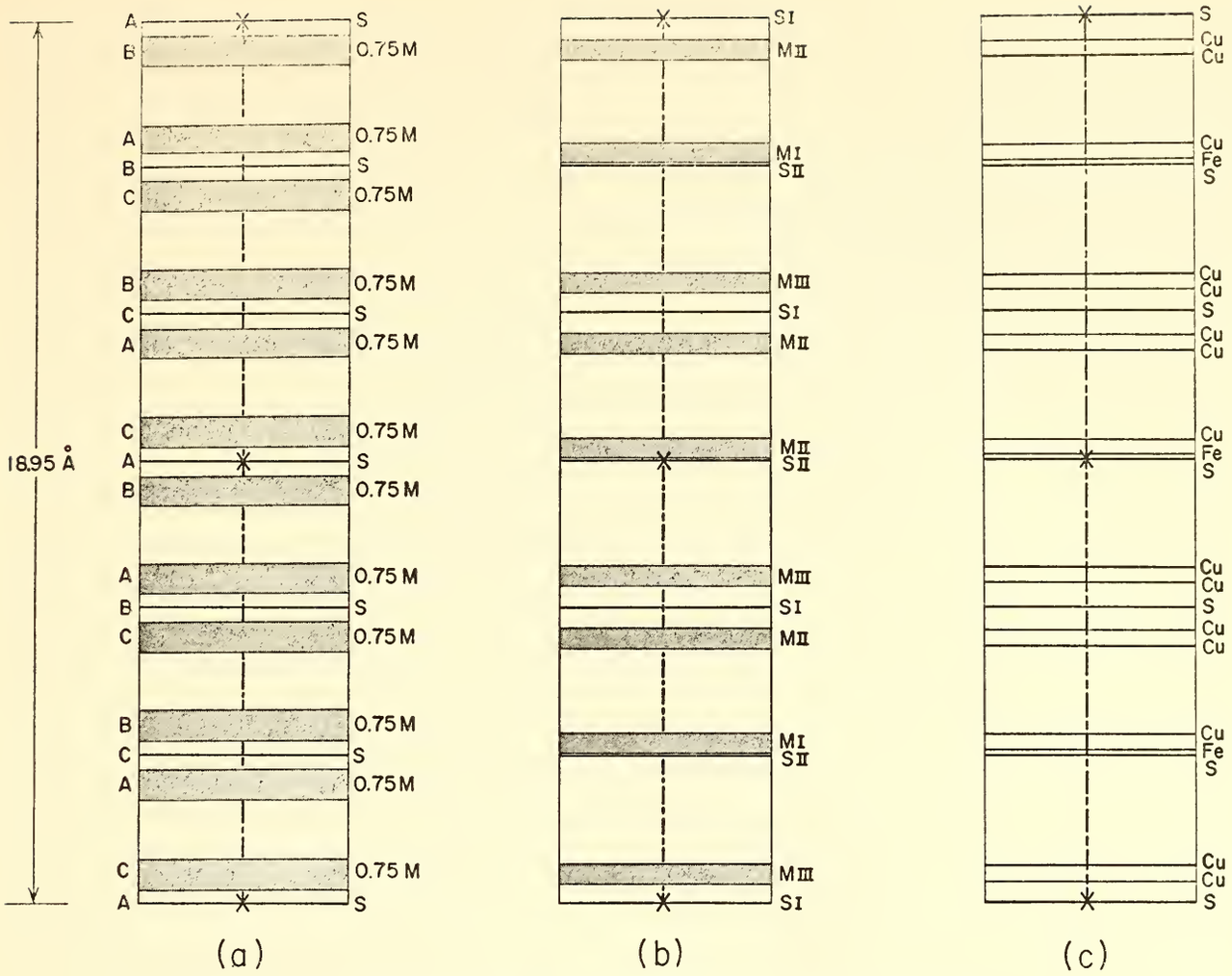

Fig. 45. Layer structures of the three polymorphic forms of bornite.

low-temperature form was not actually determined, it seems likely that the metal atom, statistically distributed at the four corners of a tetrahedron in the metastable form, will occupy one of the four sites in the low-temperature form. The stoichiometric composition confirmed for most natural bornite suggests that the Fe atoms must take some definite positions, the M_I positions, which are closer to the sulfur atoms than other metal positions. This relation in the low-temperature form is shown by lines in figure 45.

The arrangements of the metal vacant layers change their orientations according to a simple twin law in the metastable and possibly in the low-temperature forms. Domain structures always take place on transition from the high-temperature to metastable forms. This indicates that the metal vacant layers cannot stably keep their orientation over a long distance. The diffracted X rays from each crystal (= domain orientation) are not coherent with those from other crystals. The domains themselves, however, must be small, since the twins cannot be recognized as such by direct methods of observation. The volumes of the different domain orientations must be nearly equal so as to give cubic or tetragonal symmetries.

The twinning found in the metastable bornite is different from usual twins in that most of the atoms (S_I, S_{II}, M_{II}, and M_{III}) build a continuous periodic structure throughout the whole edifice, so that the twin relations apply only to the M_I and the vacant M positions. Such slight structural rearrangements take place that the transition heat should be very small and the transition unquenchable.

ORE MINERALS

Increased emphasis has recently been placed on applications of the synthetic systems to ores. Specimens have been systematically collected in a number of mines, and their mineral assemblages have been studied in polished sections and by X rays. Employment on these mineral assemblages of the geological thermometers that we have developed in recent years has produced interesting new information about the formation of the ores but has also brought forth new problems that demand solution.

Progress in these studies frequently depends on development of new research methods and their subsequent systematic employment to produce a steady flow of new data. The investigations started with the most basic systems and have progressed step by step to those sufficiently complex to include the most important minerals of many common ore types. To date twelve binary and twelve ternary systems have been studied, and rapid progress is being made in quaternary systems.

Laboratory experimentation has recently been facilitated by the development of a simple apparatus for mixing aqueous solution in closed systems and at controlled elevated temperatures. The upper temperature limit of this method in investigated systems slightly overlaps the lower temperature limit for attainment of equilibrium in corresponding dry systems. The mineral assemblages and solid solution compositions obtained in the overlapping temperature range after years of heating of the dry systems are identical with those developed in a few hours in the aqueous systems. Therefore, when such identity can be established, this method can be employed to determine the phase relations in many important systems at low temperatures and by experiments lasting only a few hours each. This method has already been applied to parts of the Fe-Ni-S system and is now being used to clarify phase relations in the Fe-S system at temperatures below 200°C.

The mineral bravoite was found to be stable below 137°C by experiments employing the method of mixing aqueous liquids. Bravoite is a common product of alteration in numerous ores, and, since its thermal stability is now known, its presence may serve as a valuable geological indicator. These experiments also indicate that the $\alpha(Ni,Fe)_{1-x}S$ phase is stable at least down to 150°C, and they have further produced data enabling us to draw the phase diagram for the system at 150°C. Differential thermal analysis and high-temperature X-ray diffraction studies of synthetic as well as natural pentlandite $(Fe,Ni)_9S_8$ have demonstrated that pentlandite is stable only below 610°C, at which temperature it decomposes to pyrrhotite ($Fe_{1-x}S$) and the $Ni_{3\pm x}S_2$ phase. This mineral commonly occurs with pyrrhotite in ultrabasic rocks. The sulfides are believed to have segregated as liquid drops from the rock magma. Earlier interpretations of the ore assemblages were based on the assumption that the pentlandite-pyrrhotite pair is stable to at least 850°C. The new information on pentlandite stability relations necessitates reinterpretation of field occurrences and has significant effect on the theory of formation of such ores. Differential thermal analysis experiments have also demonstrated that a two-liquid + vapor region extends across the sulfur-rich part of the ternary Fe-Ni-S system at temperatures above 991°C for the Ni-S side and above 1083°C for the Fe-S side.

The upper stability curve of linneite (Co_3S_4) has been determined up to 2000 bars. In the presence of vapor this mineral decomposes to $Co_{1-x}S$ and CoS_2 at 665°C. This study, similar to the study of polydymite, is of interest because the reaction $Co_3S_4 \rightleftharpoons Co_{1-x}S + CoS_2$ involves only solids.

In the literature several phases have been reported to exist in the Mo-S

system. However, only hexagonal molybdenite (MoS_2) has been established as a mineral species. Two MoS_2 forms were made synthetically, one rhombohedral at low temperatures and one hexagonal at elevated temperatures. Apparently the rhombohedral form is metastable. The only other phase obtained in the system is a monoclinic compound of approximately Mo_2S_3 composition. This phase is not stable below 610°C, where Mo and MoS_2 are stable together.

The phase relations in the Cu-Ni-S system have been studied at 600°C. No ternary compound occurs, and solid solutions extend only very short distances into the ternary system from the binary joins.

The Fe-Ni-As system has been studied at 800°C. Two ternary phases occur: $(Fe,Ni)As_3$ solid solution, which is not found in nature, and an intermediary solid solution, $(Ni,Fe)_2As$, corresponding to the mineral oregonite. Extensive solid solutions exist between some of the phases, for instance between FeAs and NiAs.

Studies of the Fe-Mo-S system have centered on the stability relations of the pyrite (FeS_2)-molybdenite (MoS_2) mineral pair. These two minerals are stable together below 726°C. At this temperature invariant conditions exist in the system, and the five phases pyrite, molybdenite, pyrrhotite, liquid, and vapor are all stable. Above the invariant point pyrite is no longer a stable phase in the presence of molybdenite, and pyrrhotite-molybdenite becomes the stable mineral pair.

Investigations of the complicated system Cu-Fe-S have shown that at various temperatures the pyrrhotite compositions of the pyrite-pyrrhotite-chalcopyrite assemblage are significantly different from those of the pyrite-pyrrhotite assemblage. These results indicate that pyrrhotite temperatures determined on ores containing chalcopyrite as well as pyrrhotite and pyrite are from 45° to 60°C lower than those that would have been obtained had chalcopyrite not been present.

Exsolution textures developed on cooling of synthetic bornite-type solid solutions have been correlated to those found in ores. This study indicates that the thermal history of an ore body cannot be surmised from the presence of exsolution lamellae of one mineral in another. Exsolution lamellae, as shown in laboratory experiments, may indicate rapid cooling, which probably does not take place in ore deposits, or they may originate from a solid solution of low concentration that cooled at a slow rate so that the degree of supersaturation was always relatively low.

Chalcocite-chalcopyrite assemblages are sometimes observed in ores. These minerals are incompatible at high temperatures but, owing to the variation in the metal-to-sulfur ratio in the chalcopyrite field, may form a stable assemblage at very low temperatures.

The phase relations determined on synthetic systems have been applied to systematically collected ore specimens from many localities. Polished-section studies of minerals from the copper deposits of the Keweenaw peninsula revealed the presence of several important minerals not previously reported from this district. These mineral associations give valuable information about the phase relations in the ternary system Cu-Ni-As.

Sphalerite-pyrrhotite and pyrrhotite-pyrite temperatures have been determined from numerous samples from the Brabant Lake, Saskatchewan, ores; from the Ducktown, Tennessee, mines; from the Elisabeth Mine, Vermont; from the Outukompu district in Finland; and from Sulitjelma, Norway.

The Mo-S System

N. Morimoto and G. Kullerud

Study of the Mo-S system by quenching, microscope, and X-ray methods was initiated primarily to elucidate the phase relations between molybdenite (MoS_2),

the most important source of molybdenum, and the other phases reported in the system. The information obtained will serve as a necessary basis for studies of more complicated systems involving molybdenum and sulfur, such as Mo-Fe-S.

Among the many reported phases in this system, only molybdenite, the hexagonal form of molybdenum disulfide, is established as a mineral species. Recently, molybdenum sesquisulfide (Mo_2S_3) was confirmed as a stable phase above 1000°C, coexisting, depending on the composition, with Mo or MoS_2 and sulfur vapor (McCabe, 1955; Stubbles and Richardson, 1960). A new form of MoS_2 with rhombohedral symmetry was synthesized at about 900°C (Bell and Herfert, 1957).

Rhombohedral MoS_2 has the cell dimensions $a = 3.16 \pm 0.1$ Å and $c = 18.37 \pm 0.03$ Å. The c translation is 1½ times as long as that of the hexagonal form. The crystal structure, given by Bell and Herfert and later revised by Semiletov (1962), has the same kind of layer structures as the hexagonal form, where Mo atoms are in triangle prisms of S atoms. Mo_2S_3 has monoclinic symmetry with $a = 8.6335$, $b = 3.208$, and $c = 6.092$ Å, and $\beta = 102°43'$. In this compound, however, Mo atoms are coordinated by octahedral arrangements of S atoms (Jellinek, 1961).

Mo_2S_3 appears to be stable only above 610° ± 5°C. When the elements are used as starting materials, Mo_2S_3 appears above 610° ± 5°C. Below this temperature Mo and MoS_2 are obtained. But when Mo and MoS_2 are the starting materials, Mo_2S_3 is not obtained even after 30 days at 650°C. On the other hand, once Mo_2S_3 is formed it does not break down even after being heated for 1 month at 600°C. The reaction rates of the system are so slow that equilibrium assemblages are not obtained even at 800°C in a reasonable time. Above 900°C, however, equilibrium is usually established in less than 1 week. The exact composition of the Mo_2S_3 phase, determined at 935°C, was found to be $Mo_{2.06}S_3$, which deviates slightly from the stoichiometric ratio. Measurements of the positions of reflections in X-ray powder patterns of "Mo_2S_3" grown in equilibrium with Mo and of those of "Mo_2S_3" grown in equilibrium with MoS_2 give identical results, indicating a very limited solid solution, if any, in this phase at 935°, 800°, and 700°C, the temperatures of these experiments.

According to Semiletov, the structural differences between the hexagonal and the rhombohedral forms of MoS_2 can be explained by assuming different stacking orders of S-Mo-S layers. MoS_2 synthesized below 900°C gives X-ray powder diffraction patterns with broad peaks, and, in general, the lower the temperature of synthesis the broader are the peaks. These poorly defined peaks do not fit exactly either with those of the hexagonal form or with those of the rhombohedral form and are on the whole similar to diffraction effects commonly attributed to stacking faults in layered structures. Above 900°C the peaks become sharp and distinctly show the hexagonal pattern. Natural MoS_2 always shows the hexagonal form, and, once synthesized, the hexagonal form of MoS_2 does not change to the rhombohedral form or to any intermediate form even at low temperatures or after prolonged heating. We believe that the rhombohedral form is metastable throughout the entire temperature range.

Experiments designed to determine possible solid solution on either side of MoS_2 composition showed that, within the limits of error of our methods, MoS_2 is stoichiometric.

The Fe-Ni-S System

G. Kullerud

Liquid immiscibility. Liquid immiscibility between sulfides and silicates has been postulated as a mechanism for the enrichment of many important ores through magmatic segregation with the sulfides separated from the silicate magma

by gravity settling. These sulfides consist mainly of mixtures of pentlandite $(Fe,Ni)_9S_8$ and pyrrhotite ($Fe_{1-x}S$), which are sulfur poor compared with sulfides of other types of deposits. It was suggested in last year's report that the metal-rich sulfide drops not only separate by gravity settling from a silicate magmatic solution but may, even before this event, have formed through liquid immiscibility among the sulfide phases. This view is supported by results of recent investigations in the ternary system Fe-Ni-S.

A region of liquid immiscibility was found by Kullerud and Yund (1962) to exist in the Ni-S system above 991°C and over a composition range from 54.5 to more than 97 weight per cent S. Kullerud (*Year Book 60*) reported the existence of a liquid immiscibility region above 1083°C and over a composition range from 46.2 to more than 95.5 weight per cent S in the binary system Fe-S. Additional differential thermal analysis experiments on ternary compositions have now shown that the liquid immiscibility region extends across the Fe-Ni-S system. Figure 46 shows the results obtained for various amounts of sulfur in a section in which the Fe/Ni ratio is constant (61.4 Fe, 38.6 Ni weight per cent).

In all experiments with more than

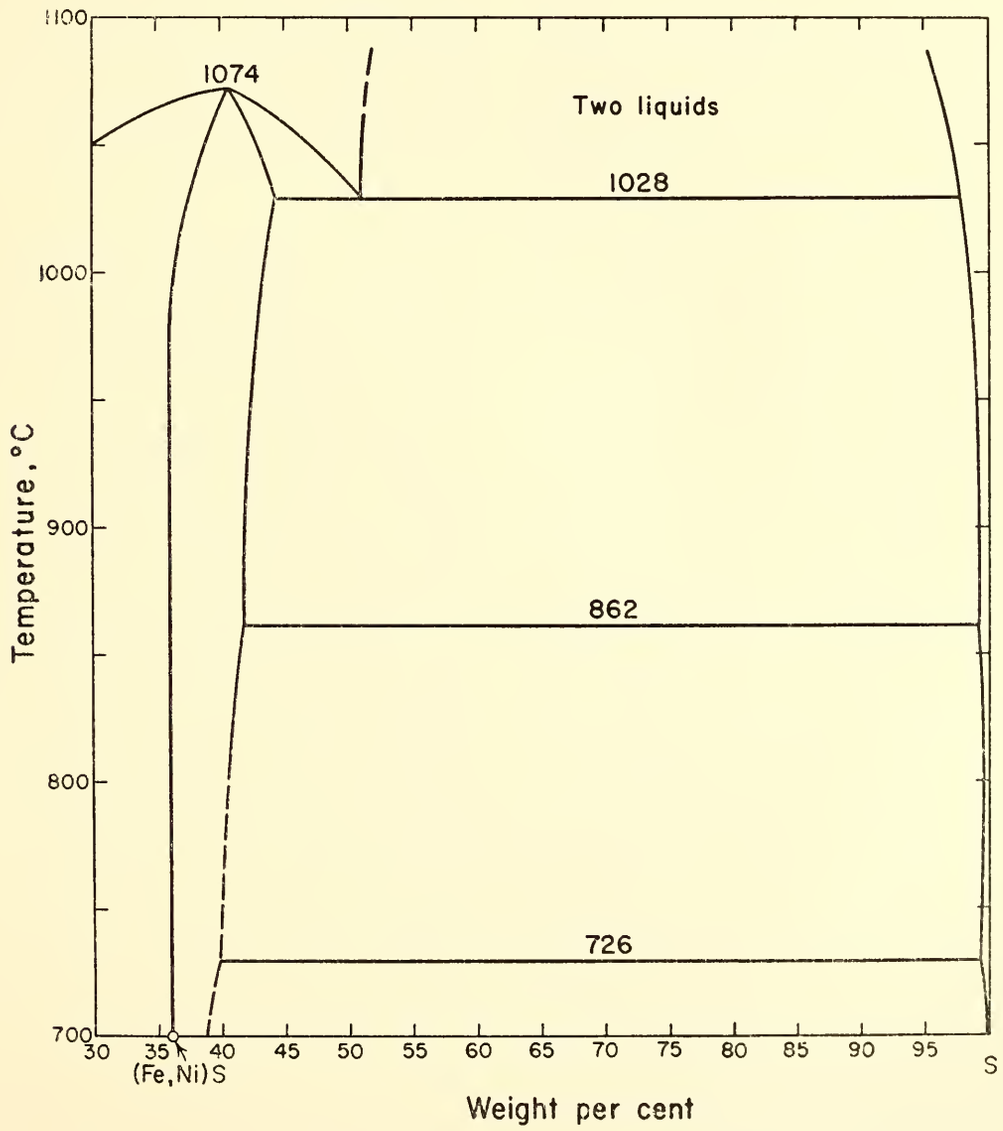

Fig. 46. Phase relations in the section from Fe,Ni alloy with 38.6 per cent nickel to sulfur. Only the part containing more than 30 per cent sulfur is shown.

41.5 weight per cent S a thermal effect, caused by the breakdown of nickel-bearing pyrite (Clark and Kullerud, *Year Book 58*), was observed at 729°C. At this temperature invariant conditions exist, and the five phases, nickel-bearing pyrite, iron-bearing vaesite, hexagonal $(Fe,Ni)_{1-x}S$ solid solution, liquid, and vapor, are all stable. A second heat effect observed at 862°C in all experiments with 41.8 weight per cent or more sulfur demonstrated the disappearance of iron-bearing vaesite from the section.

Above this temperature divariant conditions exist. The phases are now $(Fe,Ni)_{1-x}S$ solid solution, liquid, and vapor. Since the liquid and vapor both contain more than 99.9 weight per cent sulfur the section is now binary. Below 862°C it is, of course, not binary but represents only a projection onto a phase.

Stoichiometric (Fe,Ni)S in the section is stable below 860°C. Above this temperature the solid solution becomes metal deficient even in the presence of excess (61.4 Fe, 38.6 Ni) alloy. The melting relations are similar to those of the $Fe_{1-x}S$ and $\alpha Ni_{1-x}S$ solid solutions.

The maximum melting point is at 1074°C, where the solidus and liquidus curves intersect at about 40.5 weight per cent S. Mix crystals of this composition are the only ones that melt directly to a liquid of the same composition as the solid. The corresponding maximum melting point in the Fe-S system is at 1192°C and about 38.1 weight per cent S, and in the Ni-S system at 992°C and about 38.2 weight per cent S. Thus in the ternary system a curve marking maximum melting of the $(Fe,Ni)_{1-x}S$ solid solution series is slightly concave toward the sulfur corner (see fig. 47). In a *T-X* plot this curve also slopes uniformly without a maximum or a minimum. In all experiments with 49.1 weight per cent or more sulfur a heat effect was also recorded at 1028°C. The liquidus curve on the sulfur side of the maximum melting point recorded for various compositions was found to reach 1028°C when there was about 51.0 weight per cent S. In all experiments with 51 to 97 weight per cent S a single strong peak was recorded at 1028°C in addition to the heat effects at 726° and 826°C.

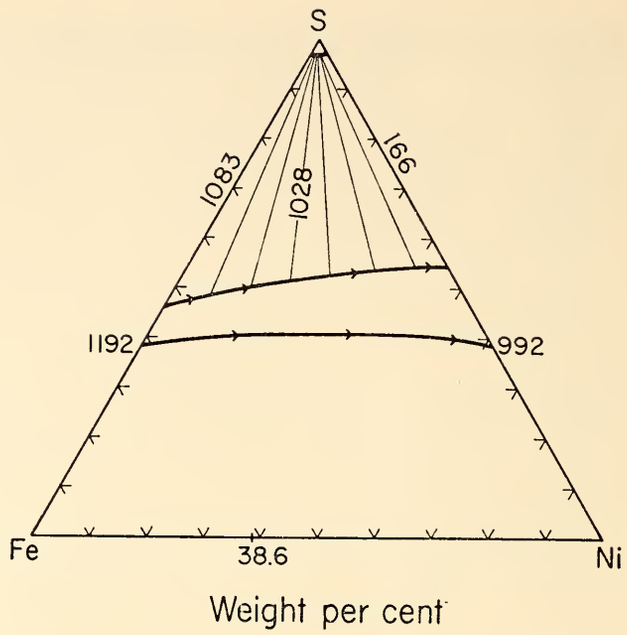

Fig. 47. Liquid immiscibility in the Fe-Ni-S system is shown in the upper part of the diagram. The heavy line extending across the system from 1192°C on the left to 992°C on the right indicates compositions and temperatures of maximum melting of the $(Fe,Ni)_{1-x}S$ solid solution series.

The liquid immiscibility region in this section, therefore, exists above 1028°C and over a composition range extending from about 51 to more than 97 weight per cent S.

Pentlandite stability relations. This mineral, our most important source of nickel, usually occurs in intimate association with pyrrhotite, often in oriented intergrowths that presumably are produced by exsolution. It is found in basic rocks like norites and may well be derived from such rocks by magmatic segregation. Pentlandite, $(Fe,Ni)_9S_8$, has cubic symmetry. The literature reports its melting point at about 875°C. It is readily synthesized in quenching experiments in closed, evacuated silica tubes at temperatures above 500°C. Below this temperature reaction rates are slow and considerable time is required to obtain a homogeneous

product. X-ray diffraction patterns of these materials are invariably identical to the X-ray diffraction pattern of natural pentlandite regardless of the temperature of synthesis. On studying synthetic pentlandite in polished sections and by using oil immersion, however, pronounced differences in textures were observed between those synthesized at 500° to 600°C and those synthesized at 700° to 800°C. The lower-temperature products appeared homogeneous when studied by means of both X rays and the microscope, whereas the higher-temperature materials displayed distinct textures due either to inversion or to breakdown in the solid state.

Since microscopical studies alone could not explain the texture variations, differential thermal analyses were tried; the results are given in figure 48. A few milligrams of Lake Toxaway quartz served as internal standard. The high-low inversion in this material appears at 573°C both on heating and on cooling. On the left side of figure 48 are shown the heating curves (bottom) and cooling curves (top) recorded for synthetic pentlandite of $(Fe,Ni)_9S_8$ composition in which the Fe:Ni ratio equals 1. A very strong thermal effect appears at 610°C on heating and at 609°C on cooling. A second strong peak was recorded at 862°C on heating and at 863°C on cooling. The temperature at which this peak occurs coincides more or less with the melting-point temperature of about 875°C given for pentlandite in the literature. Comparison of the two peaks shows that the one at 610°C is at least as strong as that produced by the melting process. Therefore, the lower-temperature effect cannot readily be explained as the result of a polymorphic inversion but rather indicates the breakdown of the pentlandite phase.

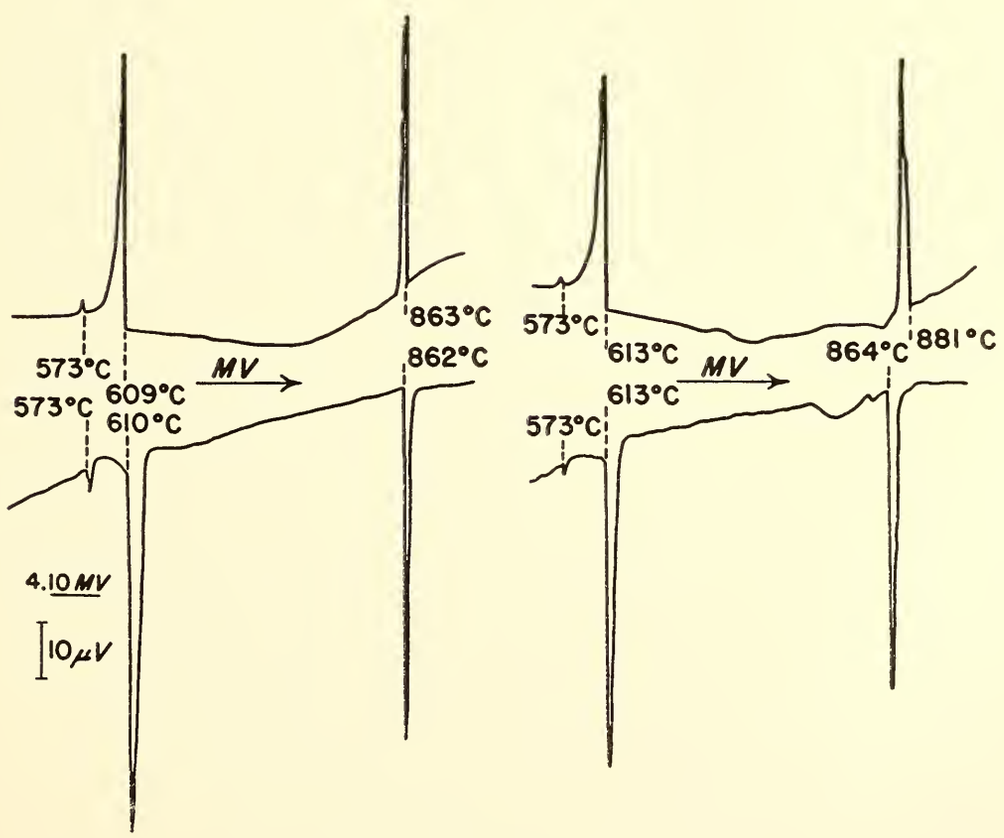

Fig. 48. Differential thermal analysis curves of synthetic pentlandite, heating curve bottom left and cooling curve top left; and of natural pentlandite, heating curve bottom right and cooling curve top right. Heating and cooling rate was 3°C per second in all experiments. The small peak at 573°C is due to inversion in quartz, which was used as internal standard.

DTA curves on pentlandite from the bottom of Frood Mine, Sudbury, are shown on the right side of figure 48. The heating curve is on the bottom and the cooling curve on the top. Lake Toxaway quartz was again used as internal standard. The heating and cooling rates of 3°C/min and all other experimental conditions were the same as those for the synthetic material.

The first strong exothermal peak, which in the synthetic material appeared at 610°C on heating, is recorded at 613°C on both heating and cooling of the natural pentlandite. The second heat effect is recorded at 864°C on heating and at 881°C on cooling. The small disturbances recorded between these peaks (see right side of fig. 48) may be due to accessory minerals in the natural sample.

To determine whether breakdown actually occurs at 610°C, X-ray diffraction films were made with a high-temperature X-ray camera first at room temperature, then at about 600°C, then at about 650°C, and finally again at room temperature. To avoid oxidation the pentlandite specimen was kept in a sealed silica tube constructed for this purpose. The first exposure gave the pentlandite pattern with no other reflections. The second also gave the pentlandite pattern but the reflections were considerably displaced from their positions on the film taken at room temperature. The displacements indicate a much larger unit-cell size at 600°C than at room temperature. The thermal expansion of pentlandite appears significantly larger than that reported for any other sulfide. The exact thermal expansion is being determined. The films made at 650°C contained none of the pentlandite reflections. Instead they showed all the stronger reflections of hexagonal pyrrhotite and all the reflections of the high-temperature $Ni_{3\pm x}S_2$ phase described by Kullerud and Yund (1962). On cooling to room temperature the pattern obtained was again that of pentlandite; no other reflections appeared.

In the Fe-Ni-S system pentlandite lies on a straight line from the $(Fe,Ni)_{1-x}S$ to the $Ni_{3\pm x}S_2$ solid solution. In figure 49, which shows the breakdown of pentlandite schematically, $(Fe,Ni)_{1-x}S$ is on the left side. The Ni content of the $(Fe,Ni)_{1-x}S$ mix crystals and the Fe content of the $Ni_{3\pm x}S$ phase at the temperature of the breakdown have not yet been accurately established. This section is not binary because of the variable metal-to-sulfur ratios of the end members. Pentlandite and pyrrhotite are stable together below 610°C. Pentlandite and heazlewoodite are stable together below about 550°C. Stability depends in part on the Ni-to-S ratio: if this ratio is high the phase may invert to $Ni_{3\pm x}S_3$ at

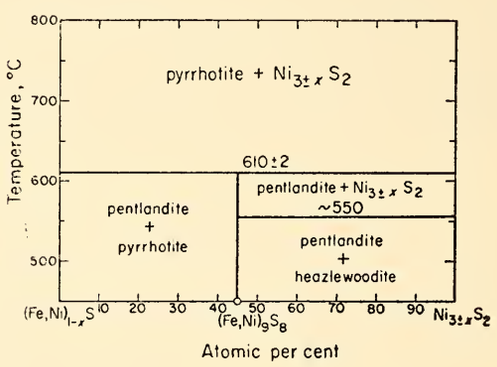

Fig. 49. Schematic illustration of the breakdown of pentlandite to pyrrhotite + the $Ni_{3\pm x}S_2$ phase at 610° ± 2°C.

535°C; if it is low, inversion may take place at 524°C (Kullerud and Yund, 1962). Solid solution of iron in heazlewoodite may also affect the temperature of inversion significantly. Above the temperature of inversion but below 610°C pentlandite is stable with the unquenchable $Ni_{3\pm x}S_2$ phase. Above 610°C pyrrhotite and the $Ni_{3\pm x}S_2$ phase are stable together. The binary $Ni_{3\pm x}S_2$ phase melts incongruently at 806°C (Kullerud and Yund, 1962) to liquid + $\alpha Ni_{1-x}S$.

Very slight disturbances are noticed in the heating and cooling curves in the 820° to 830°C region. They are too small to be caused by incongruent melting of $Ni_{3\pm x}S_2$. In DTA experiments on synthetic mix-

tures of FeS and $(Fe,Ni)_9S_8$ in the 1:1 weight per cent ratio the heat effects were again recorded at 610° and 862°C.

It is probable that a considerable amount of pyrrhotite is soluble in the $Ni_{3\pm x}S_2$ phase and that the melting temperature of that phase increases to 862°C with increasing pyrrhotite content.

Many ores containing pentlandite formed originally much above 600°C. Pentlandite, therefore, is a phase that must have formed during the cooling of the ore bodies. This new information has important bearings on the interpretation of mineral assemblages containing pentlandite.

Bravoite stability relations. Bravoite, $(Fe,Ni)S_2$, is a typical low-temperature mineral. It is commonly found as an alteration product of pentlandite, and it often forms by alteration of linnaeite. It occurs as pore fillings and in cavities in many lead-zinc ores; it occurs in certain sediments; and it has recently been reported (Ramdohr and Kullerud, *Year Book 60*) as a secondary phase in certain chondritic meteorites. In hydrothermal-type deposits bravoite is one of the youngest minerals and often is associated with older minerals like pyrite, chalcopyrite, millerite, linnaeite, and polydymite. In ores believed to have formed through magmatic differentiation of sulfide melts bravoite, one of the youngest minerals, is associated with older pyrrhotite, chalcopyrite, pentlandite, platinum minerals, etc. Bravoite in such ores is formed by alteration of older minerals through the action of water.

It was important to investigate the stability field of bravoite because of its wide geological range of occurrence. This was first attempted (Clark and Kullerud, *Year Book 59*) by the dry method involving the heating of mixtures of iron, nickel, and sulfur in silica tubes. But bravoite did not form in these experiments, which owing to slow reaction rates could not be performed below 200°C. Next, wet chemical methods were attempted. Bravoite was precipitated at room temperature with ammonium polysulfide from aqueous solutions containing weighed amounts of dissolved ferrous ammonium sulfate and nickel sulfate. These precipitates, which were exceedingly fine grained, were next heated in silica tubes with a slight excess of ammonium polysulfide at specified temperatures and for specified periods of time. The products of the heating experiments were readily identified in X-ray diffraction patterns and, much less readily, in polished sections.

The results of dozens of experiments are shown in figure 50. On heating,

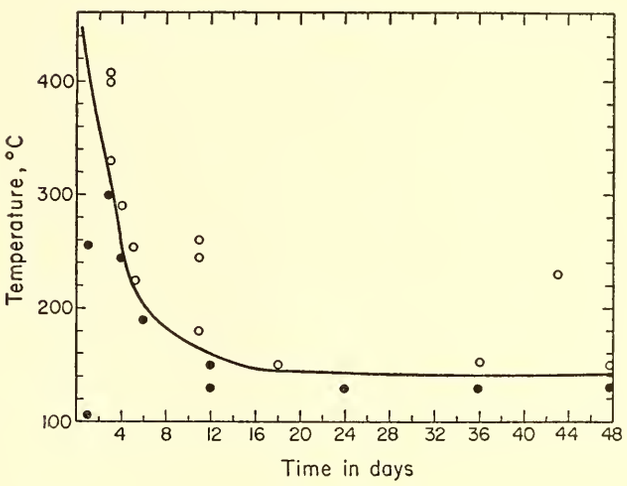

Fig. 50. The curve shows the rate of breakdown of precipitated bravoite at various temperatures. It is practically parallel to the horizontal axis after 36 days and indicates that bravoite is stable below about 140°C.

bravoite breaks down to pyrite + vaesite; the rate of breakdown is given by the curve. Below the curve bravoite persists, and above it, has decomposed. Extrapolation of this curve to the point where it parallels the time axis indicates that bravoite is stable below approximately 140°C. This method is very time-consuming, and since the reactions cannot be reversed the temperature derived by extrapolation of the rate curve may be significantly too high. To save time in this kind of experimentation and to assure equilibrium conditions, a simple method

was devised by which the two solutions could be heated separately to the desired temperature and then mixed; it is described in a separate section. The first experiments were performed at 200°C. The solutions were heated separately to this temperature and then mixed. The immediate reaction taking place on mixing was strongly exotherm and manifested itself by raising the temperature in the reaction vessel by about 5°C. The temperature gradually decreased to 200°C, and the vessel was thereafter kept at that temperature for 1 hour. The products were pyrite and vaesite, which by X rays were found to have identical cell dimensions and the same compositions as pyrite and vaesite synthesized together at 200°C by dry experimentation over a period of 550 days. The identities of the products obtained by these two methods at one and the same temperature are encouraging and indicate that equilibrium data may be obtained by the mixing-of-solutions method at temperatures too low for dry synthesis. Additional experiments showed that bravoite is stable below 137° ± 6°C. The reversibility of the reaction $2(Fe,Ni)S_2 \rightleftharpoons FeS_2 + NiS_2$ was demonstrated. In one experiment performed for this purpose the liquids were mixed at 150°C, where pyrite + vaesite form. Then the temperature was lowered to 130°C and maintained for 72 hours. After this period of time, bravoite was detectable in X-ray diffraction powder patterns.

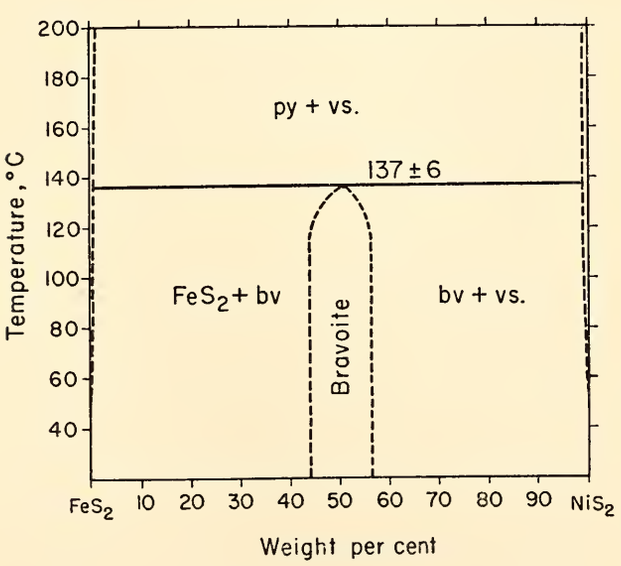

Fig. 51. Bravoite is stable below 137° ± 6°C as determined in experiments involving mixing of liquid at elevated temperatures. Below 137°C bravoite is stable with FeS_2 (pyrite or marcasite) or with vaesite, depending on the bulk composition. Above this temperature pyrite and vaesite form a stable mineral assemblage.

In figure 51 the stability of bravoite is shown in relation to pyrite and vaesite. Bravoite and FeS_2 (marcasite or pyrite) are stable together below 137°C and form a common mineral assemblage in nature. Bravoite and vaesite are also stable together below 137°C. This assemblage was previously not known to exist in nature, but we have now found it in specimens from southeast Missouri.

The Fe-Mo-S System

G. Kullerud and Peter R. Buseck

Minerals in the Fe-Mo-S system are pyrite (FeS_2) and pyrrhotite ($Fe_{1-x}S$) along the Fe-S join, and molybdenite (MoS_2), our most important source of molybdenum, on the Mo-S join. In addition, a phase of approximately Mo_2S_3 composition occurs in the synthetic system but has not been established as a mineral.

The phase relations between pyrite and molybdenite are of immediate interest because these two minerals occur together in the majority of the ores mined for molybdenum. Pyrite is stable to 743°C, where it melts incongruently to pyrrhotite + liquid. Pure molybdenite is stable to about 1350°C.

Since pyrite and molybdenite are stoichiometric compounds as closely as can be determined by our methods, the join FeS_2-MoS_2 is essentially binary even in the presence of excess sulfur. This sulfur is added to avoid decomposition of FeS_2 at temperatures below its stability limits through loss of sulfur to the vapor phase. Mixtures containing excess sulfur were heated at 700°C and lower tempera-

tures for extended periods of time. Pyrite and molybdenite remained stable together in all these experiments. Determinations of the cell dimensions by means of X-ray diffractometer methods of both phases before and after heating showed no measurable change in either pyrite or molybdenite. In polished sections pure synthetic pyrite appears identical with pyrite heated together with molybdenite, and pure synthetic molybdenite appears identical with molybdenite heated with pyrite.

To determine the solubility of pyrite in molybdenite and that of molybdenite in pyrite, synthetic FeS_2 and MoS_2 were heated together at 724°C for 11 days. Subsequent measurements of d_{311} of the pyrite with Si internal standard gave $a_0 = 5.418 \pm 0.002$ Å, which is identical with the values given by Swanson, Gilfrich, and Ugrinic (1955) and Kullerud and Yoder (1959) for pure FeS_2. Measurements of d_{006} of molybdenite after being heated with FeS_2 using SiO_2 as internal standard gave $c = 12.294$ Å, which is identical with the value of $c = 12.295$ Å given by Swanson, Gilfrich, and Ugrinic for pure MoS_2. These results indicate that very little if any MoS_2 is soluble in FeS_2 at 724°C, and that very little if any FeS_2 is soluble in MoS_2 at the same temperature. This conclusion is based on the assumption that if solid solubility existed in either phase it would have measurable effects on the lattice dimensions of the host materials.

In DTA experiments on various FeS_2-MoS_2 mixtures, all with excess sulfur, a strong thermal effect was recorded at 726° ± 3°C both on heating and on cooling. This is the maximum temperature at which pyrite and molybdenite can coexist as a mineral pair in the presence of vapor. Above this temperature pyrrhotite and molybdenite form the stable mineral association. The five phases pyrrhotite, pyrite, molybdenite, liquid, and vapor are all stable at 726°C, and invariant conditions, therefore, exist in the ternary system at this temperature. This invariant point is situated on the FeS_2-MoS_2 join at about 95 weight per cent FeS_2. This join is binary below the 726°C invariant temperature.

The Cu-Ni-S System

G. Moh and G. Kullerud

The phase relations in this system have been studied at 600°C in evacuated, sealed silica tubes. The phases that occur are chalcocite (Cu_2S), digenite (Cu_9S_5), and covellite (CuS) along the copper-sulfur join; heazlewoodite (Ni_3S_2) and the high-temperature $Ni_{3\pm x}S_2$ phase as well as Ni_7S_6, millerite (NiS), $\alpha Ni_{1-x}S$, polydymite (Ni_3S_4), and vaesite (NiS_2) on the nickel-sulfur join. At 600°C the only stable binary phases are chalcocite, digenite, $Ni_{3\pm x}S_2$, $\alpha Ni_{1-x}S$, and NiS_2. There are no ternary compounds. The limited solid solutions among the stable phases and their stability relations are shown in figure 52. At 600°C complete solid solution exists between digenite and chalcocite, which we will refer to as the chalcocite solid solution. However, this solid solution does not extend very far into the ternary system. Experiments with mixtures of members of the chalcocite solid solution and $Ni_{3\pm x}S_2$, $Ni_{1-x}S$, or NiS_2 showed that the ternary solid solution extends much less than 0.5 per cent toward $Ni_{3\pm x}S_2$ and less than 1 per cent toward both NiS and NiS_2. NiS_2 is a stoichiometric compound (Kullerud and Yund, 1962) that takes 1.0 per cent Cu_9S_5 into solid solution at 600°C. The $\alpha Ni_{1-x}S$ phase forms solid solution with the chalcocite solid solution. This solubility is very low in the nickel-deficient part of the $Ni_{1-x}S$ solid solution but increases as the nickel deficiency decreases and is about 1.3 per cent at the point of stoichiometry. The $Ni_{3\pm x}S_2$ phase that forms the most extensive binary solid solution of all the compounds in this system also, expectedly, forms the largest ternary field. It extends about 3.5 per cent toward Cu_2S and 2.5 per cent toward Cu. The solubility of sulfur is too small to be

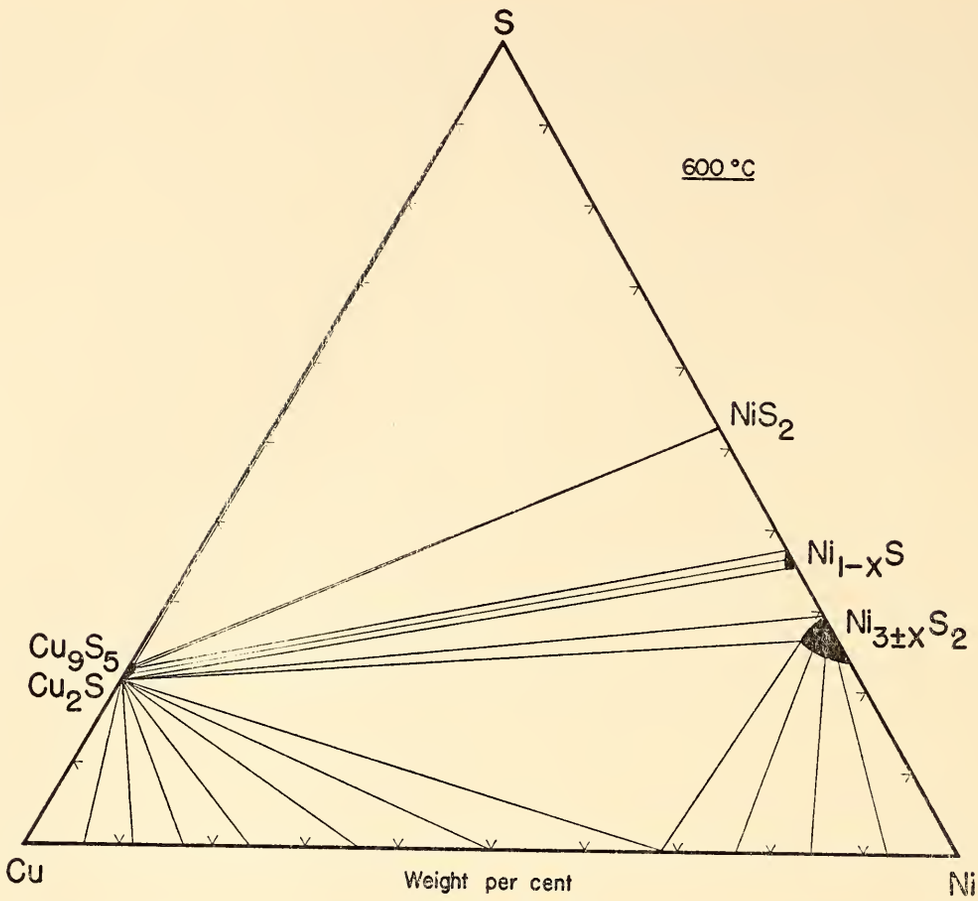

Fig. 52. Phase relations in the Cu-Ni-S system at 600°C. All phases or phase assemblages coexist with vapor, and the vapor pressure is that of the system.

measured in Cu or Ni or in the Cu-Ni solid solution series.

Since all experiments were performed in rigid tubes vapor is present in equilibrium with all phases or phase assemblages given in figure 52. The univariant assemblages are: chalcocite s.s., vaesite, liquid, and vapor; chalcocite s.s., vaesite, $\alpha Ni_{1-x}S$ s.s., vapor; chalcocite s.s., $\alpha Ni_{1-x}S$ s.s., $Ni_{3\pm x}S_2$ s.s., vapor; chalcocite s.s., $Ni_{3\pm x}S_2$ s.s., CuNi alloy, vapor. This CuNi alloy contains about 68 per cent Ni as determined from tie-line intersections and by X rays. Divariant regions are digenite, liquid, vapor; chalcocite s.s., vaesite, vapor; chalcocite s.s., $\alpha Ni_{1-x}S$ s.s., vapor; chalcocite s.s., $Ni_{3\pm x}S_2$ s.s., vapor; chalcocite s.s., CuNi alloy (ranging in composition from pure Cu to CuNi with about 68 per cent Ni), vapor; $Ni_{3\pm x}S_2$ s.s., NiCu alloy (ranging in composition from pure Ni to NiCu with about 32 per cent Cu), vapor.

The Fe-Ni-As System

Peter R. Buseck

Eleven binary compounds occur in the system Fe-Ni-As. Along the nickel-arsenic join these include $Ni_{5-x}As_2$ in both a stable (β) and a metastable (β') form; a low-temperature, possibly metastable, phase of approximate composition Ni_2As (Heyding and Calvert, 1957); $Ni_{11}As_8$, corresponding to the mineral maucherite; $Ni_{1-x}As$, corresponding to the mineral niccolite; and $NiAs_2$ in two polymorphs that correspond to the minerals rammelsbergite and pararammelsbergite. The compounds Fe_2As, FeAs, and $FeAs_2$ are stable along the Fe-As join; of these compounds only $FeAs_2$ has an established mineral equivalent, loellingite. Complete solid solution exists above 912°C between Fe and Ni. Below this temperature the solid solution is limited, with stable

α (bcc) and γ (fcc) phases and a metastable α_2 (bcc) phase occurring. A binary phase, Ni_3Fe, is stable below 503°C (Hansen and Anderko, 1958); awaruite is its mineral equivalent. At least two ternary compounds exist in the system. Of these, the $(Fe,Ni)As_3$ phase is not established as a mineral species, whereas the other, having an approximate composition $(Fe,Ni)_2As$, evidently corresponds to the mineral oregonite. Knowledge of the phase relations in this system is an important step in our efforts to gain understanding of the conditions prevailing during formation of the "magmatic segregate" type of ore. Moreover, this is one of the bounding systems of the very important quaternary system Fe-Ni-As-S, which includes many minerals of common occurrence both in arsenide and sulfide-type deposits, and thus provides a link between the two.

The system was studied at 800°C in evacuated, sealed silica glass tubes in which vapor was always present. Owing to the very slow reaction rates of the arsenides, high temperatures are required for the attainment of equilibrium within a reasonable period of time. All the results were obtained from experiments involving heating for at least 1 month. During this period the materials were reground one or more times to facilitate the reactions.

Because of the sluggish reaction rates and the appreciable solid solution between many of the phases some of the tie lines have so far been located only approximately. Much effort was devoted to verifying the stability and determining the composition of the "intermediary" or $(Fe,Ni)_2As$ solid solution.

Both ternary phases form extensive solid solution. The field of stability of the $(Fe,Ni)As_3$ phase does not extend to either of the binary end members. Its stability range has been investigated by Roseboom (1962) and Pleass and Heyding (1962). The compound $(Fe,Ni)As_3$ is analogous to the mineral skutterudite, though apparently skutterudite always contains Co. The composition of the other ternary phase, the "intermediary solid solution," is not so well known, primarily because of the difficulty of detecting it optically. In reflected light it is white, has a high reflectivity, is weakly anisotropic, and is practically indistinguishable from synthetic maucherite and $\beta Ni_{5-x}As_2$. For this reason the determination of its solid solution range is based largely on X-ray diffraction studies. Unfortunately, this method is not precise because a phase present in only a few per cent cannot be detected. However, the (Fe + Ni)/As ratio is about 2, and its solid-solution range extends from at least Ni/Fe = 5 to Ni/Fe = 1.

The X-ray pattern of the "intermediary solid solution" corresponds closely to that published by Ramdohr and Schmitt (1959) for the mineral oregonite, found with awaruite in Josephine County, Oregon. The composition given by Ramdohr and Schmitt for oregonite, however, does not lie within the range of the synthetic solid solution field at 800°C, but it is known only from X-ray fluorescence. At the low temperature at which the oregonite presumably formed, the "intermediary solid solution" may extend to the oregonite composition.

A number of the tie lines for the system Fe-Ni-As that have been located are listed below. Those described by Roseboom (1958) for the higher arsenides are not included. Tie lines run from α_2Fe-Ni to Fe_2As, to the "intermediary solid solution," and to $\beta Ni_{5-x}As_2$. Others extend from the "intermediary solid solution" to maucherite, $\beta Ni_{5-x}As_2$, Fe_2As, FeAs, to both the Fe-rich and Ni-rich sides of the solvus for the solid solution series between FeAs and niccolite, and toward niccolite solid solution.

There are several univariant regions. Those containing α_2Fe-Ni also include αFe and Fe_2As, Fe_2As with "intermediary solid solution," and "intermediary s.s." with $\beta Ni_{5-x}As_2$. Other univariant regions containing the "intermediary s.s." are those with Fe_2As and FeAs, $\beta Ni_{5-x}As_2$

and maucherite, and two phases of the FeAs-niccolite solid solution series.

There are also a number of prominent divariant regions such as the one between $\beta Ni_{5-x}As_2$ and γFe-Ni and those between the "intermediary s.s." and Ni-bearing FeAs as well as Fe-bearing niccolite.

Grains whose compositions lie within the two-phase regions between the "intermediary s.s." and Fe-bearing niccolite or Ni-bearing FeAs commonly display a distinctive myrmekitic texture. It is extremely fine grained and visible only under very high magnification; even then the phases present cannot be optically identified. However, it appears that in some samples both host phases contain these intergrowths, whether as the result of decomposition of a solid solution on quenching or as the result of a liquid's having been present is not clear.

Evidence, as yet inconclusive, has been found for the existence of a third ternary phase situated in the field bounded by the univariant region containing α_2Fe-Ni and the "intermediary s.s." together with Fe_2As on the one hand and $\beta Ni_{5-x}As_2$ on the other.

At lower temperatures awaruite (Ni_3Fe) appears as a phase. Oregonite, in its only known occurrence, is associated with awaruite. As the tie lines at 800°C run from α_2Fe-Ni to $\beta Ni_{5-x}As_2$, a switch in tie lines is necessary to establish oregonite and awaruite as a stable mineral pair at low temperatures.

The Cu-Fe-S System

Pyrrhotite-Pyrite-Chalcopyrite Relations

K. v. Gehlen[8] *and G. Kullerud*

The composition of pyrrhotite when deposited in equilibrium with pyrite is a useful indicator of the temperature conditions that existed when the assemblage formed, provided that the pyrrhotite maintained its original iron-to-sulfur ratio during the subsequent cooling process. Most ores that contain these two minerals also contain additional minerals such as chalcopyrite, sphalerite, galena, and often small amounts of magnetite, some of which are known to form measurable solid solution with one or both members of the pyrrhotite-pyrite pair. The pyrrhotite solid solution is then no longer binary but becomes ternary or even more complex, and application of the phase relations in the strictly binary synthetic system Fe-S to such ores becomes hazardous. Chalcopyrite is perhaps the most common mineral occurring with the pyrrhotite-pyrite pair, and pyrrhotite can take significant amounts of copper into solid solution. Therefore, it was of interest to investigate whether pyrrhotite compositions, as determined by d_{102} measurements, in ternary pyrrhotite-pyrite-chalcopyrite assemblages at controlled temperatures would coincide with the values given by Arnold for binary pyrrhotite-pyrite assemblages. Mixtures of iron, copper, and sulfur with bulk compositions inside the univariant pyrrhotite-pyrite-chalcopyrite field were heated in evacuated, sealed silica tubes at various temperatures. Measurements of the d_{102} value of the pyrrhotite synthesized in equilibrium with pyrite and chalcopyrite at 600°C gave 2.0532 ± 0.0017 Å. Pyrrhotite synthesized in equilibrium with pyrite in the absence of chalcopyrite, at the same temperature, gives d_{102} = 2.0497 ± 0.0007 Å according to Arnold (1962). This value is identical, within the limit of error of our measurements, with our results on pyrrhotite synthesized in equilibrium with pyrite at 600°C.

The difference in d values of pyrrhotites synthesized with and without chalcopyrite present is significant to geological thermometry. Application of the d-versus-composition and T-versus-composition curves by Arnold would only in the second case lead to the correct temperature estimate of 600°C. The d value of pyrrhotite coexisting with chalcopyrite and pyrite would indicate a

[8] University of Erlangen-Nürnberg.

temperature of only about 550°C. A positive correction of about 50°C would therefore be required at this temperature.

The magnitude of this correction depends on the variation with temperature of the copper content of the pyrrhotite phase and the composition of the chalcopyrite phase. That the required correction diminishes with decreasing temperature is probable but remains to be shown.

The solubility of copper in pyrrhotite exceeds 3 weight per cent at 700°C (Yund and Kullerud, *Year Book 59*), and we have now determined the solubility at 600°C to be about 2 weight per cent.

Sphalerite commonly coexists with the pyrrhotite-pyrite mineral pair; however, the solubility of ZnS in pyrrhotite is negligible even at very high temperatures (Kullerud, 1953). For this reason the presence of sphalerite should not measurably affect the pyrrhotite-pyrite solvus. A situation very similar to that for sphalerite exists for galena, which is also a common mineral in pyrrhotite-pyrite ores.

On the contrary, the presence of iron oxides with the pyrrhotite-pyrite assemblage may affect the pyrrhotite composition significantly since pyrrhotite is very susceptible to oxidation (Kullerud, *Year Book 56*). Numerous such ores contain small amounts of magnetite. The effect of its presence on the pyrrhotite geological thermometer may be significant.

Exsolution Textures and Rates in Solid Solutions Involving Bornite

P. R. Brett

Exsolution textures. Very little is known of the contribution of diffusion and exsolution to the formation of textures during the cooling of ores. There has been little systematic experimental work to back up the interpretation of such textures.

All previous work on exsolution textures in sulfides, mainly on bornite-chalcocite and bornite-chalcopyrite, suggests that lamellae may be retained as an exsolution texture only when the solid solution is cooled from above the solvus in relatively short periods. Complete migration to grain boundaries or a mutual boundary texture results if the solid solution is cooled over longer periods. The common occurrence of exsolution lamellae in ore sulfides has led some recent investigators (e.g., Lyon, 1959) to conclude that some ore bodies cooled from 600° to 200°C in a matter of minutes or hours. Clearly, masses of ore, some comprising millions of tons, cannot cool by conduction at so rapid a rate.

The present study was initiated to investigate this paradox and to gain a better understanding of exsolution textures.

A study of the literature of metallurgy and solid-state physics (e.g., Geisler, 1951; Baker, Brandon, and Nutting, 1959) reveals that exsolution lamellae need not necessarily be formed only by rapid cooling. Lamellae are retained as the stable exsolution texture if the degree of supersaturation is low, in other words, if the solid solution is initially dilute or the cooling rate is slow.

The solid solutions involving bornite (digenite-bornite, chalcocite-bornite, and chalcopyrite-bornite) in the system Cu-Fe-S were chosen for experimental study because phase relations are fairly well understood, because extensive solid solution occurs, and because exsolution textures in this system are commonly seen in ores.

Lamellae have now been obtained in runs cooled at rates as low as 3°C per day for 6 months. As a general rule, the more concentrated the initial solid solution, the less common are lamellae as the final exsolution product. Lenses or mutual boundary textures are the end products of exsolution in the more concentrated solid solutions. Lamellae were often observed in combination with a mutual boundary texture, suggesting that either some of the lamellae did not coalesce to

form irregular grains or there was a late stage of formation of lamellae.

In addition to lamellae and mutual boundary textures, "veining" and "replacement" relations were occasionally observed. The "veining" textures result from the exsolved phase depositing along a continuous series of grain boundaries (fig. 53, pl. 2). The "replacement" textures, in which the exsolved phase appears to replace the host phase, were observed in chalcopyrite exsolved from bornite (fig. 54, pl. 2). Eutectoid textures observed as products of exsolution in the bornite-digenite and bornite-chalcocite pairs are similar to those commonly present in these minerals in ores.

The results of this study indicate that few interpretations from textural evidence may be made on the thermal history of minerals that form solid solution pairs. Exsolution lamellae can indicate extremely rapid cooling (which is not to be expected in mineral deposits) or cooling of a solid solution of a relatively low initial concentration, in which the cooling rate was such that the degree of supersaturation was never high. Veining, pseudo-replacement, and mutual boundary textures can occur as products of exsolution. The utmost caution must be taken in the interpretation of the textural relations between any minerals that may form solid solution pairs.

Rates of exsolution. It has long been suspected that many ore minerals and mineral assemblages remain unchanged during the cooling period, in this way retaining the evidence required to infer the conditions during ore deposition. It is on the supposition that many systems do not equilibrate with falling temperature that the principles of geothermometry are based.

An understanding of the extent of equilibration of mineral systems can be obtained only by the study of reaction rates at different temperatures and pressures. Unfortunately, data on rates of solid-state reactions such as exsolution cannot be considered in terms of a rigorous theory of kinetics, for a reaction rate in the solid state is dependent not only on such variables as concentration, temperature, and pressure but also on the rate of nucleation, diffusion, recrystallization, etc. Moreover, the difficulties involved in determining the exact time for a reaction to proceed to a certain point are considerable. The composite rate cannot be quantitatively considered by separate treatment of each process, for the effect of individual variables cannot be isolated. Nevertheless, such studies can at least ascertain whether reequilibration in the system studied can be expected during the slow cooling of natural mineral assemblages. The time taken for a reaction like exsolution to proceed to a certain point can be determined only by measurement of the change in a composition-dependent property such as cell edge, hardness, or magnetic susceptibility.

The rates of exsolution in the solid solution field bornite-digenite-chalcopyrite in the system Cu-Fe-S were chosen for the present study. This system was selected because phase relations are relatively well known and because there have been suggestions in the past that solvi in this system, when determined, would be useful for geologic thermometry.

The change in composition during exsolution could be ascertained by measuring the a cell edge of exsolved bornite. This cell edge varies markedly with the Cu/(Cu + Fe) ratio. Solid solutions of various compositions about bornite along the bornite-digenite and bornite-chalcopyrite joins were prepared at 700°C. They were then annealed or cooled to 400°, 300°, 200°, and 50°C in various times. If the cell edge of the bornite was constant for a given temperature below the solvus regardless of its original composition, equilibrium was assumed to have been attained.

All runs above 50°C were held at temperature for 2 months or more, and equilibrium was attained in all. When the solid solutions were cooled to 50°C in 3

months, equilibrium was also attained; the same was observed when they were cooled from 600° to 50°C in 1 hour.

When solid solutions of chalcopyrite in bornite were annealed at 100°C for 2 weeks, equilibrium was attained, whereas digenite in bornite, held at 50°C for 3 months, approached equilibrium within approximately 3 weight per cent. When solid solutions of either chalcopyrite or digenite in bornite were cooled from above the solvus to 50°C in 7.5 minutes, equilibrium was closely approached, the variation in cell edge being 10.944 to 10.950 Å (±0.005 Å).

All the runs in which disequilibrium was most pronounced were those in which the original solid solution was dilute. In many runs the dilute solid solution exhibited no exsolution at all after being cooled or annealed in spite of the fact that more concentrated solid solutions exsolved to equilibrium. Doubtless a nucleation problem is involved; the more concentrated solids have a greater degree of supersaturation at the annealing temperature, hence have a greater tendency to nucleate.

It may be concluded from the study that rates of exsolution (and indirectly of solid diffusion) are rapid in this part of the Cu-Fe-S system. Complete equilibration would be expected in nature in times of the order of 1 hour.

To check the extent of equilibration of natural bornites the *a* cell edges of thirteen bornites from nine different localities and environments were measured by means of the X-ray diffractometer. Except for the anomalous red bed bornites mentioned elsewhere in this report, all cell edges correspond to those of stoichiometric bornite (10.950 ± 0.005 Å). This is further evidence that equilibrium is complete in nature.

The fact that equilibration can occur in so short a time casts grave doubts on the use of this part of the system for geothermometry. The majority of sulfide systems, like the minerals in the system Cu-Fe-S, also equilibrate with great rapidity in nature. Unfortunately, these systems are useless as potential geothermometers, because the exsolved phase consistently migrates out of the host mineral, making reconstitution of the original solid solution impossible. The minerals with greatest potential as geothermometers are therefore the most refractory ones (such as arsenides, oxides, pyrite, and sphalerite) and the ones most difficult to study.

Chalcocite-Chalcopyrite Assemblages

P. R. Brett

In the course of the investigation of bornite-chalcopyrite exsolution textures, bornites with maximum sulfur content were prepared along the bornite-chalcopyrite join at 700°C (see *Year Book 59*, figs. 43–45). Chalcopyrite exsolved from the bornite on annealing or cooling to 50°C; another phase was seen in amounts insufficient for determination by X-ray diffraction. By reason of the geometry of the Cu-Fe-S phase diagram, the appearance of the phase, and the composition of the runs, this phase is probably chalcocite.

The tie line chalcopyrite-chalcocite is possible only because chalcopyrite always contains less sulfur than is indicated by its stoichiometric formula, so that chalcocite, bornite, and chalcopyrite are not exactly collinear.

Chalcopyrite formed at 700°C is more deficient in sulfur than chalcopyrite formed at lower temperatures (Yund and Kullerud, *Year Book 59*). Accordingly, chalcopyrite exsolving from a chalcopyrite-bornite solid solution must become more sulfur rich as cooling proceeds. The bornite in equilibrium with the exsolving chalcopyrite must therefore become increasingly poor in sulfur (and iron) as exsolution proceeds, and must form a chalcocite-bornite solid solution that breaks down at low temperature.

Chalcocite-chalcopyrite has often been observed as a natural assemblage, particularly under supergene conditions. In view of the collinearity mentioned above,

it has invariably been taken to be a disequilibrium assemblage. The present work suggests that this need not necessarily be so.

Studies along the join bornite-digenite previously reported by Kullerud were continued down to 50°C. The join bornite-digenite was found to exist at least to 50°C. Therefore, a pyrite-chalcocite join is impossible below the bornite-digenite immiscibility gap. Above this solvus bornite, digenite, and chalcocite form a complete solid solution field (Yund and Kullerud, *Year Book 59*), again prohibiting a chalcocite-pyrite join.

The assemblage pyrite-chalcocite has commonly been reported in copper-iron sulfide deposits. Although much of this chalcocite may be misidentified digenite, there are some well authenticated examples of chalcocite-pyrite. The assemblage must be due to the breakdown of a chalcocite-digenite-bornite solid solution that formed in grain boundary equilibrium with pyrite. A chalcocite-pyrite hypogene assemblage should therefore always be accompanied by bornite and/or digenite, as at Butte.

Assuming equilibrium during deposition, a pyrite-chalcocite assemblage without accompanying digenite or bornite is thus an indication that the two minerals were not formed simultaneously. The chalcocite was probably formed at low temperatures where reaction rates are slowest.

The persistence of pyrite with chalcocite in both hypogene and supergene ores can only be ascribed to the lack of reactivity of pyrite. In view of this inertness, all sulfide assemblages involving pyrite cannot definitely be regarded as equilibrium assemblages until more conclusive evidence is available.

The relationships between compositions and the *a* cell edge of bornite solid solutions were determined by measuring the 2θ values of the (440) reflection on the X-ray diffractometer. Provided that the bornite solid solution is of maximum sulfur content, the relation between *a* and Cu/(Cu + Fe) is linear. This was demonstrated by Kullerud in his studies on the bornite-digenite join (*Year Book 59*). The relationship has now been verified for bornite containing chalcopyrite in solid solution (fig. 55). However, the present investigation revealed that a decrease in sulfur content of less than 0.5 weight per cent can increase the cell edge by as much as 0.015 Å. The cell edge of sulfur-deficient bornite is dependent on (1) the Cu/(Cu + Fe) ratio, (2) the sulfur content, (3) the quenching procedure,

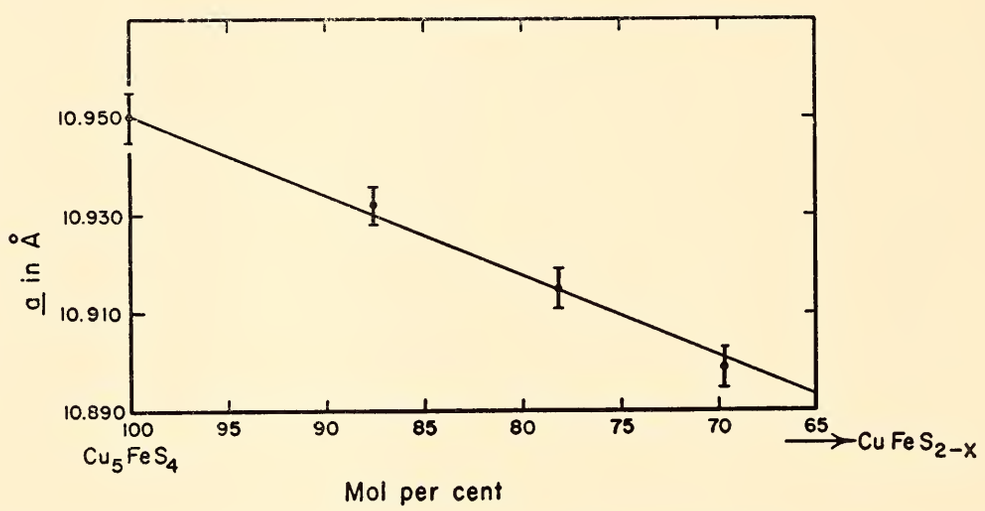

Fig. 55. Variation in the *a* cell edge of bornites with maximum sulfur content in the bornite-chalcopyrite solid solution field at 700°C.

(4) the type of grinding used in preparation of the diffractometer mount. For this reason caution should be exercised in applying data published on bornite cell edges to natural bornite.

Heating Experiments on Natural Bornites

P. R. Brett

Bornite can take some chalcopyrite into solid solution, but this is totally exsolved on cooling, even if the cooling time is only some few minutes (Brett, this report). When heated to temperatures below 400°C, however, certain natural bornites exsolve up to 25 volume per cent chalcopyrite (Wandke, 1926; Takeuchi and Nambu, 1956; Prouvost, 1960; McCauley, 1961). This situation is anomalous in view of the slow cooling that presumably occurs in nature.

Bornite from various localities was heated in evacuated silica glass tubes at temperatures ranging from 75° to 600°C. Bornites from Moonta, South Australia; Messina, South Africa; Bristol, Connecticut; and Magma, Arizona, remained unchanged, but those from Similkaween, B. C., Beaverdell, B. C., and red bed copper localities in Utah exsolved chalcopyrite up to 25 volume per cent. The Utah bornites exsolved chalcopyrite after only 10 minutes at 400°C, but no exsolution occurred even after 10 days of heating at temperatures lower than 75°C. The exsolved phase was identified as chalcopyrite both optically and by its three principal X-ray diffraction peaks.

Anomalous unheated bornite was studied by means of an electron microscope (at the National Bureau of Standards), but no submicroscopic phase of the type suggested by Takeuchi and Nambu (1956) was noted.

In general, bornite cannot be chemically analyzed accurately because small inclusions of other sulfides cannot be eliminated. A sample of the "anomalous" bornite was examined on the electron probe at the U. S. Geological Survey; this technique gave analyses with such a large standard deviation that the results could not be applied in the present study.

The possibility that oxidation could be responsible for the formation of chalcopyrite in some heated bornites seemed worth examining. Two samples of Bristol bornite were finely ground and exposed to the air for 5 days and 4 months, respectively. There was no detectable change in weight on oxidation. Bornite from Bristol was chosen because of its purity and because it did not exsolve chalcopyrite on heating. Approximately 2 volume per cent chalcopyrite was found as irregular blebs when the oxidized bornite was heated at 270°C for 1 hour. After 1 hour at 300°C synthetic stoichiometric bornite oxidized in the same way contained small amounts (less than 1 per cent) of a very fine-grained second phase, possibly chalcopyrite.

The cell sizes of all bornites were measured both before and after heating, with results shown in table 21. It is apparent from table 21 that the normal bornites have a close to that of stoichi-

TABLE 21. Cell Edge of Bornite from Various Localities before and after Heating

Locality	a before Heating, ±0.005 Å	a after Heating, ±0.005 Å
Magma, Ariz.	10.950	10.950
Bristol, Conn.	10.945	10.945
Messina, South Africa	10.956	10.956
Red bed bornite,* Utah	10.932	10.950
Red bed bornite,* Utah, second locality	10.906	------
Oxidized Bristol, Conn., bornite*	10.958	10.950
Synthetic Cu_5FeS_4	10.950	10.950

* Exsolves chalcopyrite on heating.

ometric bornite, whereas bornites that exsolve chalcopyrite may have a considerably smaller cell edge before heating and on heating revert to a cell size similar to that of stoichiometric bornite. The small cell cannot be ascribed to oxidation, because the cell edge of oxidized Bristol bornite before heating is greater than that of the unoxidized bornite.

When heated at 700°C and quenched the Utah bornite contained no exsolved chalcopyrite, but the cell size had reverted to that of stoichiometric bornite. The low value of a for the anomalous bornite evidently cannot be ascribed to a high iron content. It has been mentioned elsewhere in this report that the lower the sulfur content of a bornite the greater the cell edge. Possibly the anomalous bornites contain more sulfur than stoichiometric bornite. Heating at 700°C would probably expel the excess. At any rate, this is a reasonable explanation of the behavior of the Utah bornite.

It is suggested that at temperatures between 75° and 400°C these sulfur-rich bornites break down to chalcopyrite, stoichiometric bornite, and chalcocite. The chalcocite compensates for the increased copper content of the bornite caused by the exsolution of chalcopyrite. A phase resembling chalcocite or digenite has been observed with chalcopyrite in heating experiments on anomalous bornites by Greig (personal communication, 1961) and Prouvost (personal communication, 1962).

Yund (personal communication, 1962) reports that synthetic sulfur-rich bornites with Cu/Fe ratio equal to or less than the stoichiometric ratio exsolve chalcopyrite when heated. This is additional evidence that the anomalous bornites are anomalous not because they are iron rich or contain oxygen but because they are rich in sulfur. The conclusion is by no means proved, however.

There is good evidence that the anomalous bornites never attained the temperature of approximately 75°C during their formation or later, as it is possible to cause chalcopyrite to exsolve from them at this temperature.

Method for Mixing Liquids at Controlled Temperatures

G. Kullerud

In the past, studies of mineral assemblages in aqueous solutions have ordinarily been conducted by mixing the liquids at 25°C then slowly heating the mixture to a specified elevated temperature at which it was kept for desired periods of time. In this procedure precipitation often takes place as soon as the solutions are mixed, and the phases formed at room temperature or during the heating period may persist metastably for considerable lengths of time. A disadvantage of the method is that equilibrium cannot be proved to have existed in any one experiment. The results of many earlier studies on the pyrite-marcasite relations in which this method was used are probably examples of the shortcomings of the procedure.

Progress in our studies of dry systems is commonly hampered by slow reaction rates. At low temperatures these rates are usually so slow that dry experimentation is out of the question because of the time involved in obtaining equilibrium. Since many minerals in nature form at low temperatures and are often stable only in the region not available to dry synthesis, it is desirable that we develop other methods by which equilibrium can be obtained in a short time.

Bravoite is an example of a low-temperature mineral that we could not synthesize by the dry method. Phase equilibrium in this part of the Fe-Ni-S system could be obtained only down to 200°C, and this accomplishment required 18 months.

The new method allows separate heating to a preassigned temperature of two or if necessary more liquids. One liquid is sealed into a long evacuated Pyrex tube; the other is poured directly into a cylindrical Teflon container that

has a pressure-tight closure. The Pyrex tube is then also inserted into the Teflon tube, and the pressure seal is closed. This unit, which has a thermocouple well similar to that of cold seal and Tuttle bombs, is next placed in a horizontal preheated furnace and heated to the temperature of the experiment. Teflon softens on heating but readily withstands the internal pressure to 200°C under these conditions. When the temperature of the experiment has been reached the liquids are mixed by exerting pressure on the Teflon tube, by means of a specially constructed pair of pliers with jaws, to the point where the Pyrex tube shatters. The reaction that immediately takes place is recorded by the thermocouple, which shows a rapid increase in temperature of as much as 5°C. Thereafter the temperature drops back over the next 10 minutes or so to that recorded before the liquids were mixed. The Teflon tubes are kept in the furnace at temperature from 1 to 100 hours, depending on the temperature of the experiment after mixing of the liquids, to produce well crystallized materials. The products are filtered, washed, and studied by means of X-ray diffraction patterns and in polished sections.

Pyrrhotite from Tem Piute, Nevada

Peter R. Buseck

The composition of hexagonal pyrrhotite when in equilibrium with pyrite has found considerable use as a geological thermometer. Efforts to apply the thermometer to pyrrhotite-pyrite assemblages from the Tem Piute district encountered difficulties because at this locality the pyrrhotite is monoclinic. Arnold and Reichen (1962) suggested that the thermometer may be valid even for such assemblages and that the composition of monoclinic pyrrhotite can be determined by the standard X-ray method if the specimen is first inverted to the hexagonal form by heating in vacuo.

The tungsten-copper deposits at Tem Piute, Lincoln County, Nevada, occur in a skarn aureole surrounding a small granodiorite stock. One of the mines, the Free Tunnel, was studied in detail because of its rather complex and varied ore assemblages. All the metallic minerals occur in a diopside, andradite skarn, which separates the barren intrusion from unmineralized limestone and, locally, hornfels.

The metallic minerals occur as disseminations or small lenses, veining being extremely minor. With the exception of pyrite, chalcopyrite, and scheelite, all of which occur throughout the aureole, the minerals are roughly thermally zoned. In the "inner," formerly hotter, portions of the aureole, in approximately paragenetic sequence are molybdenite, pyrrhotite, magnetite, and marcasite. Near the limestone in the "outer," formerly cooler, parts, are sphalerite, galena, galenobismutite, cosalite, and native bismuth. Except for sphalerite these minerals are sparse.

Pyrrhotite was one of the first metallic minerals to form, and, as such, it was deposited during the earliest, hottest stages of the mineralization period. It is most prominent along the granodiorite-skarn contact but occurs in decreasing amounts farther from the granodiorite. The pyrrhotite is commonly associated with and generally surrounds euhedral crystals of pyrite, thereby indicating its later origin. In an attempt to determine its temperatures of formation all available pyrrhotite was sampled and examined in the laboratory.

Arnold (1962) demonstrated that the d_{102} spacing of hexagonal pyrrhotite is a function of its composition. The composition is dependent on the temperature of formation provided that the pyrrhotite formed in equilibrium with pyrite and that it did not reequilibrate with decreasing temperatures. Most of the Tem Piute pyrrhotite was sampled within 1 mm of pyrite, and all such samples have similar d_{102} values. It is therefore assumed

that these pyrrhotites formed in equilibrium with pyrite. Pyrrhotites that did not form close to pyrite have different compositions from those in contact with it. This would presumably not be so had all the pyrrhotite reequilibrated as temperatures fell during cooling.

In the inversion of monoclinic pyrrhotite to the hexagonal form the time and temperature allowed for annealing are critical; with too long an annealing period or too high a temperature the pyrrhotite reequilibrates, and with too short an annealing time or too low a temperature it does not invert. To determine the optimum time and temperature for annealing, natural pyrrhotite was finely ground under acetone and concentrated magnetically; replicate runs prepared from this material were heated in evacuated silica glass tubes.

Runs were annealed for 0.1 hour at different temperatures. At 700°C and 555°C the pyrrhotite-pyrite reaction is sufficiently rapid for the samples to have reequilibrated. Two runs heated at 346° and one at 455°C inverted but did not have time to reequilibrate. They indicate the same composition within the limits of error of the method. A sample heated at 300°C did not invert in 0.1 hour but did in 1½ months. Likewise, one at 260°C did not invert within 1½ months but did within 1 year. Clearly the temperature of the hexagonal-monoclinic transition lies below 260°C, but at the same time the reaction is too slow for quick annealing at temperatures below 300°C. As the samples inverted rapidly but did not reequilibrate in 0.1 hour at 346°C the other Tem Piute specimens were annealed under these conditions.

Several pyrrhotites were sampled from specimens containing no pyrite. In specimens that contained appreciable pyrite the pyrrhotite was extracted with a dentist's drill kept in contact with pyrite at all times so that no pyrrhotite was collected farther than 1 mm from pyrite.

Table 22 lists the results of X-ray measurements on a number of Tem Piute pyrrhotites, all of which were originally monoclinic. Numbers 3 and 4 are from the same sample; they provide almost identical results. Likewise, all the pyrrhotite samples that were adjacent to pyrite have very similar d_{102} values and, had they been hexagonal when collected, would indicate temperatures between 455° and 510°C. Those that were not in contact with pyrite have consistently larger d_{102} values and, had they also been originally hexagonal, would correspond to minimum temperatures between 390° and 450°C. Although very reasonable for contact metasomatic deposits such as Tem Piute, these temperatures must be regarded as tentative. At present it is not

TABLE 22. Average $2\theta^{(102)}$ and Corresponding $d_{(102)}$ of Tem Piute Pyrrhotite

Sample	$\overline{2\theta}_{(102)}$	$s_{\overline{2\theta}}$*	$d_{(102)}$	Comments
1	44.03_6	0.0041	2.0563 ± 0.0002	
2	43.96_6	0.0097	2.0594 ± 0.0004	
3	44.00_2	0.0033	2.0578 ± 0.0002	
4	44.01_7	0.0053	2.0572 ± 0.0002	
5	43.95_1	0.0044	2.0601 ± 0.0002	
6	43.90_0	0.0109	2.0623 ± 0.0005	Pyrite absent
7	43.87_1	0.0067	2.0636 ± 0.0003	Pyrite absent
8	44.01_1	0.0046	2.0574 ± 0.0002	
9	43.93_9	0.0082	2.0606 ± 0.0004	Pyrite absent
10	43.88_2	0.0042	2.0631 ± 0.0002	Pyrite absent
11	43.95_1	0.0053	2.0601 ± 0.0002	

* Standard error of eight or more successive oscillations.

clear that the relations between hexagonal and monoclinic pyrrhotite are such that measurements on inverted monoclinic material yield sound estimates of temperatures of formation.

STONY METEORITES

P. Ramdohr[9] *and G. Kullerud*

During this past year more than a hundred stony meteorites have been studied in polished sections in addition to those described in last year's report. The following opaque and semiopaque minerals have been identified: Minerals containing elemental iron include α iron (kamacite) with variable Ni content, Fe-Ni solid solutions (taenite) with the structure of γ iron, and intergrowths of the α and γ phases, plessite. Cohenite (Fe_3C) occurs only in a few stony meteorites and in small amounts. Schreibersite (Fe_3P) is widely distributed in small amounts. A new mineral, which by synthesis was found to have the composition $(Ni,Fe)_2S$ and which we refer to as the Henderson phase, was observed in three meteorites. Graphite (C) occurs in about one-tenth of the specimens. Native copper (Cu) is commonly observed, but in trace amounts. Native gold was observed in only one specimen. Troilite (FeS) is present in all specimens examined and is frequently the most abundant opaque mineral. Chalcopyrrhotite, (Fe, Cu, Ni, Zn)S, a cubic high-temperature solid solution, was observed in about one-third of the specimens. Valleriite occurs as a disintegration product of chalcopyrrhotite and as an exsolution product of pentlandite. Pentlandite, $(Fe,Ni)_9S_8$, is present in about one-fourth of the meteorites examined. Oldhamite, (Ca, Fe, Mn)S, is limited to meteorites that are highly reduced or that have a high sulfur content. A new (Fe, Mg, Mn, Ca)S phase similar to oldhamite but with much higher reflectivity is rather common. Alabandite, MnS, was not observed. A new mineral with a hexagonal layer structure and containing Fe-C-S was observed in 10 per cent of the meteorites. Daubréelite, $FeCr_2S_4$, is also present in about 10 per cent of the specimens. Sphalerite, ZnS, occurs in trace amounts only. Chalcopyrite, $CuFeS_2$, was observed in a few meteorites, and pyrite, FeS_2, was identified only once.

Besides these minerals a number of new ones were observed in small amounts and mostly in single meteorites. These phases are referred to by the letters A through L. For most of them the compositions are partly or completely unknown although their major constituents can often be deduced from the mineral assemblages with which they are associated. Mineral A is strongly anisotropic and has a dark yellow-green color. It almost invariably occurs as lenses or lamellae in daubréelite and only rarely is found independent of troilite. Its optical properties indicate that it has a pseudohexagonal orthorhombic symmetry, and it may be a transformation product of daubréelite. Mineral B occurs interlayered with mineral A and appears to have formed from it, not directly from daubréelite, with which it is also closely associated. This mineral may be a terrestrial alteration product, although the neighboring minerals, some of which are very susceptible to weathering, show no sign of alteration.

Mineral C is olive-brown, weakly reflecting, and apparently isotropic. It is commonly, but not always, associated with daubréelite. Mineral D is colorless and transparent with high refractive index. It replaces ilmenite and chromite and is always associated with chromite. Mineral E is dark brown and occurs with troilite. It is relatively soft, is isotropic,

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and shows traces of internal reflections. It contains exsolution bodies of troilite. Mineral F is white and resembles certain terrestrial arsenides and sulfarsenides such as loellingite and arsenopyrite. This mineral is isotropic and has good cleavage parallel to (111). It probably contains arsenic. Mineral G, a light blue mineral, occurs in association with troilite. It is relatively soft, is isotropic, and shows traces of internal reflections. It contains troilite exsolution bodies and is presumably a sulfide, but is optically different from any known sulfide. Mineral H has a yellow-gray color and almost metallic characteristics. It is anisotropic with greater reflectivity than chalcopyrite but with paler colors. Its properties differ from those of any previously described mineral. Mineral I is colorless; it is of the spinel type and often contains ilmenite exsolution lamellae. It is transparent, its refractive index is in the range 1.8–1.9, and its hardness is greater than that of olivine but lower than that of pure Mg-Al spinel. This mineral was found by synthesis to have the composition Mg_2TiO_4. Mineral K has a very dark gray color and is definitely isotropic. It is sometimes partly rimmed by troilite, and its optical properties indicate that it is a sulfide. It may possibly be a member of the (Fe, Cu, Zn)S mineral group. Mineral L is strongly pleochroic and is commonly intergrown with mineral A. This intergrowth indicates that mineral L may be hexagonal. Its hardness is moderate and similar to that of troilite.

In contrast to the numerous sulfides observed in stony meteorites the common oxides are limited to chromite, magnetite, and ilmenite.

With few exceptions the stony meteorites are more uniform in their silicate mineralogy than terrestrial rocks. The major silicates are olivine and pyroxene (usually orthopyroxene), with minor amounts of plagioclase. Glass is frequently present in small amounts; quartz and tridymite are rare. The individual meteorites commonly display complex mineralogical relationships. A chondrule or even a zone within a chondrule may represent local equilibrium. Sometimes reactive gases apparently brought about changes in mineralogy. Evidently an increasing degree of reduction was accompanied by increasing temperature, resulting in changes of both mineralogical composition and grain size and in elimination of brecciation. Complex genetic histories are displayed in many meteorites through spontaneous melting processes resulting in droplets of nickel-iron, sulfides, and probably glass. In many specimens the mineralogy indicates that sulfur has been introduced in some form. In such meteorites FeS is observed to replace Fe, sometimes even taenite. Since nickel is less reactive than iron with respect to sulfur, the introduction of sulfur leads to relative enrichment of nickel with a consequent decrease in the amount of kamacite. Continued introduction of sulfur leads to complete disappearance of the metal phase and often produces pentlandite. Further complications include the presence of reactive hydrocarbons in many meteorites.

Noteworthy structural and textural phenomena are: fusion on "dislocations," manifested by the occurrence of droplets of fused troilite and iron in varying amounts in veinlets; spontaneous melting, resulting in patches of glass with fused droplets of sulfide or iron in the interior of many meteorites, not related to the fusion crust, to heat developed on impact, or to brecciation or sintering and, therefore, distinctly different from fusion on "dislocations"; exsolution, which is common, as for instance ilmenite from chromite, chalcopyrite from troilite, magnetite from olivine; mechanical distortion and recrystallization, evident in very many meteorites; terrestrial weathering effects, observed in numerous stony meteorites, the products of which, such as magnetite, can sometimes be mistaken for primary components.

The new minerals discovered in stony meteorites occur in amounts too small to

permit standard chemical analysis and almost invariably also in too small amounts to allow investigation by X-ray powder diffraction methods. The electron probe, however, shows promising potentialities in this field. The new phases can also be synthesized if the major constituents can be surmised from the mineral paragenesis, and our efforts in this direction are increasingly successful.

IRON METEORITES

S. P. Clark, Jr.

During the past several years, work has been done in the systems Fe-Ni-S and Fe-Ni-P for the primary purpose of finding the compositions of troilite, (Fe,Ni)S, or schreibersite, $(Fe,Ni)_3P$, in equilibrium with both kamacite (α alloy) and taenite (γ alloy) at various temperatures and low pressures. Knowledge of these compositions as a function of temperature provides an indication of the temperature of formation of iron meteorites which supplements the one provided by the Fe:Ni ratios of the two alloy phases. Disagreement between these thermometers might indicate a lack of equilibrium among the phases present. This, coupled with textural observations, might give important information about the cooling histories of the meteorites. Alternatively, such a discrepancy might result because the iron meteorites were formed at high pressure, which would be interesting to know.

Besides the work on schreibersite, X-ray studies of the higher phosphides of iron, Fe_2P and FeP, have been made. They were stimulated by the discovery by Chao, Adler, Dwornik, and Littler (1962) that metallic spherules in some tektites consist of kamacite plus a second phase, which they tentatively identified as a phosphide. Under the microscope this other phase is highly anisotropic, which is inconsistent with the optical properties of ordinary schreibersite. The studies of the higher phosphides were made in order to help in the identification of this phase.

The System Fe-Ni-S

As was stated in last year's report (*Year Book 60*, p. 184), the amount of nickel in the troilite in equilibrium with both kamacite and taenite is small. Since the γ structure in the alloy cannot be quenched in the range of temperatures over which investigations have been made, the composition of the troilite cannot be closely determined by the standard methods of phase equilibria. If the charge contains the metastable α_2 phase, which forms from the γ alloy on quenching, an upper limit to the possible nickel content of the troilite is found by projecting the line connecting the bulk composition of the charge with the composition of the α alloy to the FeS-NiS join. The composition of the α alloy is taken from the known system Fe-Ni, assuming it to be unaffected by sulfur. This is plausible because sulfur is practically insoluble in nickel and raises the α-γ transition in iron by only 3°C.

The sensitivity of the method depends on the limiting amount of the α_2 phase that can be detected. The distinction between α and α_2 is based on the sharpness of the X-ray reflections in the back-reflection region. For the α phase, the K_{α_1} and K_{α_2} reflections of (220) are easily and sharply resolved; for α_2 they are blurred into a single diffuse reflection. Since the cell dimensions of the two phases are nearly the same, it is difficult to detect even moderate amounts of one

in the presence of the other. The X-ray reflections are superimposed.

Runs that limit the possible compositions of troilite in equilibrium with kamacite and taenite at 800° and 700°C and low pressure are shown in table 23. The run at 800°C limits the NiS content of the troilite at that temperature to 0–0.3 weight per cent. Similarly at 700°C the amount of NiS present in the troilite must lie between 0 and 0.5 weight per cent. These quantities of nickel are so low that troilite does not appear to be suitable for use as a thermometer. The accuracy with which the desired equilibrium compositions can be determined in the laboratory is too low for this purpose, although the picture could possibly be changed by use of the electron probe microanalyzer.

TABLE 23. Data Fixing the Compositions of Troilite in Equilibrium with Kamacite and Taenite

T, °C	Bulk Composition of Run, wt. %			Phases Present	Duration of Run, days
	Fe	Ni	S		
800	68.6	0.4	31.0	α_2	184
700	68.5	0.5	31.0	$\alpha(+\alpha_2)$	226
700	68.2	1.0	30.8	α_2	204

The System Fe-Ni-P

The nickel content of the schreibersite in equilibrium with kamacite and taenite is much larger than that of the comparable troilite, and it changes demonstrably with temperature. At 800°C the Ni/(Fe + Ni) ratio of this schreibersite is between 0.065 and 0.10, and at 700°C it lies between 0.125 and 0.15. Further work is required to fix these compositions more closely. Present results are consistent with the assumption that the phase diagram of the Fe-Ni system is significantly affected by phosphorus. This is known to be so for iron; the system Fe-P is of the "γ-loop" type.

An interesting result of these investigations is that within an experimental error of less than 0.01 the ratio Ni/(Fe + Ni) is the same in the schreibersite as in the γ alloy with which it is in equilibrium. This is true for ratios up to at least 0.25 and at temperatures of both 700° and 800°C. It is somewhat surprising in view of the strong segregation of nickel in the metal phase in alloy-sulfide and alloy-silicate systems of iron and nickel. A possible interpretation is that the metal-phosphorus bond is nearly metallic in schreibersite. That a metallic form of phosphorus can be made at high pressures is at least consistent with metallic behavior of the phosphorus atom in the schreibersite lattice.

Higher Phosphides in the System Fe-P

In addition to the work on schreibersite, some of the properties of Fe_2P and FeP have been investigated. The powder diffraction patterns of these phases have been completely indexed out to the minimum d values observed with Fe K_α radiation, and the optical properties of the solid phases formed by quenching liquids in this system and those formed by growth in the solid state have been observed.

Fe_2P grown in equilibrium with FeP at 1000°C has unit cell parameters measurably smaller than those of Fe_2P equilibrated with Fe_3P at the same temperature. This indicates that at high temperature Fe_2P departs from stoichiometry. The possibility of lack of stoichiometry in FeP has not yet been investigated.

The fact that liquids in this system are known to be easily supercooled suggested

that metastable solid phases might be formed on quenching liquids. Charges of composition 89.5 weight per cent Fe, 10.5 weight per cent P, and 73.3 weight per cent Fe, 26.7 weight per cent P, were fused at 1070° and 1300°C, respectively, and quenched by dropping into ice water. These compositions are close to the eutectics between Fe and Fe_3P, and Fe_2P and FeP, respectively. The temperatures are a few tens of degrees above the eutectic temperatures of 1050° and 1262°C (Hansen and Anderko, 1958).

These runs yielded the phases to be expected if equilibrium had been reached, as shown by the X-ray patterns of the charges. The first produced Fe_3P and metal, and the second Fe_2P and FeP. The optical properties of the quenched charges, however, are strikingly different from those of the same phases when grown by combination of the elements at subsolidus temperatures. Both Fe_3P and FeP were highly anisotropic under the microscope and exhibited properties corresponding to the description of the unknown phase in the metallic spherules in tektites described by Chao, Adler, Dwornik, and Littler (1962). These properties are in sharp contrast to the properties of these phases when grown at lower temperatures. The difference is possibly due to strains in the lattice. It does not appear to affect the X-ray properties.

On the basis of its X-ray properties, the unknown phase in the tektites can be identified as an iron-rich schreibersite. The *d* values of the reflections observed by Chao, Adler, Dwornik, and Littler (1962) are compared with those of Fe_3P in table 24. The spacing of the (411) reflection indicates a Ni/(Fe + Ni) ratio of about 0.05 according to the determinative curve given in *Year Book 60,* page 184. This estimate may be somewhat high because of absorption.

The agreement between the *d* values in columns 1 and 3 of table 24 is excellent. The identification of the last reflection in the table as (402) must be considered somewhat uncertain, however, since this is one of the weaker reflections in the schreibersite pattern. It is surprising that Chao et al. should observe this reflection and not stronger ones such as (510) or (132). The other reflections listed in the table are among the strongest ones in the schreibersite pattern. None of the lines of Fe_2P or FeP has *d* values close to 1.600 Å.

TABLE 24. Comparison of the X-Ray Properties of the Unknown Phosphide and Fe_3P

Unknown Phase (Chao et al., 1962)		Fe_3P	
d	*I*	*d*	*hkl*
2.19	w	2.1984	321
2.14	vw	2.1455	330
2.11	vw	2.1090	112
1.973	vw	1.9787	411
1.600	vvw	(1.5936)	(402)

These results indicate that little new information about the origin of tektites can be inferred from the presence of optically anisotropic schreibersite. It is clear from the glassy nature of these bodies and the spherical shape of the metallic particles that they have been melted and then relatively rapidly quenched. These seem to be the conditions necessary to produce the observed phosphide. Of greater interest is the new evidence that the unknown phase is indeed schreibersite. Tektites are commonly thought to be the result of the "splash" produced by the impact of an iron meteorite. The principal disagreement about their origin centers around whether the impact occurred on the earth or the moon. In either event the metallic spherules are presumably part of the meteorite itself, and as such they may be virtually identical in all tektites produced by a given impact. Hence the content of minor elements like sulfur, phosphorus, or carbon in the spherules should help in determining whether or not there was a multiplicity of falls in regions of complex strewn fields such as southeast Asia and Australia.

GEOTHERMAL CALCULATIONS

S. P. Clark, Jr.

The past year has witnessed a striking reawakening of interest in terrestrial heat flow stimulated in part by geothermal investigations in the Pacific Ocean basin. This work, which in recent years has been carried on mainly by R. P. von Herzen at the University of California at La Jolla, has shown much fine-scale irregularity, which must in part at least be real. For example, the extremely high heat flows on the East Pacific Rise now appear to be confined to *two* relatively narrow zones trending parallel to the crest of the Rise. The sharpness of these features is suggestive of volcanic origin; in any event their cause must lie at very shallow depths. The interesting question whether similar features exist in continental regions cannot be answered with present data. A number of proposals for drilling holes for geothermal purposes have been submitted to the National Science Foundation, and it is to be hoped that the observational basis of this subject can be greatly broadened in the next few years.

Theoretical investigations of subjects related to earth temperatures, such as those described in *Year Books 59* and *60*, have been continued. This type of work forms an essential background for the interpretation of geothermal results. The studies have been facilitated by the replacement of the IBM 704 digital computer at the National Bureau of Standards by the more powerful IBM 7090, decreasing the expense and labor involved in treating the rather cumbersome problems that have been considered. Most investigative effort has been devoted to the effect on surface heat flow of very high thermal conductivity at depth and to the cooling of a uniform, nonradioactive earth.

In some of the cases considered below, radioactive generation of heat is involved. It is assumed that 40 per cent of the present heat production is by uranium, 40 per cent by thorium, and 20 per cent by potassium—proportions similar to those commonly observed in terrestrial rocks. Account is taken of radioactive decay by fitting the decay curve of such an assemblage of radioactive isotopes with a single decay constant. This approximation, which is amply accurate for present purposes, leads to a fourfold reduction in machine time.

The effect of high thermal conductivity at depth has been investigated as described in *Year Book 59* (p. 144). We consider a sphere composed of an outer shell with finite and constant thermal conductivity surrounding a central region with infinite conductivity. This gives a rough upper limit to the effect of such processes as radiative transfer, which lead to high conductivities at high temperatures and hence imply high conductivity at great depths. The model has obvious imperfections: the thickness of the outer shell must be set arbitrarily, the thermal gradient must vanish in the central region, and a discontinuous change in properties is introduced at a level where the properties of the real earth are likely to be continuous. But this approach has the great advantage that it leads to linear equations, and the radiogenic heat and heat flow can be clearly and uniquely separated from the thermal effects of initial heat.

The first theoretical investigation of heat flow involved calculations of the thermal flux from an earth of constant properties with radioactive elements distributed uniformly throughout a surface layer of variable thickness (Clark, 1961). It was found that the flux, when regarded as a function of thickness of the radioactive layer, passed through a broad maximum at moderate thicknesses and decreased when the thickness exceeded 500 km. MacDonald (1961) later published a similar calculation for a non-

linear model in which radiative transfer was taken into account. His results show a monotonic rise in heat flow with thickness of the radioactive layer. Since there is no satisfactory way of separating radiogenic flux from that due to initial heat in MacDonald's problem, and since he took very high initial temperatures (1880°C at a depth of 100 km), it seems worth while to examine further the case of an earth with a perfectly conducting center. This examination should reveal the reasons for the qualitative differences between the uniform and nonlinear models.

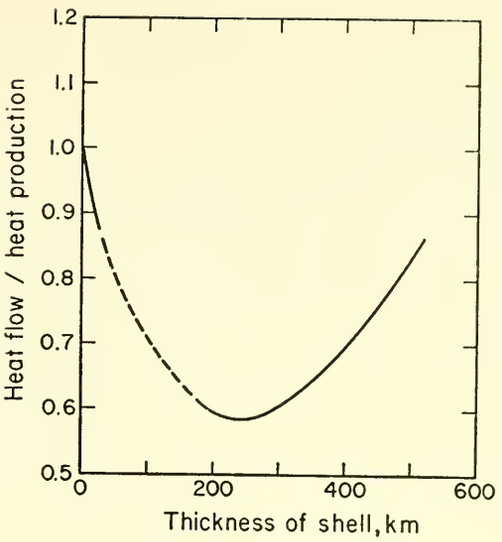

Fig. 56. Ratio of surface heat flow to present heat production as a function of thickness of outer shell of finite conductivity. Radioactivity uniformly distributed through outermost 500 km.

In the first problem considered it was assumed that radioactivity was uniformly distributed through the outermost 500 km of the earth. The initial temperature was taken to be zero, and the thickness of the outer shell of finite conductivity was allowed to range from 200 to 500 km. Results are shown in figure 56; as the thickness of the outer shell is increased, the curve levels off and asymptotically approaches a value of about 1.1. The pronounced minimum in the curve of figure 56 is perhaps surprising at first. It results from conduction of heat toward the earth's center as well as toward the surface. Downward conduction is most efficient if the shell is 200 to 300 km thick. The temperature reaches a fairly pronounced maximum at shallow depths (fig. 57), but this maximum does not

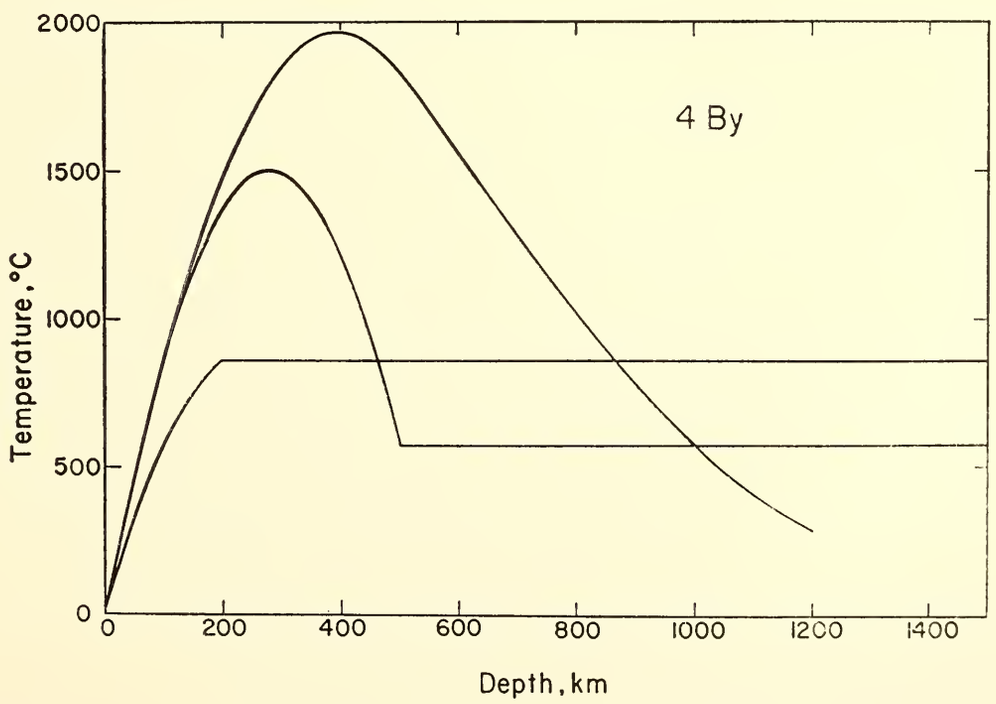

Fig. 57. Temperatures for outer shells 200, 500, and 6371 km thick. Initial temperature zero Radioactivity uniformly distributed through outermost 500 km.

exist if plausible nonzero initial temperatures are adopted. In that case the concentration of radioactive heat production near the surface tends to lessen the amount of cooling at shallow depths; the consequences are discussed below.

In another set of calculations, the effect of a central region of infinite conductivity on the cooling of a nonradioactive earth was investigated. The initial temperature was assumed to be of the form $T_0 + mx$, where x is depth and T_0 and m are constants. The thermal conductivity does not enter this problem explicitly, and it is convenient to consider the thermal gradient at the surface rather than heat flow as a function of the thickness of the outer shell. Results are shown in figure 58, where the various curves are labeled with appropriate values of T_0 and m.

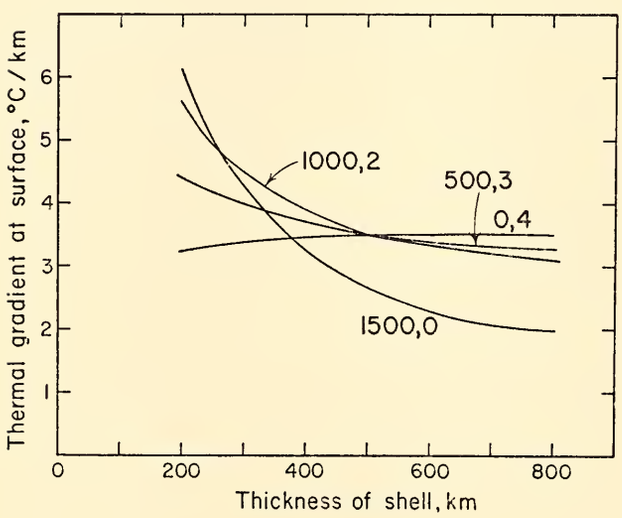

Fig. 58. Thermal gradient at the surface as a function of the thickness of the outer shell of finite conductivity. No heat production. Initial temperature $T_0 + mx$. Numbers beside curves give values of T_0 and m.

From figure 58 we see that the thermal gradient at the surface, and hence the heat flow, is sensitive to the thickness of the outer shell if T_0 is large and m is small. The gradient is large for small thicknesses of the shell and decreases markedly as the thickness is increased. For small T_0 and large m there is a very much weaker effect in the opposite sense.

In the foregoing examples the problem is put somewhat differently from the way it appears in the published work cited above. Here the independent variable has been the thickness of the outer shell, a parameter most closely related to the assumed effectiveness of processes such as radiative transfer which lead to high conductivity at high temperatures. Figure 59, however, shows heat flow as a function of thickness of the radioactive layer for several thicknesses of the outer shell. T_0 was taken to be 1800°C, and m 0.8°C/km; these constants lead to initial temperatures close to those tabulated by MacDonald.

These results indicate that MacDonald's findings of an increase in heat flow with increasing thickness of the radioactive layer is due to nonlinearity in his earth model. It appears to result from the reduced cooling caused by shallow radioactivity. Thickening the radioactive layer maintains near-surface temperatures at higher values, causing high thermal conductivity because of radiative transfer. This produces an effect analogous to reducing the thickness of the outer shell of finite conductivity. Radioactive heating in effect promotes the escape of initial heat.

In the linear case the T_0 term contributes between 30 and 40 per cent of the total flux, the lowest proportion corresponding to the thickest outer shells. If this term were cut in half, which is a plausible adjustment, the extreme ratios of heat flow to heat production shown in figure 59 would be reduced to about 0.6 and 1.1. MacDonald's estimates of the contribution of initial heat to the flux at the surface led to values less than 25 per cent, which seem too low by contrast with the present results.

The results given above extend the previous study of the effect of depth of burial of radioactivity on surface heat

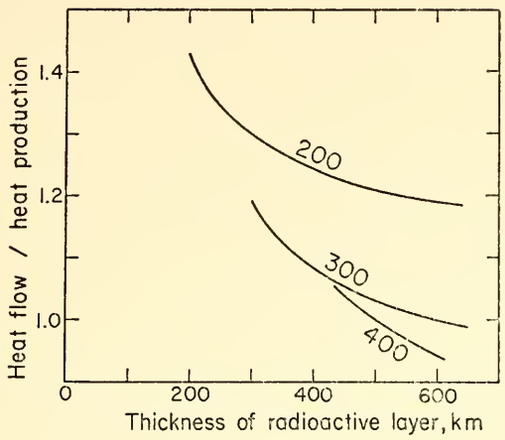

Fig. 59. Ratio of heat flow to heat production as a function of the thickness of the radioactive layer. Initial temperature $1800 + 0.8x$. Numbers beside the curves give thicknesses of the outer shell of finite conductivity in kilometers.

flow to cases involving further variable parameters. The effect of high thermal conductivity at depth proves to be greater than that of changing the thickness of a surface layer of radioactivity, especially if the initial temperature is high. These results point up our inability to find a connection between radioactive heat production and heat flow at the surface without precise hypotheses about thermal properties and initial temperatures in the mantle.

A second major field of investigation has been the cooling of a uniform, nonradioactive earth. Interest in this problem arises mainly from its geomagnetic implications, discussed below. Initial temperatures in the earth are assumed to be of the form $m(R^n - r^n)$, where R is the radius of the earth, r is the radial coordinate, and m and n are constants. Initial temperatures for n ranging from 1 to 4 and for a central temperature of 500°C are shown in figure 60, and the amount of cooling in 5×10^9 years is shown in figure 61. For the higher values of n, the cooling is greatest near the surface and becomes small at great depths. For $n = 1$, however, the cooling is nearly independent of depth, and even increases slightly toward the center. These results agree with the earlier conclusions (*Year Book 59*, p. 146) that the cooling of the deep layers cannot exceed 100° or 200°C on this model. These data show that cooling cannot be large at depth unless initially the thermal gradient was relatively steep.

The geomagnetic importance of this problem arises from the fact that forceful arguments can be made in support of the notion that the earth's magnetic field results from fluid motions in the outer core. The simplest way to produce such motions proves to be thermal convection. The temperature at the boundary of the inner core is probably fixed by latent heat of crystallization, and it becomes necessary to find conditions under which the temperature at the outer boundary of the core remains steady or decreases slightly with time. If this condition is not met, an adiabatic gradient will not be maintained and thermal convection will cease.

Two processes tend to heat the lower mantle: conduction of heat from the core down the adiabatic gradient, which is presumed to exist; and residual radioactivity in the mantle itself. If conditions can be found under which cooling more than offsets these sources of heating, an obstacle in the path of dynamo theories of the magnetic field will be removed.

The usual way of estimating initial temperatures in the mantle is to assume that they correspond to some melting curve. Empirically the most satisfactory such curve is given by the Simon equation, which predicts a very small thermal gradient in the lower mantle. But this prediction does not take account of the transition zone between 400 and 1000 km. Evidence that phase changes are responsible for this region is accumulating, and, if they are, the melting curve should steepen in this range of depth. Further work will be required to show whether steep gradients can persist throughout the lower mantle and prevent any rise in temperature near the boundary of the core.

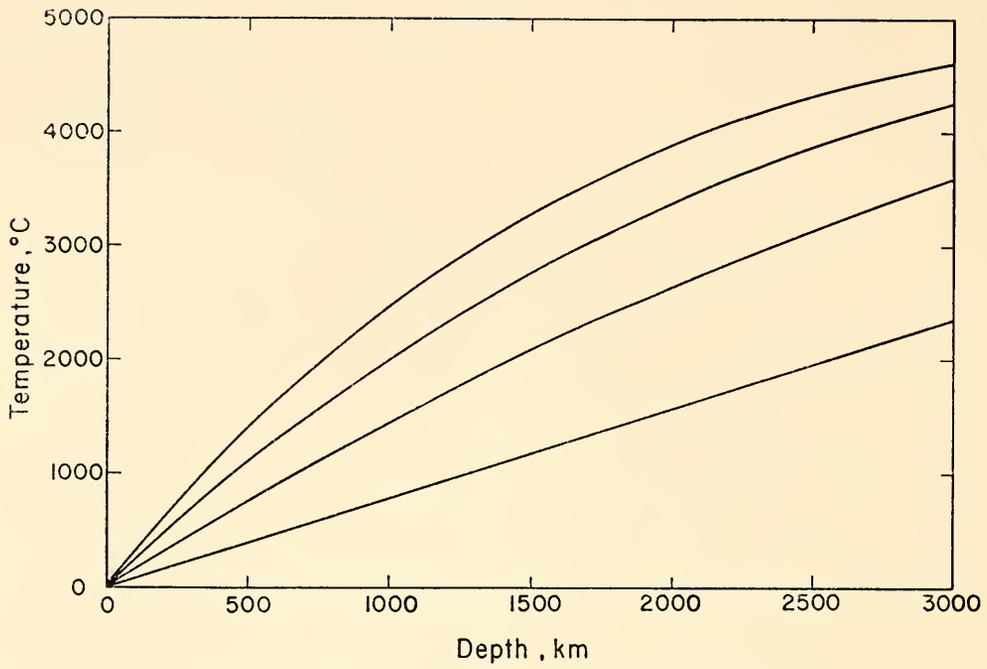

Fig. 60. Four cases of initial temperature considered. Reading from bottom to top, curves are for $n = 1, 2, 3$, and 4, respectively.

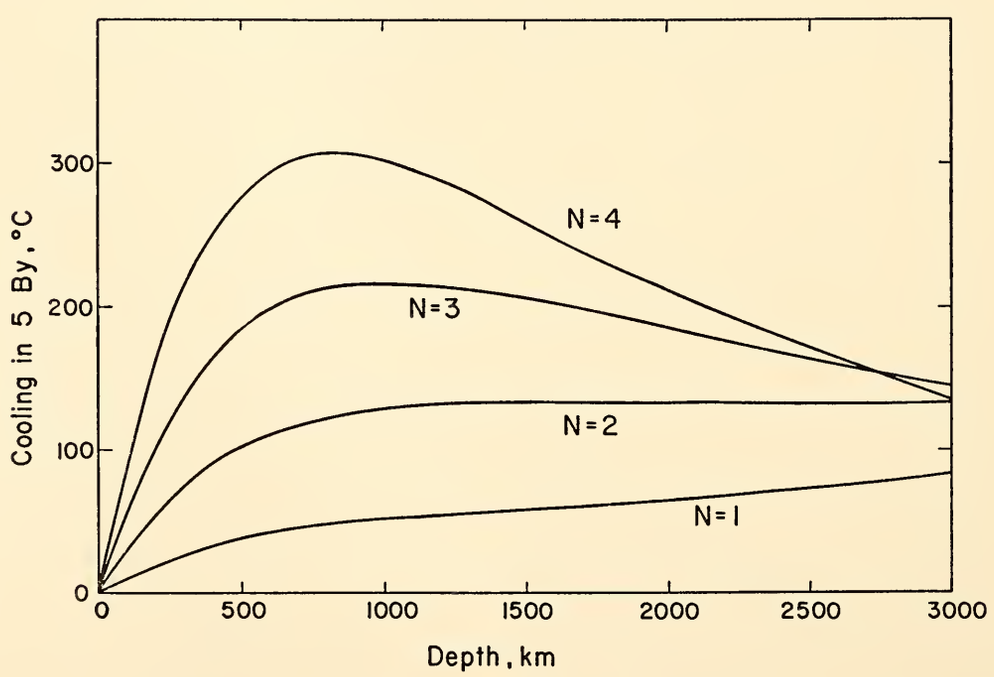

Fig. 61. Cooling of nonradioactive earth. Initial temperatures shown in figure 60.

THE AGES OF ROCKS AND MINERALS

G. R. Tilton, G. L. Davis, S. R. Hart,[10] *and L. T. Aldrich*[10]

> Some drill and bore
> The solid earth, and from the strata there
> Extract a register, by which we learn
> That he who made it, and revealed its date
> To Moses, was mistaken in its age.
>
> Cowper, *The Task*

Knowledge of the earth's crust has been extended into the past by interpreting the isotopic ages of minerals. Some age measurements give new dimension to existing geological concepts; others may allow a choice between conflicting ideas or provide a basis for new consideration unhampered by preexisting conceptions. An example of the extension of knowledge is afforded by an outline map of the central part of the North American continent. A regular pattern of ages has been developed from the age measurements by a number of laboratories in the United States and Canada. No similar regularity is apparent on other continents. Geophysical implications of these results await further study. The relation of measured ages to a geologic problem at Rainy Lake on the Minnesota-Ontario boundary is being studied. Zircon measurements are being made in an attempt to find ages predating a 2600-million-year-old metamorphic event. In Finland the approximate contemporaneity of the Karelian and Svecofennian orogenies has been established, although geologic evidence had earlier been interpreted to show that the Svecofennian belt is the older.

Geographic Distribution of Mineral Ages in the Central Portion of North America

One important application of the dating of rocks is to ascertain the ages and their geographic distribution in the crystalline basement rocks of the continent of North America. Information about this problem has been accumulating from many laboratories at an ever-increasing rate over the past decade. In *Year Book 57* a map was given showing the extent of the observations as of 1957. So much more information is now available that it seems appropriate to discuss the problem again.

Our interest has been particularly stimulated by additional age investigations in the southwestern United States. The results summarized in table 25 and figure 62 indicate that crystallization of igneous rocks occurred approximately 1300 to 1500 m.y. ago in an area extending from southeast Missouri to eastern New Mexico. The earlier investigations of Aldrich, Wetherill, Davis, and Tilton (1958) and Giletti and Damon (1961) have reported similar ages in western Arizona and Colorado.

When these ages are compared with others from central North America it is seen that older ages occur to the north and west of the 1300–1500 m.y. rocks; younger ages, to the south and east. The distribution of ages is shown in figure 63, based on a survey of ages in the literature. Some of the localities have been more thoroughly studied than others. In favorable places ages from zircon, mica, and feldspar are in agreement; in less favorable ones, only K-Ar or Rb-Sr ages have been measured for a single mineral. The Paleozoic ages (200–450 m.y.) from the Appalachian chain that serve to define the <0.5-m.y. zone have been omitted for simplification; likewise the post-Precambrian ages from the Rocky Mountain area in the western United States have not been shown.

Figure 63 shows that the age measurements in central North America can be

[10] Department of Terrestrial Magnetism.

TABLE 25. Mineral Ages from the Central and Southwestern United States

No.	Locality	Mineral and Rock*	Age, million years					
			$\frac{Sr^{87}}{Rb^{87}}$	$\frac{Ar^{40}}{K^{40}}$	$\frac{Pb^{206}}{U^{238}}$	$\frac{Pb^{207}}{U^{235}}$	$\frac{Pb^{207}}{Pb^{206}}$	$\frac{Pb^{208}}{Th^{232}}$
	St. Francis Mts.							
M-5	Fredericktown, Mo.	Muscovite (P)	1430	1405				
		Zircon (G)			970	1120	1425	1230
M-16	Granite, Mo.	Microcline (G)	1300					
		Biotite (G)	1320	1280				
	Decaturville Uplift							
M-20	Decaturville, Mo.	Muscovite (P)	1445	1290				
	Arbuckle Mts.							
M-23	Tishomingo, Okla.	Zircon (G)			970	1080	1320	1200
		Biotite (G)	1350	1250				
	Sandia Mts.							
A-26	Albuquerque, N. M.	Zircon (G)			1120	1250	1475	1290
		Biotite (G)	1340	1300				

* P, pegmatite; G, granite.

Fig. 62. Locations of samples from the central and southwestern United States.

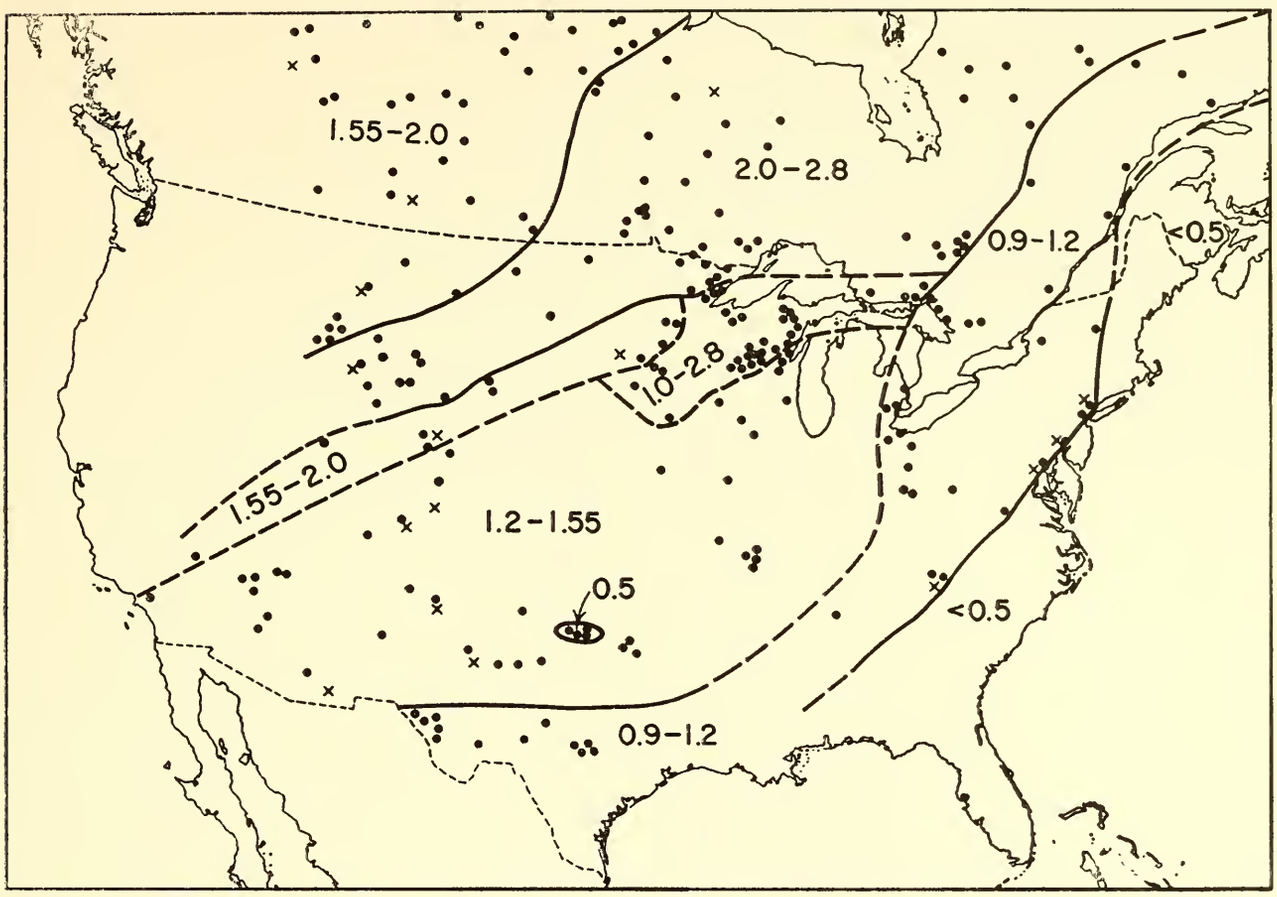

Fig. 63. The distribution of ages in crystalline rocks from the central part of North America. Circles represent ages that are within the limits specified on the map for a particular zone. Crosses are ages that are not within the limits.

grouped by age and geographic location in such a manner that few exceptions are found. In some cases, such as the 300–350 m.y. ages for biotite from Precambrian gneisses in the western part of the Appalachian belt, the exceptions have obvious explanations. These ages reflect Appalachian metamorphism. Others, such as the 1100-m.y.-old granite at Pikes Peak, Colorado, seem to represent isolated intrusions of younger bodies of rock. The area comprising much of the states of Minnesota and Wisconsin, the northern peninsula of Michigan, and part of Ontario is a "mixed age zone" in which ages similar to those in each of the surrounding zones can be found. In general, the present results confirm and extend the pattern of age distribution given in *Year Book 57*.

There is as yet no evidence that the regularities in the occurrence of age zones found for the central part of North America will be found on other continents. On the contrary, such data as exist for Europe, Africa, and Australia, although perhaps less extensive than those for North America, indicate rather complex patterns of age distribution.

The geophysical significance of the age distribution in figure 63 is a matter for further study. Taken at face value the pattern suggests that the continent has increased in extent over geologic time, but the results do not constitute proof of this. For a land mass to grow at the expense of an ocean basin it is necessary to form a crust some 30 to 40 km thick. At present it is not certain that the age distribution in North America applies to such a thick layer of rock. This factor, together with the lack of similar regularity in age distribution on other continents, indicates a need for caution in

interpreting the results. Whatever the interpretation, the distribution pattern shows an impressive regularity.

Ages of Minerals from the Coutchiching Sediments, Rainy Lake, Ontario

In the vicinity of Rainy Lake, at International Falls and Fort Frances on the Minnesota-Ontario border, A. C. Lawson mapped a series of metamorphosed sedimentary strata that he named Coutchiching, lying below a series of metamorphosed volcanic rocks (Keewatin). Circular bodies of granite-gneiss are enclosed by the Coutchiching. There is lack of agreement among geologists whether they are intrusive granites, mantled gneiss domes, or paragneisses derived by intense metamorphism of Coutchiching sediments.

In *Year Book 59* the ages measured for a single zircon sample from the Coutchiching metasediments at Rainy Lake, Ontario, were reported. The age pattern was very discordant, so much so that when examined from the viewpoint of continuous loss of lead by solid diffusion (Tilton, 1960, and *Year Book 59*) the very old age of 3800 m.y. was derived—older than any age found previously. That the zircon is old was indicated by the Pb^{207}-Pb^{206} age of 2760 m.y. The search for very old rocks, as well as the need to establish the mechanisms of loss of daughter elements, was stimulated by these results, and rocks of the area were collected.

New age measurements have been made in an effort to determine the time intervals involved in the formation of this rock sequence. The initial studies have been on the mineral zircon, because past work has shown that zircon ages are but little affected, if at all, by the forces attendant upon regional metamorphism. Consequently, zircons preserve a record of an initial crystallization. They survive detrital cycles because of their physical properties, thus providing some clues to the source of the sediments. The ages of micas, feldspars, and amphibole minerals are much more sensitive to the effects of geological cycles.

The ages measured are given in table 26. The first sample is the one to which reference has already been made. An even more discordant pattern was found for sample CC 35, implying an impossibly

TABLE 26. Zircon Ages, Ontario

No.	Rock and Location	U, ppm	Th, ppm	Age, million years			
				$\frac{Pb^{206}}{U^{238}}$	$\frac{Pb^{207}}{U^{235}}$	$\frac{Pb^{207}}{Pb^{206}}$	$\frac{Pb^{208}}{Th^{232}}$
RL 109	Coutchiching, Rainy Lake, Ont. (impure)	648	858	1150	1840	2750	1250
CC 21	Coutchiching?, Rainy Lake (or granite gneiss)	874	877	1870	2280	2670	1470
CC 22	Coutchiching?, Rainy Lake (or granite gneiss)	823	613	2340	2540	2700	2040
CC 26*	Coutchiching, Rainy Lake	1134	n.d.	2280	2460	2620	—
CC 29	Gneiss, Rainy Lake	264	n.d.	2450	2600	2730	—
CC 20	Keewatin, Rainy Lake (metavolcanic)	121	n.d.	1960	2300	2630	—
CC 35	Granite, Bad Vermilion Lake (impure)	1460	3380	520	1080	2500	290
CC 33	Granite, Bad Vermilion Lake	263	n.d.	2140	2450	2730	—
CC 43	Granite, Saganaga Lake, Minn.	261	n.d.	1540	2020	2550	—

* Biotite from CC 26: Rb-Sr age, 2510 m.y.

old age when corrected for loss of lead by continuous diffusion. Careful study of this sample, as well as the earlier one, revealed the presence of an impurity in both (15 per cent in CC 35, 5 per cent in RL 109). The impurity did not yield an X-ray pattern, and positive identification has not yet been made. The ages of these two samples result from the analysis of a mixed system, not comparable with that of the other zircons. That this can be so is evident from figure 64, representing all the zircon data on a concordia diagram.

The preliminary conclusions that can be drawn from the results of the zircon analyses are:

1. The Pb^{207}-Pb^{206} values for all the Rainy Lake zircons lie within the range 2620–2750 m.y., a very narrow range in view of the geological complexity of the area. The ages are only a little greater than the mica ages from the area.

2. The source rocks for the zircons in the sediments crystallized earlier than 2600 m.y. ago, and possibly earlier than 2750 m.y.

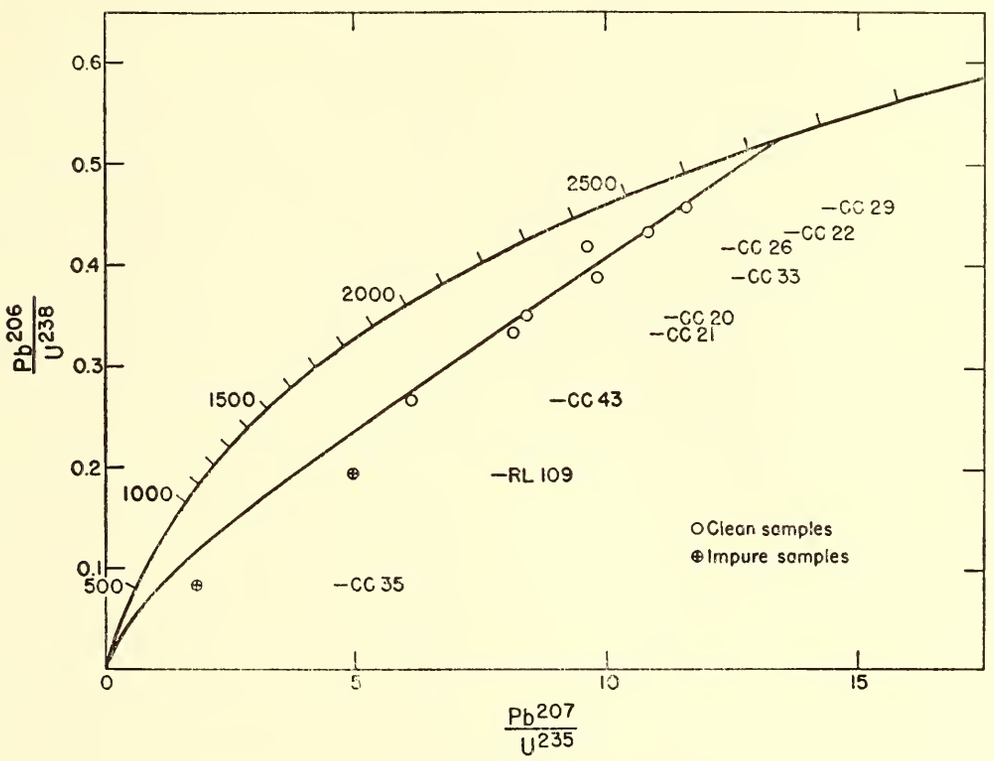

Fig. 64. Parent-daughter ratios for zircons from Rainy Lake, Ontario, compared with the curve calculated for loss of lead by continuous diffusion for 2750 m.y.

The least-squares line of best fit to the points, excluding the two questionable samples, coincides with the essentially linear part of the continuous diffusion loss curve for zircons crystallizing 2750 m.y. ago. The impure samples lie off this line. Another sample of the Bad Vermilion Lake zircon, CC 33, obtained from a different part of the granite, gave a pattern conforming to that of the rest of the zircons.

3. A single Keewatin sample is not significantly different in age from the Coutchiching zircons.

4. The discordant ages of the pure samples can be explained by loss of lead by continuous solid diffusion.

The results can be explained in two ways. The zircons may have crystallized at a single time about 2700 m.y. ago in the source for all the Coutchiching samples, or else older rocks or sediments

were so strongly metamorphosed that the zircon "clocks" were completely reset 2600–2700 m.y. ago.

Age Relation between the Karelian and Svecofennian Orogenies in Finland[11]

Two orogenic belts with distinctly different trends have long been recognized in the Precambrian rocks of Finland. The Karelian belt extends with a north-northwest trend from Lake Ladoga in southeastern Finland to Finnish Lapland, whereas the Svecofennian belt extends across southern Finland in a general east-west direction. Many geologists believe that the Svecofennian belt is older than the Karelian. The evidence in support of this view has recently been summarized by Eskola (1961). The principal observations are that the trend of the Karelian belt seems to interrupt that of the Svecofennian belt and that granites and granite-gneisses similar to those found in the Svecofennian belt occur as blocks in the basement complex of the Karelian belt. The basement complex in the Svecofennian belt is nowhere recognized with certainty by geologists.

Kouvo (1958) reported several mica and zircon ages for the intrusive rocks in both belts and found ages of 1750 to 1850 m.y. in each, in agreement with the few measurements of earlier workers. Some geologists have accepted the viewpoint that intrustion of rocks occurred simultaneously in the two belts; others, notably Eskola (1961), have advanced another interpretation. Eskola suggested that the influence of metamorphism on the "older" Svecofennian belt at the time of intrusion of rocks of the "younger" Karelian belt was sufficiently strong to erase the existing age record in the different minerals. This will be called a "rejuvenation hypothesis."

Wetherill, Kouvo, Tilton, and Gast (1962) found a Pb^{207}-Pb^{206} age of 2240 m.y. for zircon from a Svecofennian schist near Tampere, 100 miles northwest of Helsinki, showing that complete erasure of ages had not taken place 1800 m.y. ago in the Svecofennian belt. Since no age determinations had been made on zircon from the intrusive rocks in this area, the question of rejuvenation throughout much of the Svecofennian belt was not resolved by this result. The possibility existed that neither the sediments nor the intrusives were completely regenerated 1800–1900 m.y. ago in the Tampere area, but were in the other areas studied. This postulate has been shown to be most unlikely by work in the past year.

At Tampere a Svecofennian granodiorite intrudes graywacke and phyllitic schists. The body is approximately 20 km in diameter with numerous dikes and stringers cutting the surrounding sediments. Many large outcrops of granodiorite occur in the area, so that it is possible that this body is part of a considerably larger mass. Zircon age determinations have been made on three samples: a specimen of granodiorite taken about 1 km from the observed contact with the schists; schist A, collected about 2 km from the contact with the granodiorite; and schist B, collected 5 km from the contact. Zircons from these specimens differ in appearance in that rounding is more frequent in the samples from the schist than from the granodiorite. Observations on 200 crystals from each sample indicated that 75 to 85 per cent were rounded in the schist samples, only 5 per cent in the granodiorite. The granodiorite contained several per cent of crystals with length-to-breadth ratios of 3 to 5; such elongated crystals were not observed in the schists. The zircons from the granodiorite and schist are dissimilar in form and appear to represent two distinctly different populations. The age of the granodiorite zircon should not be appreciably influenced by zircon from the schist.

The age results are given in table 27.

[11] In collaboration with Olavi Kouvo, Geological Survey of Finland, Otaniemi.

TABLE 27. Ages for Zircon from a Svecofennian Intrusive and the Neighboring Schists

Rock	Concentration, ppm		Age, million years			
	U	Th	$\frac{Pb^{206}}{U^{238}}$	$\frac{Pb^{207}}{U^{235}}$	$\frac{Pb^{207}}{Pb^{206}}$	$\frac{Pb^{208}}{Th^{232}}$
Granodiorite	524	105	1710	1810	1920	1900
Schist A	476	220	1850	2030	2220	1580
Schist B	465	214	1790	2000	2230	1720

The zircon from the granodiorite has nearly concordant age values compatible with a time of crystallization about 1900 m.y. ago. This is in agreement with the results of Kouvo on other Svecofennian intrusive rocks. On the other hand, the Pb^{207}-Pb^{206} age values for the zircons from the schists are distinctly older, showing that the rejuvenation hypothesis does not apply in this area. These ages are strong evidence that intrusion of igneous rocks occurred about 1900 m.y. ago in both the Svecofennian and the Karelian belts and that the two orogenies are therefore approximately contemporaneous.

The data are also pertinent to the problem of discordant lead ages for zircons. The temperature conditions under which zircons lose lead are not well understood and are based on studies of zircons that have undergone regional metamorphism. Here the magnitudes of time and temperature are poorly known. The present observations show that intrusion of sizable masses of rock can occur without completely erasing the age record in zircon in the immediately surrounding rock. Knowledge of the true age of the zircons would permit a more restrictive statement to be made about the amount of lead loss. At present these data do not uniquely determine the age or ages of the zircons from the schists. The zircons may have been derived from a source somewhat older than shown by the Pb^{207}-Pb^{206} age values, perhaps 2300 m.y. old. Alternatively, if some loss of lead from the zircons did occur at the time of granodiorite intrusion 1900 m.y. ago, or if sources of more than one age contributed zircon to the schists, some or all of the zircons might be considerably older than 2300 m.y. Wetherill, Kouvo, Tilton, and Gast (1962) found 2700-m.y.-old rocks in the pre-Karelian basement complex; rocks of this age might have contributed zircons to the schists at Tampere.

ORGANIC GEOCHEMISTRY

Paleobiochemistry

The fatty acids are major components of all living matter and are among the more thermally stable organic substances. In principle, they can survive at low temperatures for billions of years and thus might be found in sedimentary rocks that have been deposited since the origin of organisms employing fatty acids. Fatty acids have been postulated to be the major material from which some petroleum hydrocarbons are formed.

We have extracted and identified fatty acids from recent and ancient rocks. In an attempt to provide a background for interpreting our observations we have also conducted laboratory studies of the stability of the crude components of living matter at elevated temperatures. These will be described first.

Thermal Stability of Algae

P. H. Abelson

When organic detritus is deposited in anaerobic sediments a number of mechanisms act to alter or destroy it, including biological activity and chemical changes

resulting from interaction among the components and degradation due to the intrinsic instability of organic matter. Unusual circumstances may shield the detritus from most of these factors except intrinsic chemical instability, which thus sets an upper limit to the long-term survival of components. Thermal stability can be estimated by laboratory experiments on pure compounds at elevated temperatures coupled with application of the Arrhenius equation to extrapolate to ambient temperatures. For saturated fatty acids this procedure yields decomposition times of many billions of years. In nature, however, most of the organic matter is degraded more rapidly, and chemical interactions surely play an important role. To investigate them, work was started last year on thermal stability of the components of algae. These studies have been extended to include an examination of the changes in major biochemical components and a more detailed look at the fate of fatty acids.

Chlorella pyrenoidosa was incubated both wet and dry in the absence of oxygen at temperatures of 190° and 142°C. The product was split into major fractions by the conventional procedure employed for fresh tissue. This has obvious drawbacks since after degradation the fractions no

TABLE 28. Thermal Degradation of Algae

	Control	190°C		142°C
		20 hours	12 days	40 days
Cold TCA (H_2O-soluble fraction)	6	9	5	9
Lipides	32	25	20	24
Hot TCA (nucleic acid)	9	2	1	2
Protein	48	33	10	31
Residue	5	31	64	34

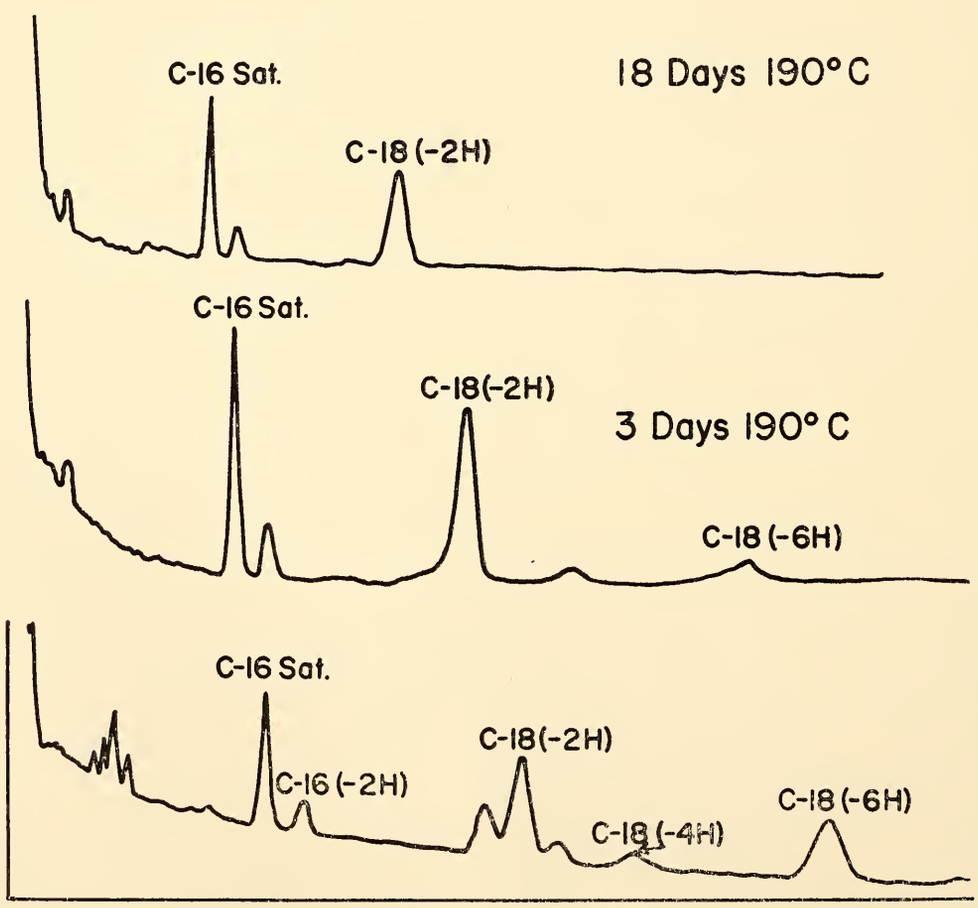

Fig. 65. Gas-liquid chromatograms of methyl esters of fatty acids extracted from *Chlorella*. The algae were exposed to heat for varying periods of time. The bottom curve is for a control specimen.

longer behave exactly like those from fresh material. However, we can make useful comparisons, obtain a feeling for what is happening, and gauge the role of chemical interactions and degradation in altering the organic sediments. Results of such experiments are displayed in table 28. It may be noted that with prolonged incubation a major amount of the organic matter is converted to a black insoluble residue similar to the kerogen of sedimentary rocks. Even with short exposures significant changes occur, including the virtual disappearance of nucleic acid. Increase in the cold trichloroacetic acid extract (water-soluble fraction) probably arises from breakdown products of other fractions. Detailed examination of the protein and lipide fractions provides more detail on what has occurred. Some of the more unstable amino acids disappear as expected, but even the more stable ones like alanine vanish at a faster rate. Thus, when incubated in dilute solution, pure alanine has a half-life of 2×10^7 sec at 190°C. When it is incubated as part of protein of algae the half-life diminishes to 10^6 sec at 190°C.

The fate of some of the individual fatty acids was also examined. The striking feature was the relatively rapid rate of disappearance of the more highly unsaturated substances. This is illustrated in figure 65, which displays chromatograms of methyl esters of fatty acids extracted from heated and from control *Chlorella*.

The chromatogram of the control specimen reveals a substantial content of an unsaturated C-18 fatty acid containing three double bonds (–6H). On heating at 190°C for 3 days this compound largely disappears, and it has practically vanished after 18 days. The C-18 compound with two double bonds is less sensitive, but it also disappears after the longer incubation. The saturated and monounsaturated acids were about equally resistant, the saturated apparently being the more enduring. Under favorable thermal conditions both these classes of compounds could last millions of years. As we shall see, these thermal tests agree only in part with what has been observed in nature, and it appears that additional mechanisms operate in the sediments to destroy unsaturated fatty acids.

Fatty Acids in Sedimentary Rocks

P. H. Abelson and P. L. Parker

Fatty acids have been extracted from rocks ranging in age from recent to 500 m.y. old. The most abundant species seen were the saturated acids, stearic (C-18), palmitic (C-16), and myristic (C-14). Large qualitative and quantitative differences between the contents of source algal detritus and the residual carbonaceous material of the sediments have been noted. Most striking is the absence of unsaturated fatty acids in even recent samples.

Many samples have been examined, of which the following are typical: (1) recent mud from Gulf Coast off Port Aransas, Texas, collected by P. L. Parker; (2) recent mud from San Nicolas Basin off Southern California collected by K. O. Emery; (3) core from Pedernales, Venezuela, 5000 years old, furnished by John M. Hunt; (4) Green River shale from Mahogany ledge about 40 m.y. old, collected near Rifle, Colorado, by P. H. Abelson; (5) sample from Alun shale, Sweden, approximately 500 m.y. old, furnished by Gösta Salomonsson of the Swedish Shale Oil Company.

Samples were ground in a ball mill if necessary to attain small particle size, treated with aqueous HCl, filtered, dried, and extracted in a Soxhlet extractor. The crude product consisted mainly of highly colored tarry materials amounting to 1 to 10 per cent of the weight of organic matter in the original sample. In turn the desired fatty acids constituted as little as 0.1 per cent of the crude extract. To obtain reasonably resolved peaks from gas-liquid chromatography, partial purification was essential. This included chemical refining and solvent extraction. The saturated fatty acids can withstand rather

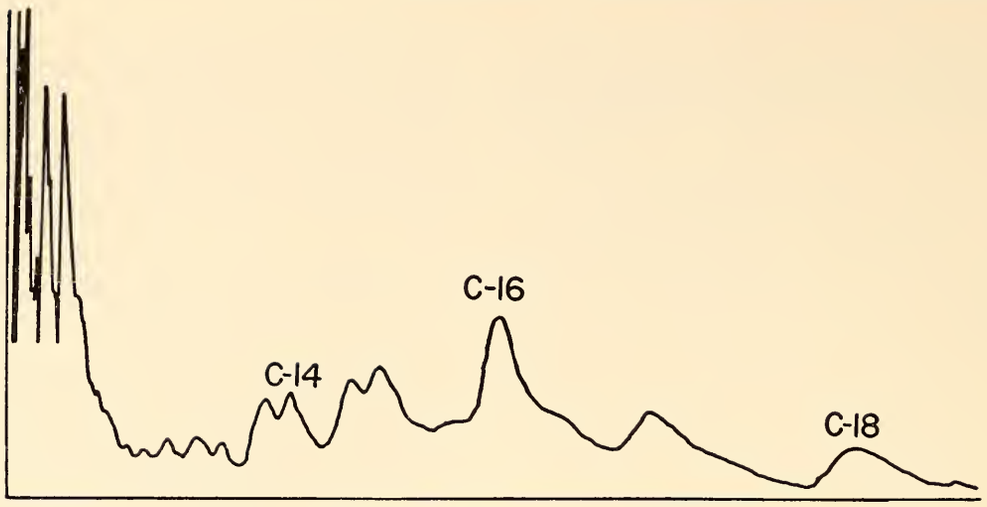

Fig. 66. Chromatogram of esters of fatty acids extracted from surface mud collected at Port Aransas, Texas.

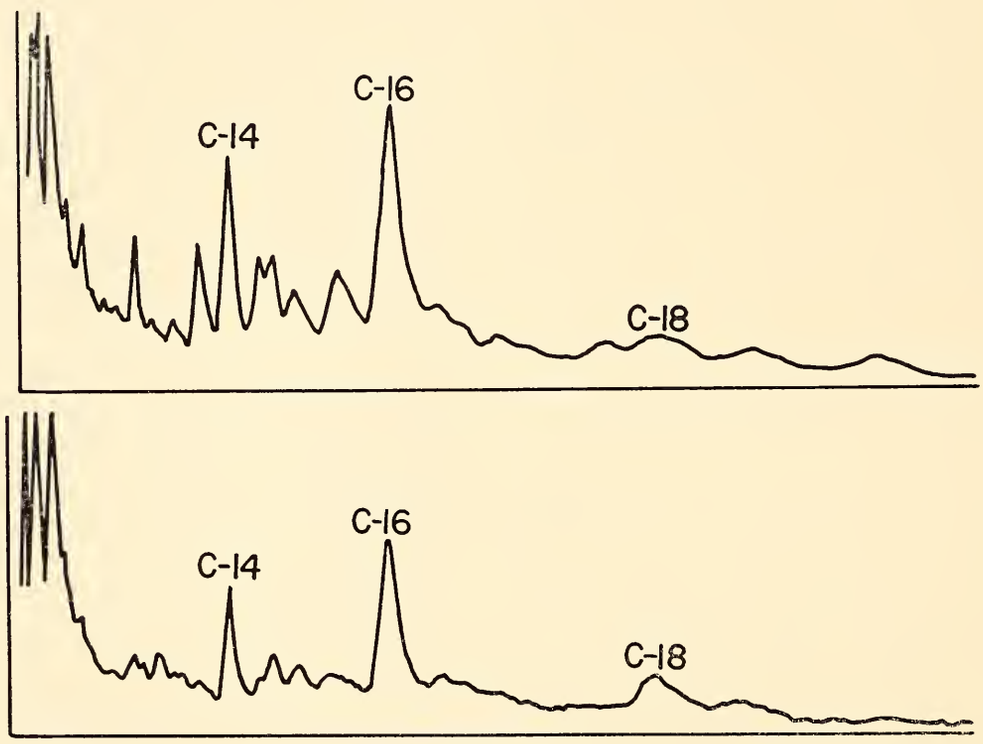

Fig. 67. Chromatograms of esters of fatty acids extracted from a grab sample from San Nicolas Basin (lower curve) and a core from Pedernales, Venezuela.

drastic oxidizing and reducing treatment, during which the tars are destroyed. The methyl esters of fatty acids are much more soluble in petroleum ether than the tars are. Various combinations of these treatments were employed, and their effectiveness was monitored by radioactive tracers. In the exploratory stages special care was taken to preserve unsaturated acids. Later it became clear that saturated fatty acids were present in substantial quantities in old rocks, and procedures were modified accordingly.

Cooper (1962), who has recently reported on the occurrence of fatty acids in rocks and petroleum reservoir waters, employed urea adduction in his studies. This procedure concentrates fatty acids with respect to unsaturates and tars but with the small quantities of saturated acids available tends to lead to relatively large losses.

In figures 66 to 69 are shown chromatograms of fatty acids obtained from rocks

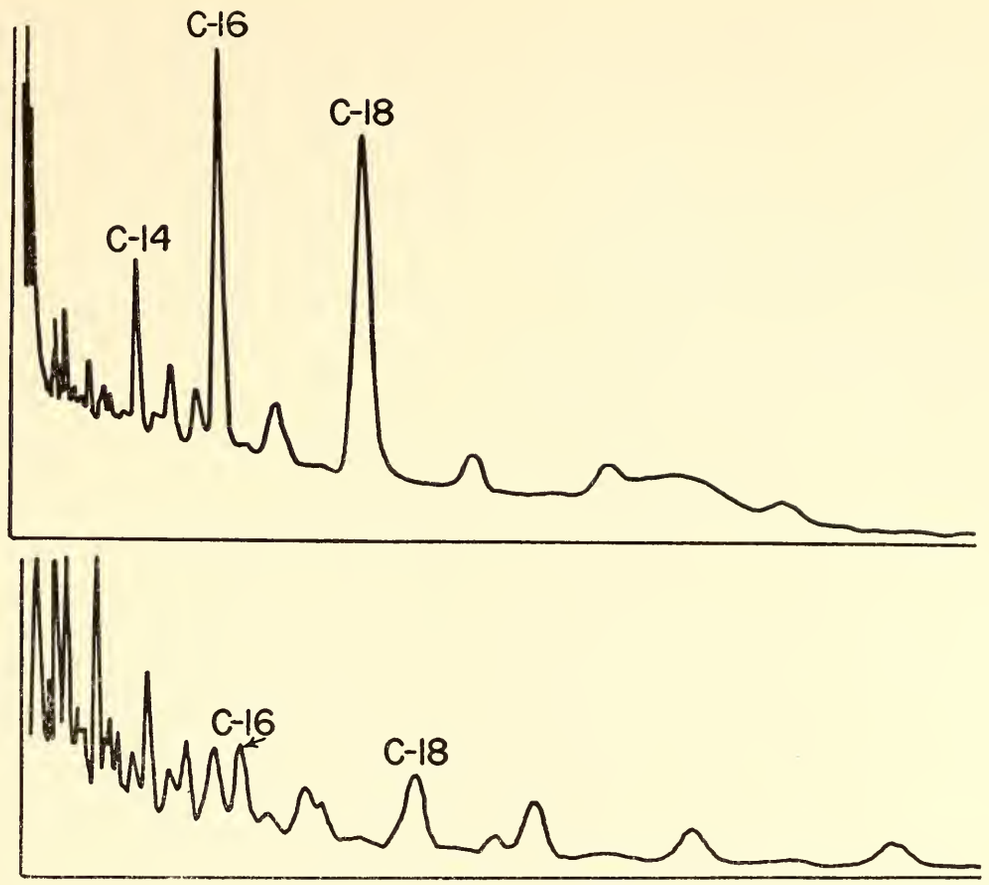

Fig. 68. Chromatograms of esters from Green River shale. The upper curve was obtained from material that had a special treatment with concentrated HI.

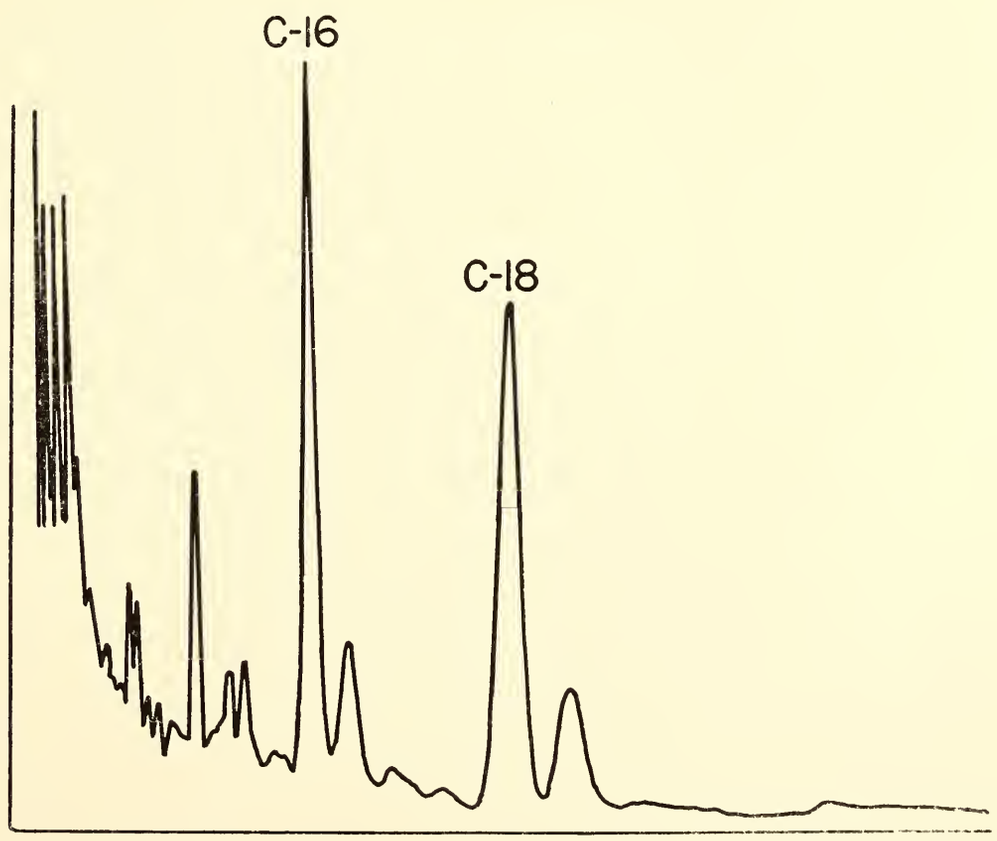

Fig. 69. Chromatogram of esters of fatty acids extracted from Alun shale. The crude acids were treated with alkaline permanganate to partly free the fatty acids of tars.

of a variety of ages ranging from a few years to 500 m.y. The chromatograms are not strictly comparable because of differences in chemical processing. Nevertheless, there is considerable similarity in these traces. All indicate the presence of C-14, C-16, and C-18 saturated fatty acids.

In the recent sediments palmitic acid (C-16) was the major constituent. This is in keeping with its ubiquitous occurrence and large abundance in present-day organisms. Oleic acid (C-18 — 2H), which is a major constituent of algae and which readily survives thermal tests, is not present. In the older rocks stearic acid (C-18) was relatively more important. We believe that this may or may not imply differences in the utilization of fatty acids at an earlier period. There are many mechanisms that could lead to relative losses of one or another of the constituents.

The amounts of fatty acids extractable from old rocks are relatively small, 2×10^{-4} to 10^{-5} gram per gram of organic matter. The same is also true of recent sediments. Since the original detritus might have contained 10 to 20 per cent fatty acids, a rather dramatic change has occurred. Only 1 part in 1000 of these acids apparently remains in an extractable form after a short period of exposure to the anaerobic environment.

To investigate this low yield, tracer experiments employing C^{14}-tagged palmitic acid were carried out. These experiments checked the efficiency of procedures once acids were freed from the matrix; they could not shed light on the efficiency of the original extraction. They showed that a labeled substance could be added to a mud and be recovered in a crude extract, and that purifications could be carried through without undue loss.

The disappearance of most of the fatty acid or its tight binding to the matrix when detritus is converted to kerogen thus remains an important but unsolved problem of organic geochemistry.

Another significant aspect is that the fatty acid content of organic matter does not change much with time from the present to the 500-m.y.-old specimen. This gives hope that even older occurrences may be found.

The Isolation of Organic Compounds from Precambrian Rocks

T. C. Hoering

The ultimate fate of most organic materials is to be oxidized to carbon dioxide. Some organic substances escape this fate by being buried in sediments. A few remain as compounds similar to those of the original living cells. Thus amino acids, carbohydrates, fatty acids, and pigments have been found in sedimentary rocks. However, the majority of the organic compounds in rocks have been converted to an insoluble substance known as kerogen.

At 25°C organic substances are unstable with respect to decomposition into methane, carbon dioxide, and graphite. At this temperature reactions leading toward these products require times as long as billions of years. The so-called "graphite" of Precambrian sedimentary rocks may contain intermediate molecules in the chemical pathways of the decomposition of kerogen.

It is the purpose of this work to consider the chemical nature of the carbon of Precambrian sedimentary rocks and to see whether any recognizable organic compounds can be isolated from it. Any such organic compounds need not bear much resemblance to the chemical components of living cells, but as the nature and transformations of kerogen are gradually understood they may give some insight into the existence and the nature of Precambrian life.

The reduced carbon of Precambrian rocks is reminiscent of high-rank anthracite coal, and therefore some of the techniques for the elucidation of coal structure were employed. The reactions used included (*a*) oxidation and recovery

of aromatic and aliphatic acids, (*b*) thermal pyrolysis and isolation of aliphatic and olefinic hydrocarbons, (*c*) reduction with anhydrous hydrogen iodide and identification of saturated hydrocarbons, (*d*) solvent extraction followed by spectroscopy of the extracts.

For experimental simplicity, much of the work was done on massive graphite of Precambrian age. The samples include the following:

1. Michigami coal from the Iron River formation of northern Michigan. It has been described by Tyler, Barghoorn, and Barret (1957). Samples were collected by E. S. Barghoorn and P. H. Abelson.

2. Anthroxolite from Sudbury, Ontario, Canada (Thompson, 1956). The samples were collected by P. H. Abelson.

3. Graphitic material from the Soudan iron mine, Oliver Mining Company, Soudan, Minnesota. Samples were collected by F. L. Klinger.

4. Carbon leader from the Main Reef series, Transvaal, South Africa. Sample was donated by P. Ramdohr.

Some work was done also on the finely dispersed carbon of the Gunflint chert, the Bulawayan limestone, and the Transvaal dolomite. These rocks are described in another section of the writer's report.

Oxidation of coal by alkaline potassium permanganate is a well known reaction. The products are a mixture of benzene polycarboxylic acids (Holly and Montgomery, 1956). Figure 70 is a drawing of a paper chromatogram of the aromatic acids isolated from the oxidation of the carbonaceous material from the Soudan iron mine. The acids on the chromatogram appeared as dark blue and fluorescent spots when viewed under ultraviolet light. The ultraviolet adsorption spectrum of an aromatic acid from one of the spots of the chromatogram is shown in figure 71; it is typical of this class of compounds. Through a comparison of the rate of migration of known substances on paper chromatograms, the presence of benzenetricarboxylic, benzenetetracarboxylic, and benzenepentacarboxylic

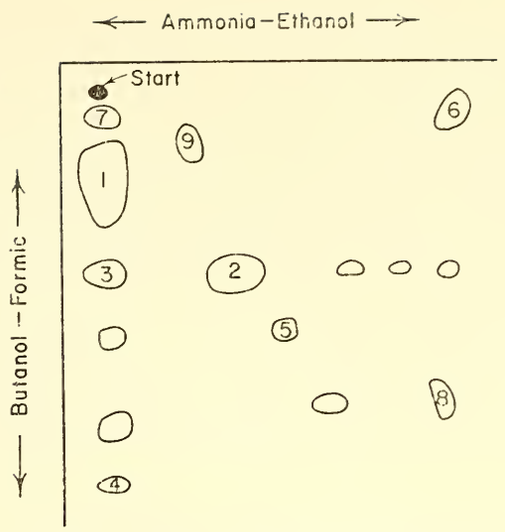

Fig. 70. Paper chromatogram of the aromatic acids from the oxidation of Michigami coal. A mixture of 1 part of coal with 1.6 parts of KOH was refluxed with excess $KMnO_4$ for 24 hours. The solution was acidified, treated with SO_2, and evaporated to dryness. The solids were extracted with diethyl ether. The extract was separated by two-dimensional paper chromatography according to the procedure of Germain (1959). The separated acids gave a deep blue color or a bright fluorescence when viewed under ultraviolet light. A comparison of R_f values and colors under ultraviolet light, with known acids, indicated the presence of benzene polycarboxylic acids.

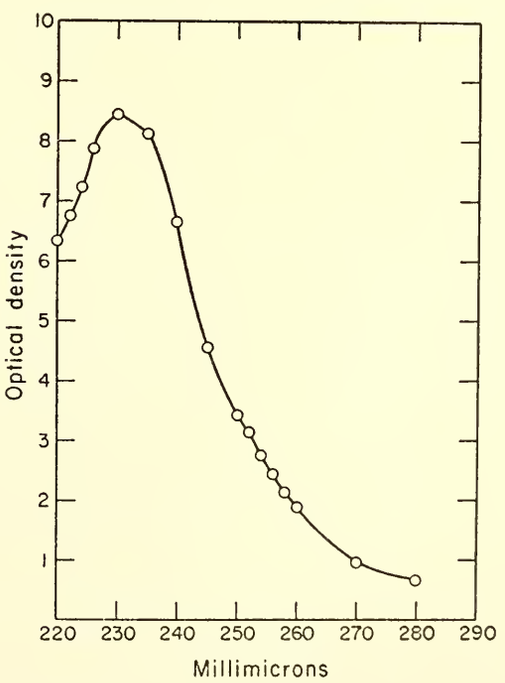

Fig. 71. The ultraviolet adsorption spectrum of an aromatic acid from the oxidation of Michigami coal. The spot numbered 2 in the paper chromatogram shown in figure 70 was eluted with dilute sodium hydroxide, and the ultraviolet adsorption spectrum was taken.

acids was indicated. Benzenehexacarboxylic acid (mellitic acid) was identified in all samples, but as this compound can be made from purely inorganic graphite its presence is of little significance to this research. The oxidation products were also examined for low-molecular-weight aliphatic acids, but only acetic acid was identified.

The pyrolysis of coals is a well studied process. Figure 72 is a tracing from the gas-liquid chromatography separation of the hydrocarbons derived from the heating of Michigami coal in a vacuum. All the hydrocarbons from methane through pentane have been identified.

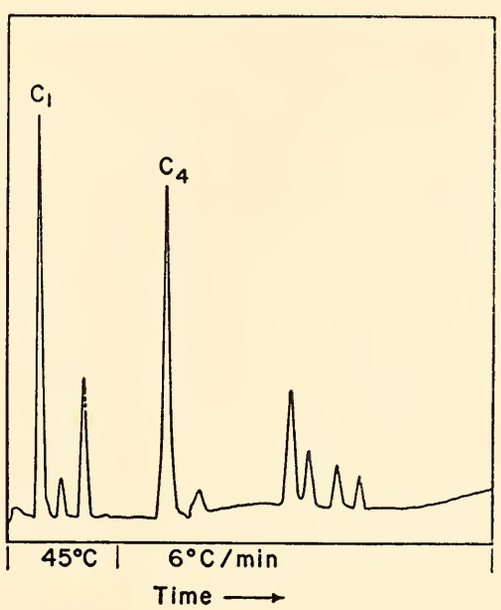

Fig. 72. Gas-liquid chromatogram of the hydrocarbons from the pyrolysis of Michigami coal. Samples of graphite were pyrolyzed in a vacuum, and the gases were pumped off for chemical analysis. The temperature was raised gradually. The gases given off below 250°C were due to adsorbed air. At 300°C hydrocarbon gases began to be evolved, and above 600°C molecular hydrogen was observed. The gases were transferred to a temperature-programmed gas-liquid chromatograph and separated with a 6-foot silicone rubber packed column. This figure shows a typical chromatogram with gases from methane through pentane being observed.

Thermal pyrolysis of coal is very destructive to any structure and does not give much insight into the nature of the organic substances present. The chemical reduction and liquefaction of coal may be more informative. Figure 73 shows a typical mass spectrometric analysis of the saturated hydrocarbons obtained from the action of anhydrous hydrogen iodide on Michigami coal. A mixture of hydrocarbons from methane through hexane is indicated. The orders of magnitude of the yields of hydrocarbons liberated by pyrolysis and reduction range from 10 to 100 parts per million of starting rock.

The exhaustive extraction of coal by basic solvents such as pyridine has long been a means of isolating organic substances. Infrared adsorption spectra of organic molecules are very specific for the types of chemical bonds contained in them. Figure 74 is an infrared adsorption spectrum of the organic substances extracted from the carbonaceous material of the Transvaal dolomite by pyridine. The presence of methylene groups ($-CH_2-$) is the most conspicuous feature of this spectrum.

The chance of contamination is an ever-present danger in the search for trace amounts of organic substances in material that has had such a long history as the rocks studied in this work. Contamination in the chemical reagents and water used for the work can be tested by running the appropriate blanks. Airborne dust or pollen is another source of contamination. By using a number of different procedures and by looking for a number of different organic substances, we can hope to decide whether laboratory contamination is a problem. Natural contamination of the rock during the long period from its deposition in the Precambrian to the present is much more difficult to evaluate.

The results obtained so far support the premise that the so-called "graphite" of Precambrian rocks was originally kerogen. If so, it is of interest to ask whether the organic compounds that formed this kerogen were the product of living cells or whether they could represent abio-

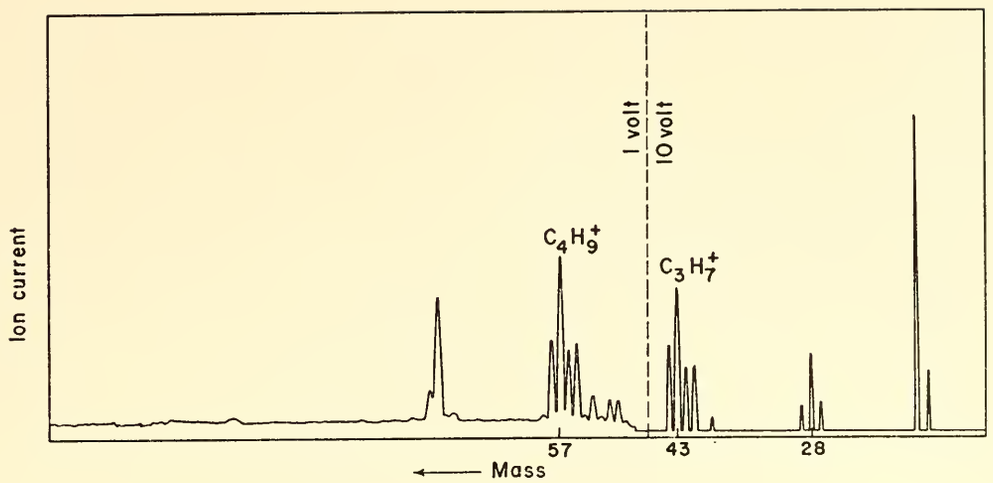

Fig. 73. The mass spectrum of the hydrocarbons from the treatment of Precambrian "graphite" with anhydrous hydrogen iodide. Ten grams of Michigami coal was placed in a bomb, and 50 grams of anhydrous hydrogen iodide was distilled in. The bomb was heated to 180°C for 16 hours. Substances volatile at 100°C were distilled off the reaction mixture, and the iodine and hydrogen iodide were removed. The gases were separated into fractions by gas-liquid chromatography, and various fractions were admitted into the mass spectrometer for analysis. This figure shows the mass spectrum of a mixture of hydrocarbons obtained in this manner from Michigami coal. A mixture of hydrocarbons from methane through pentane is shown.

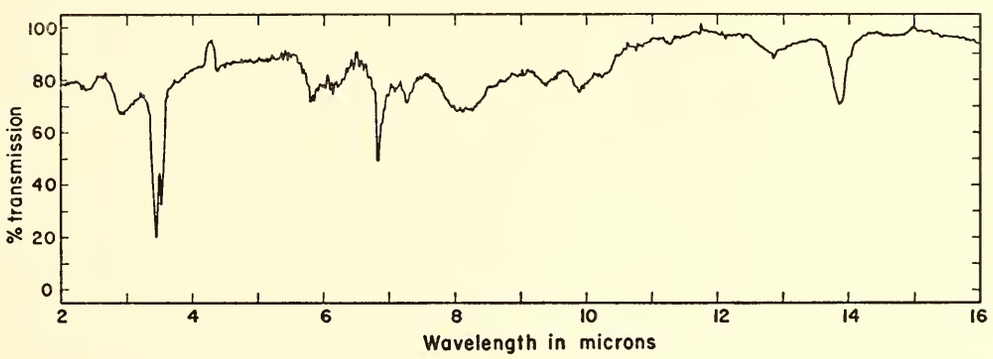

Fig. 74. Infrared adsorption spectrum of pyridine extract from Precambrian Transvaal dolomite. The "graphite" from the Transvaal dolomite was exhaustively extracted in a Soxhlet apparatus with pyridine. The pyridine was evaporated, and the resulting oil was pressed into a KBr pellet. The sharp adsorptions at 3.4–3.5, 6.8–6.9, and 14.0 microns are characteristic of isolated methylene ($-CH_2-$) groups. The broad adsorptions at 5.5–5.6 microns are suggestive of substituted aromatic hydrocarbons.

logically produced organic compounds from a period that preceded terrestrial life. A number of the rocks studied have textures that are generally described as due to colonial algae. The carbon isotope studies reported by the writer here indicate that photosynthesis was occurring during the time of their formation.

Thus the evidence is in favor of the existence of biological activity very early in the Precambrian era.

The Biogeochemistry of the Stable Isotopes of Carbon

The Isotopic Composition of the Carbon of Fatty Acids

P. L. Parker

Nier and Gulbransen (1939) first measured variations in the C^{13}/C^{12} ratios of naturally occurring carbon. They noted that plant and animal carbon was

slightly depleted in C^{13} compared with the inorganic carbon of limestone. Craig (1953) in a detailed survey of variations in the relative abundance of the carbon isotopes confirmed and expanded this observation. In both these studies the whole plant or animal was combusted to carbon dioxide, and so the measured ratio represents an average of the many different chemical compounds present in living matter. Living matter can be broken down into different types of chemical compounds and the C^{13}/C^{12} ratios of these compounds compared. Abelson and Hoering (1961) carried the study of the C^{13}/C^{12} variations to the molecular level for several amino acids isolated from a variety of photosynthetic organisms. Measurements of C^{13}/C^{12} ratios of fatty acid molecules relative to the C^{13}/C^{12} ratios of organisms from which the acids were isolated are described in the following.

The total lipide was Soxhlet-extracted from the samples with methanol and chloroform. The extract was taken to dryness on a steam bath under a stream of dry nitrogen. The residue was saponified for 2 hours with a 5 per cent solution of potassium hydroxide in methanol and acidified with sulfuric acid. A few milliliters of water were added, and the fatty acids were extracted from the mixture with chloroform which was then taken to dryness. The water-free residue was esterified by the boron trifluoride method (Metcalfe and Schmitz, 1961). This final solution was a complex mixture of the methyl esters of several fatty acids as well as any material that happened to follow the chemical procedure. Final purification and separation of the mixture of esters into specific esters was brought about by high-temperature gas chromatography.

The chromatographic analysis was performed with an 8-foot copper column packed with diethylene glycol succinate (LAC 3R 728) on acid-washed chromosorb-P. A thermal conductivity detector was used so that the samples could be recovered. A small amount of the total sample was analyzed to locate and identify the fatty acids present. Then a large sample was injected into the instrument, and the pure ester of each fatty acid was collected as it streamed out of the detector. Small glass tubes passing through a paper cup of dry ice served as collectors. To obtain 2 or 3 mg of ester it was necessary to repeat the collection two or three times. The glass tube containing the sample was placed directly in the combustion line, and the sample was burned to CO_2. This CO_2 was used for the mass analysis. The results of the mass analyses are expressed in terms of δC^{13}, the parts per thousand difference between the C^{13}/C^{12} ratio of the sample and a reference material.

$$\delta C^{13} = \frac{\left(\frac{C^{13}}{C^{12}}\right)_{\text{sample}} - \left(\frac{C^{13}}{C^{12}}\right)_{\text{reference}}}{(C^{13}/C^{12})_{\text{reference}}} \times 1000$$

A negative δC^{13} value indicates that the sample contains less C^{13} than the standard; a positive value, that it contains more.

In view of the complex physical manipulations and chemical procedure it was necessary to run a number of control experiments to ensure that the isotope fractionation measured was not thereby brought about. The esterification reaction was shown not to fractionate isotopes by a comparison of stearic acid with methyl stearate made from the stearic acid. If the acid is taken as 0.0 per mil the ester is −0.5. The isotope effect in the gas chromatography was measured by comparing the ester before and after chromatography. If the ester before chromatography is taken as 0.0 the ester after chromatography and 100 per cent collection is −0.4; after only 50 per cent collection the ester is +4.8 (100 per cent collection was used throughout this work). Isotope fractionation due to the procedure is less than 1.0 per mil. On the basis of repeated runs with the same starting material the overall error is estimated to be 1.0 per mil.

By means of these techniques the isotopic compositions of the fatty acids of two algae, a marine grass, and a plankton tow were measured. The results are given in table 29. Without exception the fatty acids were found to be significantly depleted in C^{13} as compared with the whole cell. Variations between different fatty acids from the same organism are too close to experimental error to be considered significant.

Chlorella was grown in the laboratory in a solution of the type described by Sorokin and Krauss (1958), 5 per cent CO_2, 95 per cent air, agitation, and constant illumination. Table 29 shows that the fatty acids of *Chlorella* are about 4 per mil depleted in C^{13} as compared with the total cells. The *Chlorella* used in the present work was grown in the same way and had the same carbon isotope ratio as the *Chlorella* used by Abelson and Hoering 2 years before. According to Abelson and Hoering the total amino acids of *Chlorella* are enriched in C^{13} by 3 per mil relative to the total cells. Thus the depletion in C^{13} of the fatty acids is balanced by the enrichment in C^{13} of the amino acids. The isotope variations are in the right direction and of the magnitude to yield a material balance.

TABLE 29. δC^{13} of Fatty Acids

Sample	Versus Inorganic Carbon as Reference	Versus Total Cell as Reference
Chlorella pyrenoidosa (inorganic carbon was tank CO_2)		
Total cells	−18	0.0
Palmitic	−22	−4
Palmitoleic	−22	−4
Stearic plus oleic	−23	−5
Ulva sp. (inorganic carbon was sea-water carbon)		
Total cells	−17	0.0
Palmitic	−24	−7.0
Stearic	−24	−6.8
Oleic	−24	−7
Linoleic	−24	−7.5
Linolenic	−25	−7.6
Thalassia sp. (inorganic carbon taken as sea-water carbon)		
Total cells	−6.5	0.0
Palmitic	−19	−13
Palmitoleic	−21	−15
Stearic	−21	−14
Oleic	−21	−14
Linoleic	−21	−14
Linolenic	−17	−11
Plankton, mostly euphausiids (inorganic carbon taken as sea-water carbon)		
Total cells	−20	0.0
Myristic	−24	−4.4
Palmitic plus palmitoleic	−26	−6.2
Stearic plus oleic and linoleic	−25	−5

The other three samples, from the ocean, were collected by the Marine Laboratory of the University of Miami. Again, the fatty acids of these three samples are depleted in C^{13} relative to the whole organism. *Ulva* is a marine alga that grows attached to rocks along the coast. *Thalassia* is a marine "grass" that grows in great abundance in the shallow bays of the Gulf and Atlantic coast. The euphausiids are small animals that live in the open sea.

The isotope fractionation in the formation of the fatty acids is in the same direction for all three of the photosynthetic organisms. If the δC^{13} of the feed CO_2 is taken as 0.0, the δC^{13} values of the fatty acids for all the plants fall between −17 and −25, suggesting that the biochemical reactions involving isotope fractionation in going from CO_2 to fatty acids are similar for all the plants studied.

Petroleums are depleted in C^{13} relative to modern organisms (Silverman and Epstein, 1958). Petroleum derived in large part from fatty acids, which have been shown to be generally depleted in C^{13} relative to whole organisms, might reflect this depletion in C^{13}. This is probably too simple a picture, because the organic molecules enriched in C^{13} must also be accounted for. Nevertheless, knowledge of the isotopic composition of specific types of molecules from living

organisms may give some clues about the ultimate fate of the many different types of organic molecules trapped in the sediments.

The Stable Isotopes of Carbon in the Carbonate and Reduced Carbon of Precambrian Sediments

T. C. Hoering

When inorganic carbon is fixed by plant photosynthesis there is an isotope effect and living cells have a lower concentration of the heavy isotope of carbon than the carbon dioxide or bicarbonate ion of their environment. The analyses of the C^{13}/C^{12} ratio of a large number of carbonates and reduced fossil carbons have been published. Ages of these samples range from recent back to the Cambrian. In general, the C^{13}/C^{12} ratio of carbonates is 1.02 to 1.03 times that of the associated reduced carbon, undoubtedly because of the isotope fractionation during photosynthesis.

Wickman (1941) and Rankama (1948) have proposed taking the isotopic composition of the graphite in very old rocks as an indication of biological or nonbiological origin. Their reasoning was criticized by Craig (1954) for a number of reasons, including their rather arbitrary grouping of carbon isotope ratios into biological and nonbiological.

The purpose of the present work was to measure the isotopic composition of the carbon in coexisting carbonates and reduced carbons in some of the very oldest rocks of the Precambrian. The existence of isotope fractionation between the oxidized and reduced forms of carbon in a rock that has had a mild thermal history, especially if the magnitude of the fractionation is similar to that found in rocks of known biological association, suggests that photosynthesis was occurring during the time of deposition of the rock. In Precambrian rocks, in which fossil evidence is meager or nonexistent, such geochemical studies give especially important evidence on the record of early terrestrial life.

The following criteria were set up for the selection of samples: (*a*) they are sedimentary rocks that have suffered as low a degree of metamorphism as possible; (*b*) they have presumptively remnant algal structures; (*c*) their minimum age can be estimated from the isotopic ages of neighboring igneous rocks. On this basis, the following rocks were used:

1. The Gunflint chert from near Port Arthur, Ontario. This rock, carefully described by Tyler and Barghoorn (1954), has a minimum age of 1.7 b.y. Structures contained in it have been described as filamentous blue-green algae. The samples were collected by E. S. Barghoorn.

2. The algal limestone from the Belt series of Glacier Park, Montana. They contain structures described as colonial algae and are documented by Fenton and Fenton (1937). General aspects of the geology of the region are described by Ross (1954). A minimum age of 1.2 b.y. is suggested (Tilton and Davis, 1959). The samples were collected by P. H. Abelson.

3. Domed algal growths of the Dolomite series, from near Schmidt's Drift, Union of South Africa. The structures have been described by Young and Mendelsohn (1948), and isotopic ages measured by Nicolaysen (1958) have set a minimum age of 2.0 b.y.

4. The Bulawayan limestone of the Zwankendaba series, from Bulawayo, Southern Rhodesia. The algal stromatolites in these rocks have been described by McGregor (1940), and the rocks have a minimum age of 2.7 b.y. (Holmes, 1954). These are taken by many geologists to be among the oldest known sediments. Samples were collected by I. Goldberg.

5. The Randville dolomite of the Iron River formation from near Crystal Falls, Michigan. The rocks have been described by James (1958). They have a minimum age of 1.5 b.y. The samples were col-

lected by H. James and P. H. Abelson.

Thin sections have been cut from the carbonate rocks for microscopic examination. Typically they consist of partly recrystallized calcite or dolomite with black specks of dispersed carbon in them. In some, the black particles have been concentrated along grain boundaries of the recrystallized material.

The carbon dioxide for the isotope analysis of the carbonate fraction was generated by treating with concentrated phosphoric acid. The reduced carbon fraction was isolated from the carbonate fraction by treating with hydrochloric and hydrofluoric acid. The resulting insoluble residue contained the reduced carbon, pyrite, and insoluble metal fluorides. It was combusted to give impure carbon dioxide. This gas was unsuitable for isotope analysis and was purified by gas-solid chromatography with a heated column of silica gel and with helium as the sweep gas. After the eluted carbon dioxide passed through a conductivity cell and its response was measured, it was frozen from the helium stream by passing it through a trap cooled with liquid nitrogen. This purification requires only about 5 minutes and yields very pure carbon dioxide, suitable for the mass spectrometer.

The results of this experiment are shown in table 30 and are expressed in parts per thousand difference in the C^{13}/C^{12} ratio of the sample and a standard material, NBS Isotope Reference Sample 20.

$$\delta C^{13} = \frac{\left(\frac{C^{13}}{C^{12}}\right)_X - \left(\frac{C^{13}}{C^{12}}\right)_{\text{standard}}}{(C^{13}/C^{12})_{\text{standard}}} \times 1000$$

There is clearly a large difference in the carbon isotope ratio between the oxidized and the reduced forms of carbon. The δC^{13} of the reduced carbon tends to be slightly more negative than is reported for coals of more recent ages, possibly because of isotope fractionation during the transformation of organic material while stored in the sediments.

A hypothesis is that the carbonate and the carbon are related to each other by some inorganic process. The reduction of carbon dioxide by magmatic gases to give graphite or the interaction of carbon dioxide and methane to give graphite would involve high temperatures. These processes would yield isotope fractionation, the heavy isotope concentrating in the carbon dioxide and the light isotope in the graphite phase. The rocks used in the present study, however, show no evidence of exposure to such high temperatures. It would have to be a coincidence that the distribution of the isotopes is so similar to that found in rocks of known biological origin.

The results of this work are consistent with a model of the existence of photosynthesis and biological activity in the oldest rocks of the Precambrian era. The experiments described in another part of the report on the isolation of organic compounds from the carbon of Precambrian rocks give support to the model.

TABLE 30. Isotopic Composition of the Carbon in Precambrian Rocks

Sample	δC^{13} Carbonate	δC^{13} Reduced	Difference
Gunflint chert	−4.0	−31.7	27.7
Algal limestone, Glacier Park	1.5	−23.5	25.0
Algal domes, Dolomite series, S. Africa	0.8	−29.9	29.1
Bulawayan limestone	−0.7	−29.1	28.4
Randville dolomite	2.4	−18.8	21.4

MISCELLANEOUS ADMINISTRATION

Institute on Isotopes and Radioactivity

A week-long institute, or special course, "Isotopes and Radioactivity," designed to acquaint secondary school science teachers of the Washington area with the role of radioactive isotopes in science and civil defense, was held from October 30 to November 3 at the Administration Building of the Carnegie Institution of Washington. It attracted much favorable attention from the press, radio, and television, and drew enthusiastic praise and thanks from the participants.

Conceived by Philip H. Abelson, President of the Washington Academy of Sciences, the Institute was sponsored by the Academy and the Joint Board on Science Education. At the request of Dr. Abelson, the morning-lecture and afternoon-laboratory curriculum was organized by Ralph T. Overman, Chairman of the Training Division of the Oak Ridge Institute of Nuclear Studies. About 140 teachers from parochial, private, and public schools were released from their classrooms to take this intensive course, one or two from each school. Their classes were met by scientists and engineers who had volunteered through the Joint Board to substitute for them.

The Institute is discussed in more detail in an article, by Frank L. Campbell, which appeared in the December 1961 issue of the *Journal of the Washington Academy of Sciences.*

Journal of Geophysical Research

The *Journal of Geophysical Research* is published monthly by the American Geophysical Union with P. H. Abelson (Geophysical Laboratory) and J. A. Peoples, Jr. (University of Kansas), as coeditors. About half of the editorial work, including manuscripts on upper atmosphere and space, as well as some of the papers involving geochemistry, are handled at this Laboratory. The *Journal* is regarded by many as the world's leading geophysical publication.

Though publishing about 5400 pages a year, the *Journal* has one of the fastest publication times among scientific journals. This accomplishment is due to the effective efforts of Dr. and Mrs. Peoples at Kansas, and the cooperation of Mrs. Lucile Stryker and Miss Mary Jane Miles of Carnegie Institution, and Mr. A. D. Singer and Miss Marjorie E. Imlay of the Geophysical Laboratory.

Lectures

During the report year staff members and fellows were invited to present lectures as follows:

As the recipient of the Regents' Distinguished Alumnus Award for 1961–1962, P. H. Abelson addressed a gathering at the Washington State University on April 5, 1962. At the American Association for the Advancement of Science meetings in Denver he participated in the Extraterrestrial Biochemistry and Biology Symposium and the Symposium on Geochemical Evolution—the First Five Billion Years. He delivered the Retiring President's Address before the Washington Academy of Sciences and the Sigma Xi Lecture at the Institute of Biosciences, Florida State University; and he participated in the Panel Discussion on the Chemical Origin of Life before the Chemical Society of Washington. Dr. Abelson also lectured to the Department of Botany, University of Missouri; the Applied Physics Laboratory, Johns Hopkins University; the Medical School at Georgetown University; the Institute for Space Studies, New York City; Research Associates at the National Institutes of Health; and the National Academy of Sciences at its annual meeting in Washington, D. C.

F. R. Boyd lectured at the Department of Geology, Pennsylvania State University.

C. W. Burnham gave two talks to the Geology Department at the University of Minnesota and addressed the Washington Crystal Colloquium at the National Bureau of Standards.

S. P. Clark, Jr., lectured at the College of Mineral Industries, Pennsylvania State University; the Department of Geology, University of Minnesota; the Institute of Geophysics, University of California at Los Angeles; and the National Academy of Sciences Summer Study Session on Nuclear Processes in Geology, Woods Hole, Massachusetts.

G. Donnay gave a lecture on color symmetry groups at the Mineralogical Institute of the University of Tokyo, Japan.

H. J. Greenwood delivered two lectures at the Department of Geology, California Institute of Technology.

T. C. Hoering addressed the Research and Development Laboratory of the Gulf Oil Company, Pittsburgh; the Department of Botany, University of Maryland; and the National Academy of Sciences Summer Study Session on Nuclear Processes in Geology, Woods Hole, Massachusetts. He also participated in the Symposium on the Biogeochemistry of the Isotopes of Sulfur at Yale University.

G. Kullerud lectured at the National Research Council, Ottawa, and the Departments of Geology at Lehigh University and McGill University. He also gave a series of five talks at the Department of Geology, Queen's University, Kingston, Ontario, and two lectures at the Department of Geology, University of Western Ontario.

N. Morimoto lectured at the Departments of Geology at the University of California, Berkeley, and the University of California, Los Angeles.

P. L. Parker addressed the Department of Zoology, Cornell University, and the Institute of Marine Science, University of Texas.

H. S. Yoder, Jr., gave three lectures at Clemson College and one at the Lamont Geological Observatory of Columbia University. During a visit to Japan, supported in part by the National Science Foundation, he gave lectures at the International Symposium on Volcanology held in Tokyo and the Departments of Geology of Hokkaido, Tohoku, and Kyoto Universities. He also spoke on high-pressure techniques at symposia in Kyoto and Osaka sponsored jointly by the Department of Geology of Kyoto University and the Matsushita Electric Industrial Company.

Petrologists' Club

Six meetings of the Petrologists' Club were held at the Laboratory this year. The following papers were presented:

"The system Fe-Zn-S; a preliminary report after five years," by Paul Barton and Pete Toulmin (U. S. Geological Survey).

"The petrology of the Rainier underground tests," by D. E. Rawson (Lawrence Radiation Laboratory).

"New observations on the opaque minerals of stony meteorites: Facts without hypotheses," by Paul Ramdohr (University of Heidelberg and Geophysical Laboratory).

"Petrological applications of the electron probe," by S. O. Agrell (Cambridge University).

"Field and laboratory observations pertaining to the origin of granite pegmatites," by R. H. Jahns (Pennsylvania State University).

"Some applications of sedimentary petrology to layered intrusions," by E. Dale Jackson (U. S. Geological Survey).

The Summary of Published Work below briefly describes the papers published in scientific journals during the report year. In addition, the following papers are now prepared for publication: P. H. Abelson, "Geochemistry of amino acids"; P. H. Abelson, "Paleobiochemistry"; R. G. Arnold, R. G. Coleman, and V. C. Fryklund, "Temperature of crystallization of pyrrhotite and sphalerite from the Highland-Surprise Mine, Coeur d'Alene District, Idaho"; F. Chayes, "Numerical correlation and petrographic variation"; G. A. Chinner and J. F. Schairer, "The join $Ca_3Al_2Si_3O_{12}$-

$Mg_3Al_2Si_3O_{12}$ and its bearing on the system CaO-MgO-Al_2O_3-SiO_2 at atmospheric pressure"; L. A. Clark, "X-ray method for rapid determination of sulfur and cobalt in loellingite"; S. P. Clark, Jr., "Temperatures in the continental crust"; B. R. Doe, "Relationships of lead isotopes among granites, pegmatites, and sulfide ores near Balmat, New York"; H. J. Greenwood and H. L. Barnes, "Binary mixtures of volatile components"; G. Kullerud, "Sulfide research"; G. W. Morey, "The action of water on calcite, magnesite, and dolomite"; N. Morimoto, "On the transition of bornite"; N. Morimoto and G. Kullerud, "Polymorphism in digenite"; J. V. Smith and W. Schreyer, "Location of argon and water in cordierite"; G. R. Tilton, G. W. Wetherill, and G. L. Davis, "Mineral ages from the Wichita and Arbuckle Mountains, Oklahoma, and the St. Francis Mountains, Missouri"; A. C. Turnock and H. P. Eugster, "Fe-Al oxides: Phase relationships below 1000°C"; D. R. Wones, "Phase equilibria of 'ferriannite,' $KFe_3^{+2}Fe^{+3}Si_3O_{10}(OH)_2$"; H. S. Yoder, Jr., and C. E. Tilley, "Origin of basalt magmas: An experimental study of natural and synthetic rock systems"; R. A. Yund, "The system Ni-As-S: Phase relations and mineralogical significance."

SUMMARY OF PUBLISHED WORK

(1352) Heat flow in the Austrian Alps. S. P. Clark, Jr. *Geophys. J.*, *6*, 54–63, 1961.

Data on underground temperature obtained during the construction of the Arlberg and Tauern tunnels in Austria have been combined with measurements of the thermal conductivity of 42 samples of rock from near the tunnels to calculate the terrestrial heat flow. The value in the Arlberg is found to be $(1.9 \pm 0.2) \times 10^{-6}$ cal/cm² sec; that in the Tauern, $(1.8 \pm 0.2) \times 10^{-6}$ cal/cm² sec. The new results are in good agreement with the value 1.9×10^{-6} cal/cm² sec found earlier in the Loetschberg tunnel in Switzerland, and indicate that relatively high geothermal fluxes extend into the eastern Alps. The high flux can be attributed to radioactive heat generation in a thickened crust.

(1353) Ponctualisation des charges dans les structures cristallines du type ionique. J. D. H. Donnay and G. Donnay. *Compt. Rend.*, *253*, 291–292, 1961.

Pairs of neighboring ions with the same sign are replaced by points, in which is concentrated the total charge of the two ions. Such points, regardless of their sign, are equivalent as far as morphology is concerned. This punctualization is performed on various projections of the crystal structure of barite (planar, onto coordinate planes; linear, onto coordinate axes).

(1354) A structural explanation of the polymorphism and transitions of $MgSiO_3$. W. L. Brown, N. Morimoto, and J. V. Smith. *J. Geol.*, *69*, 609–616, 1961.

Differences between the polymorphs of $MgSiO_3$ consist essentially of different ways of stacking slabs of SiO_3 chains, and transitions between the polymorphs may be effected by movements of chains by two-thirds of the *z*-axis spacing, together with associated displacements of Mg atoms by one-third of *c*. The transitions from proto- to rhombic enstatite and from proto- to clinoenstatite involve the same percentage of displaced atoms, but, because the displaced atoms are distributed more uniformly in the second transition, it is thought that a nucleus of clinoenstatite will propagate more easily than one of rhombic enstatite. This suggestion is consistent with the rapid metastable formation of clinoenstatite at low temperatures and with the sluggish formation of rhombic enstatite (often very disordered) from protoenstatite. Shearing stress should favor the formation of clinoenstatite in conformity with the experiments of Turner et al., and thus it may be a "stress mineral" in the sense of Harker. Highly complex schemes for arranging the SiO_3 chains are possible, and, as an example, three possible sequences are proposed for the enstatite with a 36 Å *a* axis described by Byström.

(1355) Compositions and structural states of anhydrous Mg-cordierites: A re-investigation of the central part of the system MgO-Al_2O_3-SiO_2. W. Schreyer and J. F. Schairer. *J. Petrol.*, *2*, 324–406, 1961.

The central portion of the system MgO-Al_2O_3-SiO_2 has been studied with the aim of determining the range of solid solution as well as the stability limits of the various structural states of the ternary compound cordierite. The previously suggested limited solid solution between cordierite of the composition $2MgO \cdot 2Al_2O_3 \cdot 5SiO_2$ (2:2:5) and SiO_2 is now believed to exist only metastably. Between 800° and 1300°C the composition of cordierite was found to be invariably $2MgO \cdot 2Al_2O_3 \cdot 5SiO_2$. Above 1300°C, however, there is evidence for the existence of limited solid solution in cordierite (2:2:5) toward a theoretical compound "Mg-beryl" (3:1:6). The existence of cordierite solid solution at liquidus temperatures has an important bearing on the melting relations of many compositions within the system. Because of this solid solution the courses of crystallization of melts consisting of normative cordierite (2:2:5) and small amounts of $MgSiO_3$, for example, have to follow parts of the boundary curve between the cordierite and spinel fields with these two phases coprecipitating over a limited range of temperatures. The dividing line between compositions that complete their crystallization at the ternary eutectic forsterite + protoenstatite + cordierite + liquid, 1364° ± 3°C, and those that complete their crystallization at the ternary eutectic protoenstatite + cordierite + tridymite + liquid, 1355° ± 3°C, was formerly considered to be the join $MgSiO_3$-cordierite (2:2:5). Because of solid solution in cordierite coexisting with liquid this dividing line is displaced slightly in the direction toward more siliceous bulk compositions. Furthermore, the temperature maximum along the boundary curve cordierite + protoenstatite + liquid cannot lie at the intersection of this boundary curve with the join $MgSiO_3$-2:2:5, but must lie with the tie line $MgSiO_3$-cordierite$_{ss}$. The position of this temperature maximum thus moves closer to the ternary eutectic protoenstatite + cordierite + tridymite + liquid. Temperatures and compositions of some of the invariant points in the system have been redetermined.

On the basis of Miyashiro's distortion index, the structural states of the cordierites synthesized are subdivided into high-cordierite, intermediate-state cordierite, and "low"-cordierite. High-cordierite was obtained in all compositions at any temperature as the first form of cordierite to crystallize. With continued heating at appropriate temperatures, this metastable high-cordierite was found to go over gradually through intermediate-state cordierite to the stable form "low"-cordierite. The rate of this transition varies with bulk composition and generally increases with temperature. In contrast to this metastable behavior are the stable relations among the polymorphs, which were found to be a function of temperature as well as total bulk composition of the cordierite-bearing mixtures. In bulk compositions with low Al_2O_3/SiO_2 ratios ($\lesssim$1:5) high-cordierite was not found to be a stable phase at any temperature; in bulk compositions with intermediate Al_2O_3/SiO_2 ratios high-cordierite is stable only in the presence of much liquid; in those with high Al_2O_3/SiO_2 ratios (>1:2:5) a stable transition from "low"-cordierite to high-cordierite takes place at subsolidus temperatures. This relationship is considered indirect evidence that Al/Si ordering is the principal cause of the transition from high- to "low"-cordierite.

Owing to solid solution the transition from "low"- to high-cordierite in the presence of liquid, for certain bulk compositions with intermediate Al_2O_3/SiO_2 ratios, takes place in a manner that cannot be described by a varying distortion index. For this reason a new variable, the intensity index, defined as $i = I_{(511+421)}/I_{(131)}$, is introduced, which is zero for high-cordierite solid solutions and 1.15 to 1.35 for "low"-cordierite.

The sensitive dependence of the structural behavior of cordierite on its chemical environment excludes the possibility of using this property as a geologic thermometer to a very large extent. Experimental investigations on cordierite-bearing synthetic "haplobuchites," as well as on a fused shale from the Bokaro coalfield in India, revealed that high-cordierite is not a stable phase for these bulk compositions at any temperature. Natural cordierites with structural states close to, or identical with, high-cordierite, which have been found in rocks formed at high temperatures (buchites, etc.), are believed to be metastable products of crystallization. They are preserved because the duration of heating was not sufficient to produce the stable low-temperature form. Petrographic and X-ray studies

show that there is a close relationship between the distortion index and the degree of perfection of the crystal form of cordierites in these rocks. On the basis of these results it seems possible to use the structural state of cordierites, at least qualitatively, as a geologic timer for the crystallization history of the enclosing rock.

(1356) A redetermination of equilibrium relations between kyanite and sillimanite. S. P. Clark, Jr. *Am. J. Sci., 259,* 641–650, 1961.

The equilibrium curve between kyanite and sillimanite has been established by quenching experiments at temperatures between 1000° and 1500°C and pressures between 17 and 24 kb. The curve is given by the expression $P = 4.1 + 13.2 \times 10^{-3}\ T$, where the pressure, P, is in kilobars and the temperature, T, is in degrees Centigrade. There is some evidence that the phase boundary may depart from linearity at low temperatures, but no quantitative estimate of the amount of curvature can be obtained from present data.

If kyanite forms stably in nature, pressures of nearly 10 kb are required. This is equivalent to the weight of about 30 km of overburden. Such great depths of burial are not required if pressure is contained by the strength as well as by the weight of the overlying rock. It is suggested that "tectonic overpressures" of a kilobar or more may exist in rocks undergoing deformation.

(1357) Metastable solid solutions with quartz-type structures on the join SiO_2-$MgAl_2O_4$. W. Schreyer and J. F. Schairer. *Z. Krist., 116,* 60–82, 1961.

Various members of a series of metastable solid solutions with a quartz-type structure and with compositions between SiO_2 and $MgAl_2O_4$ have been synthesized from glass. Increasing amounts of Mg^{+2} and Al^{+3} in the quartz structure cause a gradual contraction parallel to, and a gradual expansion perpendicular to, the c axis. Siliceous members of the series are optically positive, and less siliceous members negative; for a member with about 73 weight per cent SiO_2 the birefringence is zero. The refractive indices of the solid solutions increase with decreasing SiO_2 content. Members with less than about 92 weight per cent SiO_2 exhibit high-quartz structures even at room temperature, whereas more siliceous members go through an inversion to a low-quartz structure when quenched to room temperature. The temperature of this inversion is lower than that of pure quartz (SiO_2) as a result of the presence of Mg^{+2} and Al^{+3} in the structure.

(1358) Phase relations in the system Ni-As. R. A. Yund. *Econ. Geol., 56,* 1273–1296, 1961.

Phase relations in the system Ni-As were determined in rigid silica glass tubes, in collapsible gold tubes, and by differential thermal analyses. The system includes the well established minerals maucherite ($Ni_{11}As_8$) niccolite ($Ni_{1\pm x}As$), and the $NiAs_2$ polymorphs rammelsbergite and pararammelsbergite.

A phase with the composition of Ni_3As (dienerite) could not be synthesized, and if this phase exists it must be stable only below 200°C. $Ni_{5-x}As_2$ is stable to approximately 993°C and has a large variation in its Ni/As ratio. Maucherite, which is essentially restricted to $Ni_{11}As_8$ composition, melts incongruently at 830° ± 5°C to niccolite plus a liquid. The existence of a metastable form of $Ni_{11}As_8$ appears to be likely.

Niccolite, which is stable to 962° ± 3°C, also has a large variation in its Ni/As ratio. The niccolite solvus between NiAs and $NiAs_2$ is not useful as a geothermometer, however, since it is nearly vertical in the temperature range of geologic interest. The pararammelsbergite-rammelsbergite inversion was found to occur at 590°C under the vapor pressure of the assemblage when pure $NiAs_2$ is in equilibrium with niccolite. The inversion temperature is raised 22°C/1000 bars, giving a ΔH of 0.57 kcal/mole at 590°C. When pure $NiAs_2$ is in equilibrium with metallic arsenic instead of niccolite, the inversion temperature is approximately 8°C higher. Investigation of the inversion temperatures of natural specimens of rammelsbergite and pararammelsbergite shows that solid solution of elements such as Fe, Co, and S may lower the inversion by more than 100°C.

(1359) Molar volumes and thermal expansions of andalusite, kyanite, and sillimanite. B. J. Skinner, S. P. Clark, Jr., and D. E. Appleman. *Am. J. Sci., 259,* 651–668, 1961.

Precise measurements of unit-cell parameters of four andalusites, four sillimanites, and

five kyanites from different localities lead to the following molar volumes at 25°C: andalusite, 51.550 ± 0.011 cm^3/mole; sillimanite, 49.918 ± 0.015 cm^3/mole; kyanite, 44.116 ± 0.021 cm^3/mole.

Unit-cell parameters at high temperatures were measured with a heating stage on an X-ray diffractometer. From these data the molar volumes and thermal expansions of all three minerals were obtained between 25° and 1050°C.

(1360) Woodring Conference on Major Biologic Innovations and the Geologic Record. P. E. Cloud, Jr., and P. H. Abelson. *Proc. Natl. Acad. Sci. U. S.*, *47*, 1705–1712, 1961.

The Woodring Conference was held at Big Meadows Lodge, Skyline Drive, Virginia, June 14–16, 1961. It was attended by twenty-three biologists and geologists. The conference was a multidisciplinary approach to major biological innovations in the context of the geologic record, and with emphasis on the nature, manifestations, and timing of events leading to the first Metazoa. This report describes the proceedings of the meeting and includes an excellent bibliography.

(1361) The system $NaAlSi_2O_6$-H_2O-argon: Total pressure and water pressure in metamorphism. H. J. Greenwood. *J. Geophys. Res.*, *66*, 3923–3946, 1961.

Phase equilibrium in metamorphic rocks is affected by temperature, pressure, the proportions of nonvolatile components, and the chemical potentials of the reacting volatile components. Theory interrelating these variables has been tested by studying the reaction analcite → albite + nepheline + water in the presence of mixtures of water and argon. New data on the system Ar-H_2O permit calculation of the composition of the water-argon mixture, which should equilibrate with the phase assemblage analcite + albite + nepheline. Experimental determination of this composition as a function of pressure at constant temperature is in good agreement with the theory.

(1362) Stability relations of glaucophane. W. G. Ernst. *Am. J. Sci.*, *259*, 735–765, 1961.

Stability relations have been determined for glaucophane [$\square Na_2Mg_3Al_2Si_8O_{22}(OH)_2$] + excess vapor and for quartz + glaucophane + vapor by means of conventional hydrothermal techniques. The high-temperature stability limit of this amphibole ranges from 850°C at 175 bars vapor (= total) pressure to 868°C at 2000 bars P_{vapor}. Neither differential stress nor high pressures are necessary for the formation of glaucophane. The presence of excess silica lowers its high-temperature stability limit only 3° to 6°C.

Unusually large enthalpy values for the reactions glaucophane → forsterite + enstatite + albite + vapor and quartz + glaucophane → enstatite + albite + vapor (330 ± 60 and 320 ± 60 kcal/mole, respectively) can be explained only in part by the change in coordination of aluminum from 6 in glaucophane to 4 in albite. The entropy of glaucophane at 864°C and 1000 bars vapor pressure is 150 ± 50 cal/deg/mole.

Optical properties of synthetic glaucophane agree well with data for natural specimens. Unit-cell dimensions of the synthetic material are slightly larger than those of natural glaucophanes.

The experimental investigation indicates that glaucophane is stable over a wide range of physical conditions, given appropriate chemical conditions. Bulk compositions rich in soda and magnesia and poor in lime relative to alumina should favor production of glaucophane. The rare occurrence of such chemical environments severely restricts the crystallization of glaucophane in nature.

(1363) Annual report of the Director for 1960–1961.

(1364) Age measurements on rocks from the Finnish Precambrian. G. W. Wetherill, O. Kouvo, G. R. Tilton, and P. W. Gast. *J. Geol.*, *70*, 74–88, 1962.

New mineral age measurements are reported from several subdivisions of the Finnish Precambrian. Samples of zircon, feldspar, and muscovite collected from the gneissose pre-Karelian basement area in eastern Finland indicate an age of about 2700 m.y. for these rocks. In contrast, biotite ages from the same rocks agree at 1800 m.y., presumably representing the effect of the orogeny at this time.

Measurements on samples of mantled gneiss domes within the Karelian belt give feldspar and zircon ages supporting the correlation of these rocks with the pre-Karelian basement

to the east, and again the biotite ages represent the time of the 1800-m.y. orogeny. These results are closely analogous to data previously reported for mantled gneiss domes near Baltimore, Maryland.

Additional measurements on the younger Precambrian rocks of Finland confirm earlier data indicating an age of around 1800 m.y. for plutonic rocks associated with both the Svecofennian and Karelian orogenic belts.

(1365) Polymorphism in bornite. N. Morimoto and G. Kullerud. *Am. Mineralogist, 46*, 1270–1282, 1961.

Synthetic Cu_5FeS_4 and natural bornite were observed in three crystalline modifications: (1) a high-temperature form, face-centered cubic, with $a = 5.50 \pm 0.01$ Å, $Z = 1$, and probably antifluorite structure; (2) a metastable form, cubic, $Fd3m$ or $F\bar{4}3m$, with $a = 10.94 \pm 0.02$ Å, $Z = 8$; (3) a low-temperature form, primitive tetragonal, space group $P\bar{4}2_1c$, pseudo-$I\bar{4}2d$, with $a = 10.94 \pm 0.02$, $c = 21.88 \pm 0.04$ Å, $Z = 16$. The high-temperature form is nonquenchable and is stable only above 228° ± 5°C (for synthetic materials). The metastable form appears on rapid cooling from temperatures above that of the polymorphic inversion; it changes to the low-temperature form slowly at room temperature. The low-temperature and the metastable forms are closely related in crystal structure, as shown by their similar intensity distributions in X-ray patterns. Twinning of the tetragonal form about a threefold twin axis [221] accounts for other previously reported "modifications."

(1366) Arsenopyrite crystal-chemical relations. N. Morimoto and L. A. Clark. *Am. Mineralogist, 46*, 1448–1469, 1961.

The composition of naturally occurring arsenopyrite varies from about $FeAs_{0.9}S_{1.1}$ to $FeAs_{1.1}S_{0.9}$, as indicated by the more credible published chemical analyses and one new analysis. Analytical errors probably account for any apparent deviations of the Fe/(As+S) ratio from 1:2.

Five arsenopyrites of different compositions were studied by single-crystal X-ray methods. The changes caused by increasing arsenic content are (1) the triclinic symmetry approaches monoclinic and (2) metrically the cell approaches the orthorhombic. These pseudosymmetries give rise to two types of twinning. Although refinements of the arsenopyrite crystal structure by means of ($h0l$) and ($hk0$) data were hampered by twinning, the atomic coordinates obtained in this investigation confirm those of Buerger. The interatomic distances Fe-As, Fe-S, and As-S are 2.35, 2.25, and 2.33 Å, respectively.

Indexed X-ray powder data are given. The metrically monoclinic cell constants for six analyzed arsenopyrites relate linearly to arsenic content and inversely to sulfur content. Provided the combined minor element content is below 1 per cent, the curve $d_{131} = 1.6106 + 0.00098x$, where x is the arsenopyrite arsenic content in atomic per cent, enables rapid determination of arsenopyrite compositions to within 1 atomic per cent.

(1367) Stability relations of the ferruginous biotite, annite. H. P. Eugster and D. R. Wones. *J. Petrol., 3*, 82–125, 1962.

Annite, $KFe_3AlSi_3O_{10}(OH)_2$, a member of the iron biotites and the ferrous analogue of phlogopite, has been synthesized and its phase relations have been determined as functions of temperature, fugacity of oxygen (f_{O_2}), and total pressure ($P_{total} \approx P_{H_2O} + P_{H_2}$). A method for controlling f_{O_2} at high total pressures is described, and data for the "oxygen buffers" used are given. Buffers range from quartz + iron + fayalite assemblages (low f_{O_2}) to magnetite-hematite assemblages (high f_{O_2}). Optical properties and unit-cell dimensions of synthetic annites depend on the conditions of synthesis.

By recalculating published analyses of natural iron-rich biotites it can be shown that a constant hydrogen content cannot be assumed for such biotites. Oxidation may have occurred by drying at 115°C. Octahedral occupancy therefore cannot be calculated from such data.

Phase relations of annite are presented in 2070 and 1035 bar sections. Depending on f_{O_2}-T values, annite was found to decompose to one of the following assemblages: hematite + sanidine, magnetite + sanidine, fayalite + leucite + kalsilite, iron + sanidine. All decompositions are dehydration and redox reactions and are sensitive to changes in f_{H_2O} and f_{O_2} (or f_{H_2O} and f_{H_2}). At 2070 bars total pressure annite + magnetite + sanidine can coexist between 425° and 825°C, depending on the magnitude of f_{O_2}.

In the presence of quartz the stability field

of annite is more restricted. Phase equilibria in the system $KAlSiO_4$-SiO_2-Fe-O_2-H_2 have been summarized schematically.

Wherever possible, thermodynamic extrapolations are made to test the internal consistency of the data. Enthalpies of formation are calculated for both annite and phlogopite. Ranges of f_{O_2} values in nature as well as mechanisms for changes in f_{O_2} are investigated. It is useful to distinguish between assemblages that are internally buffered with respect to f_{O_2} changes and those that are not buffered. The applications of individual reactions involving annite to specific geologic problems are discussed with respect to igneous, metamorphic, and sedimentary rocks.

(1368) The Ni-S system and related minerals. G. Kullerud and R. A. Yund. *J. Petrol., 3*, 126–175, 1962.

The system Ni-S has been studied systematically from 200° to 1030°C by means of evacuated, sealed silica glass tube experiments and differential thermal analyses. Compounds in the system are Ni_3S_2 (and a high-temperature, nonquenchable $Ni_{3\pm x}S_2$ phase), Ni_7S_6, $Ni_{1-x}S$, Ni_3S_4, and NiS_2. The geologic occurrence of the minerals heazlewoodite (Ni_3S_2), millerite ($\beta Ni_{1-x}S$), polydymite (Ni_3S_4), and vaesite (NiS_2) can now be described in terms of the stability ranges of their synthetic equivalents.

Hexagonal heazlewoodite, which is stoichiometric within the limit of error of the experiments, inverts on heating to a tetragonal or pseudotetragonal phase at 556°C. This high-temperature phase ($Ni_{3\pm x}S_2$) has a wide field of stability, from 23.5 to 30.5 weight per cent sulfur at 600°C, and melts incongruently at 806° ± 3°C. The βNi_7S_6 phase inverts to αNi_7S_6 at 397°C when in equilibrium with Ni_3S_2 and at 400°C when in equilibrium with αNiS. Crystals of αNi_7S_6 break down to $Ni_{3-x}S_2$ + αNiS at 573° ± 3°C. The low-temperature form of $Ni_{1-x}S$, corresponding to the mineral millerite, is rhombohedral, and the high-temperature form has the hexagonal NiAs structure. Stoichiometric NiS inverts at 379° ± 3°C, whereas $Ni_{1-x}S$ with the maximum nickel deficiency inverts at 282° ± 5°C. The $Ni_{1-x}S$-NiS_2 solvus was determined to 985° ± 3°C, the eutectic temperature of these phases. Stoichiometric NiS is stable at 600°C but breaks down to $Ni_{3-x}S_2$ and $\alpha Ni_{1-x}S$ below 797°C, whereas $\alpha Ni_{1-x}S$ with 38.2 weight per cent sulfur melts congruently at 992° ± 3°C. Vaesite does not vary measurably from stoichiometric NiS_2 composition and melts congruently at 1007° ± 5°C. Polydymite breaks down to $\alpha Ni_{1-x}S$ + vaesite at 356° ± 3°C. Differential thermal analyses showed the existence of a two-liquid field in the sulfur-rich part of the system above 991°C and over a wide compositional range.

(1369) Equilibrium relations between pyrrhotite and pyrite from 325° to 743°C. R. G. Arnold. *Econ. Geol., 57*, 72–90, 1962.

The pyrrhotite solvus that represents the compositions of pyrrhotite coexisting in equilibrium with pyrite was determined in the temperature range 325° to 743°C by experiments conducted in sealed, evacuated, silica glass capsules and at pressures equal to the pressure of the vapor in equilibrium with the condensed phases. Experiments conducted in sealed, collapsible gold tubes demonstrate that confining pressures of 2000 bars do not measurably affect the position of the solvus below 670°C.

The compositions of synthetic hexagonal pyrrhotite were measured within ±0.13 atomic per cent Fe with the aid of an X-ray determinative curve that relates $d(102)$ to composition.

X-ray powder data and a general description are given for an unidentified lamellar iron sulfide phase occurring in rapidly quenched iron-deficient pyrrhotite.

Temperatures of crystallization of ten natural pyrrhotite-pyrite assemblages are estimated by means of the pyrrhotite solvus. The temperature of crystallization of sphalerite coexisting with pyrrhotite and pyrite in four of these samples was also measured. With very few exceptions the estimates obtained from the two methods agree well within the experimental error.

(1370) Measurement of the metal content of naturally occurring, metal-deficient, hexagonal pyrrhotite by an X-ray spacing method. R. G. Arnold and L. E. Reichen. *Am. Mineralogist, 47*, 105–111, 1962.

It is shown on the basis of fourteen chemically analyzed pyrrhotites that the metal content of metal-deficient natural pyrrhotites may be measured to ±0.25 atomic per cent

by means of an experimentally derived X-ray determinative curve, provided that the combined concentration of nickel, cobalt, and copper in solid solution is less than about 0.6 per cent by weight.

(1371) Metastable osumilite- and petalite-type phases in the system MgO-Al_2O_3-SiO_2. W. Schreyer and J. F. Schairer. *Am. Mineralogist, 47*, 90–104, 1962.

Two new compounds have been synthesized metastably in the system MgO-Al_2O_3-SiO_2. One has a structure similar to that of osumilite and other related phases, such as the synthetic compound $Na_2O \cdot 5MgO \cdot 12SiO_2$. It has a composition along the line SiO_2-$MgAl_2O_4$, probably close or equal to $MgO \cdot Al_2O_3 \cdot 4SiO_2$ as deduced from the phase assemblages. On the other hand, the measured mean index of refraction (1.535), according to the Gladstone-Dale relationship, suggests a composition containing less SiO_2, such as $4MgO \cdot 4Al_2O_3 \cdot 7SiO_2$. The other compound, whose composition is unknown, yields a powder X-ray diffraction pattern similar to those of petalite, $Li_2O \cdot Al_2O_3 \cdot 8SiO_2$, and lithium disilicate, $Li_2O \cdot 2SiO_2$. The two metastable phases form during devitrification of glass of certain bulk compositions at relatively low subsolidus temperatures. Upon further heating they are gradually replaced by assemblages that are more stable for these bulk compositions and include cordierite and a silica modification with or without protoenstatite.

(1372) A titaniferous basalt from the Island of Pantelleria. E. G. Zies. *J. Petrol., 3*, 177–180, 1962.

A new analysis of a highly titaniferous basalt from the Island of Pantelleria, first described by H. S. Washington, is presented. The new values for both TiO_2 and Al_2O_3 differ appreciably from Washington's and produce marked changes in the calculation of the CIPW norm. The analytical procedures by which the new values were obtained are given in outline.

(1373) Centers of charges inferred from barite morphology. J. D. H. Donnay and G. Donnay. *Soviet Phys. Cryst., 6*, 679–684, 1962.

A comparison between the crystal structure of barite and its morphological development leads to the concept of centers of charges. The centers of charges act as equivalent points in the bond assemblage that controls the morphology.

(1374) Mineral ages from the Appalachian province in North Carolina and Tennessee. G. L. Davis, G. R. Tilton, and G. W. Wetherill. *J. Geophys. Res., 67*, 1987–1996, 1962.

Age measurements are given for nine zircons and nine micas from the Appalachian orogenic zone in western North Carolina and eastern Tennessee. These measurements provide further evidence for the existence of crystalline rocks as old as 1000 m.y. in the area. A still older age of 1300 m.y. is found for zircons from two gneissic rocks; these older zircons are probably detrital. All the zircons have discordant ages. The discordances are compatible with loss of lead by continuous diffusion or episodic loss as a result of Paleozoic metamorphism. Possible difficulties in ascribing the discordances solely to episodic loss during Paleozoic metamorphism are pointed out. The problem of loss of lead during fusion of zircon has been studied; losses are shown to be negligible.

(1376) Skutterudites $(Co,Ni,Fe)As_{3-x}$: Composition and cell dimensions. E. H. Roseboom, Jr. *Am. Mineralogist, 47*, 310–327, 1962.

Skutterudites were synthesized by heating mixtures of Co, Ni, Fe, and As at 600° to 800°C in sealed, evacuated silica tubes. The resulting phases were identified by powder X-ray diffraction methods and by ore microscopy.

Analyses of natural skutterudites by numerous workers have indicated nonstoichiometric compositions with a deficiency of As. It has been suggested that the As deficiency may be due to the presence of other phases as impurities. In the present study, synthetic cobalt skutterudite was found to have a small but real As deficiency, even in the presence of crystalline As, but this deficiency is too small to account for the large deficiencies indicated in many analyses of natural skutterudites.

Natural skutterudites are known to vary widely in their Co, Ni, and Fe content, but pure Ni and Fe members are unknown. The same is true for synthetic skutterudites. The limits of solid solution vary little with tem-

perature between 600° and 800°C, and most natural skutterudites fall within the limits of solid solution observed for the synthetic phases.

The cell edges of twenty-six synthetic skutterudites with nickel content equal to or greater than iron content are related to composition by the function $a = 0.1240X - 0.0246Y + 8.2060$, where a is the cell edge in Å, X is the mole ratio Ni/(Co + Ni + Fe), and Y is the mole ratio Fe/(Co + Ni + Fe). The function describes the measurements to a standard deviation of 0.00086 Å. The cell edges of thirteen analyzed natural skutterudites of other workers show fair agreement with the synthetic ones, and are described by the above function to a standard deviation of 0.0097 Å. The deviations of the measured cell edges of natural skutterudites from cell edges computed using the function are not demonstrably due to differences either in (As + S)/(Co + Ni + Fe) ratios or in total sulfur content.

(1377) Erzmikroskopische Untersuchungen an Magnetiten der Exhalationen im Valley of the 10,000 Smokes. P. Ramdohr. *Neues Jahrb. Mineral., Monatsh.*, 49–59, 1962.

These fumaroles produced locally large quantities of loosely coherent crystals of magnetite. Analyses by E. G. Zies revealed the presence of substantial amounts of Zn, Cu, Pb, Mn, Ni, Co, Mo, and Sn. Various writers have thought of these metals as existing in an anomalous form of mixed crystals in the magnetite in spite of the fact that Zies gave evidence and expressed the opinion that most of the metallic constituents were present as sulfides. Actually the magnetite contains the following sulfides: FeS, FeS_2, $CuFeS_2$, chalcopyrrhotite, bornite, Cu_2S, Cu, ZnS, MoS_2, FeAsS. Besides that there is zincite; only a part of Zn and Mn are in magnetite itself. Paragenetically, that assemblage is of interest for ore deposits of exhalative origin in general.

(1382) Phase equilibria in silicate systems at high pressures and temperatures. F. R. Boyd, Jr. In *Modern Very High Pressure Techniques*, edited by R. H. Wentorf, Jr., Butterworths, Washington, D. C., pp. 151–162, 1962.

Studies of mineral equilibria at high pressures yield data for estimating the conditions of formation of igneous and metamorphic rocks. These data also provide a basis for speculation about the mineralogy of rocks in the earth's mantle. In the pressure range up to 50 kb, experiments are most easily and accurately made with single-stage apparatus. Pressures up to 100 kb can be obtained with two-stage apparatus in which the piston is supported by a KBr cell compressed to about 20 kb.

Most silicates whose atomic structures are relatively open networks invert or break down to denser phases in the pressure range 10 to 30 kb. The quartz-coesite inversion is a chemically simple example, and the P-T curve for this reaction in the temperature range 700° to 1700°C is given. Minerals with more closely packed atomic structures are stable to much higher pressures, but some inversions in these minerals have been discovered. The inversion of the olivine Fe_2SiO_4 to a spinel form is briefly discussed. Few measurements of the effect of pressure on the melting relations of silicates have thus far been made, although such data will have important geologic applications. A preliminary melting curve for diopside to 35 kb is given.

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[18]Resigned June 15, 1962.
[19]Appointment terminated February 28, 1962.

Fig. 34. Ilmenite-"magnetite" intergrowth made by holding a charge of pure ulvöspinel at 1000°C and the f_{O2} of the bomb walls for 3 hours. The darker gray "magnetite" host is about $Mt_{50}Usp_{50}$; the light gray $ilmenite_{ss}$ lamellae lie in the (111) planes of the host. ×2200. Photograph by Professor Paul Ramdohr.

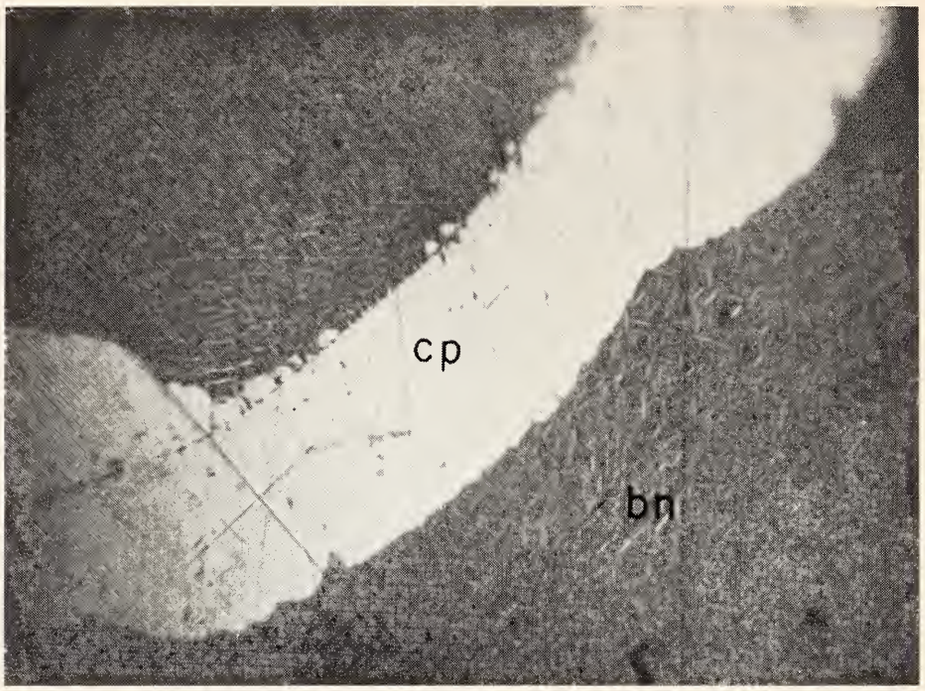

Fig. 53. Chalcopyrite (cp) exsolved from bornite (bn). Exsolved chalcopyrite is in the form of a vein and as tiny lamellae. Specimen annealed at 300°C for 4½ months. × 800 (oil).

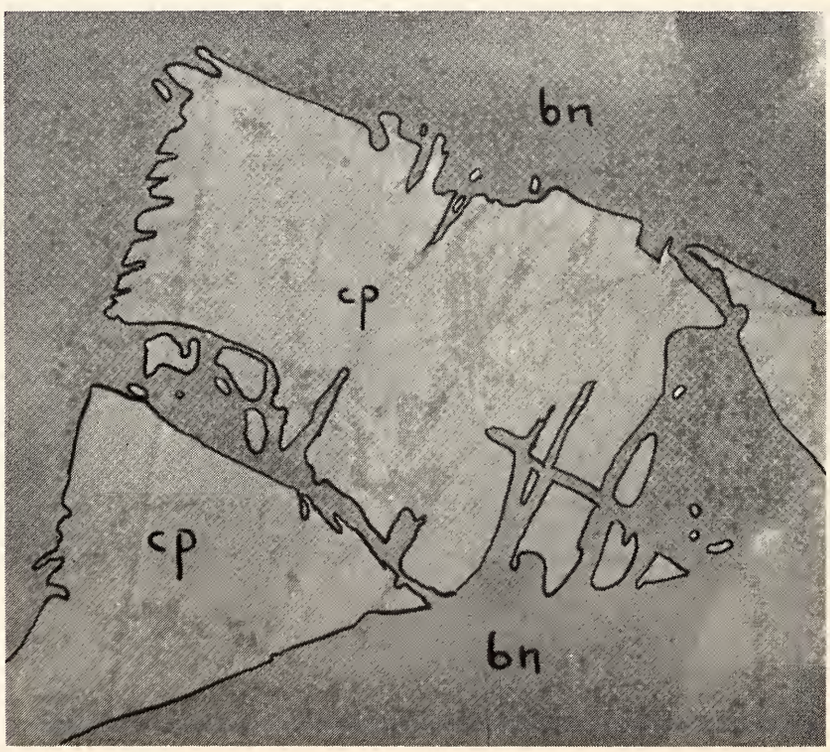

Fig. 54. Chalcopyrite (cp) exsolved from bornite (bn). Note pseudoreplacement texture. Specimen cooled from 700° to 50°C in 22 hours. × 1600 (oil). (Retouched.)

Department of Terrestrial Magnetism

Washington, District of Columbia

Merle A. Tuve,
Director

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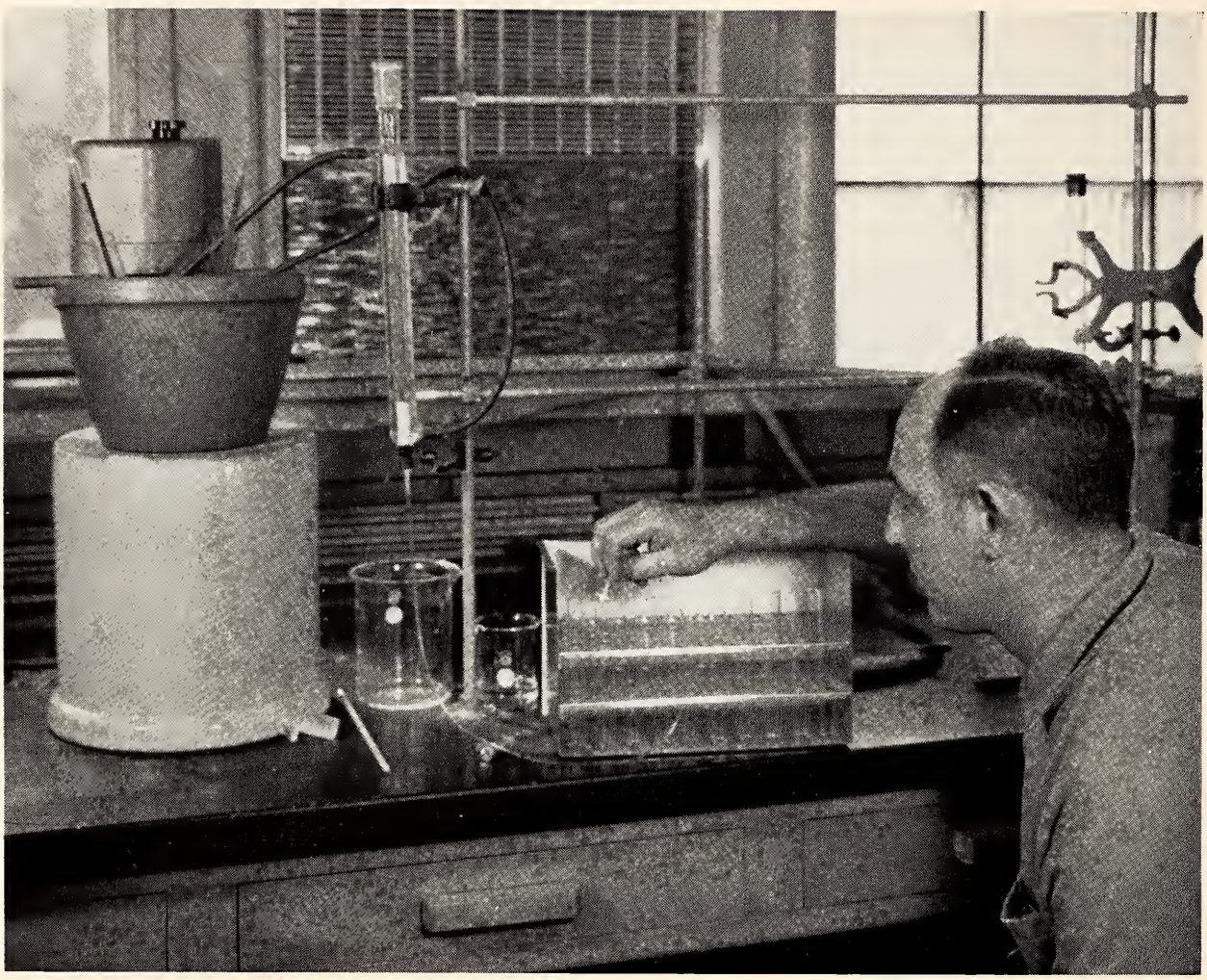

Purification of "messenger" nucleic acid. Single-stranded DNA, the genetic material of the cell, is immobilized in a bed of agar. RNA is passed through the bed, where the RNA molecules ("messengers") containing nucleotide sequences complementary to those in the DNA are specifically hybridized by means of hydrogen bonding. The hydrogen bonds are then "melted," and the purified messenger is collected for further study.

Two beautiful examples of laboratory work at the Department, each one exhibiting in conspicuous simplicity the patterns governed by the laws of nature, moved, during the year, from the idea stage into the factual stage of accomplishment.

One example was the use of a spin-polarized beam of deuterons in our 4-Mev Van de Graaff generator for studies of light-element nuclear reactions. Appropriately, in view of the Swiss emphasis, and our own, on nuclear physics as a scientific discipline (not technology), this was a joint activity of our Department with Professor P. Huber of Basel, Switzerland, and his colleagues. Two years ago, his group there developed the first successful spin-polarized hydrogen-ion source, and our high-voltage generator, now 25 years old, is the only one in the world with the obsolescent characteristic of large bulk, so that the 19-foot ball, which is the high-voltage electrode, is able to accommodate the large 6-kilowatt analyzer that provides the polarized beam. This spin-polarized beam provides a direct and simple approach to many familiar questions of spin and parity in nuclear reactions.

The second example evolved abruptly in the course of studies of nucleic acids by our biophysics group, who were examining the early products of biosynthesis and the handing-on of genetic information by "biological coding" from the DNA to RNA and protein. The idea has been familiar for several years that the double-stranded helix of DNA (as in chromosomes, etc.) must somehow separate into single strands and then transmit its genetic information (e.g., in the form of sequences of the nucleotides of adenine, guanine, cytosine, and thymine) to RNA and thence to proteins, with their many highly specific enzymatic properties. Nevertheless, the complex mixture of old and new RNA molecules, which is the end result of standard separation procedures, has meant that the "messenger RNA" which carries to the nascent protein the DNA information or code (by being a complementary sequence of nucleotides) has not been available for study as a separate item. "Messenger RNA" to date has chiefly been useful as a concept. Toward the close of the report year a new and simple technique was developed here for the adsorption of highly specific fractions of RNA on single-stranded DNA which has long sequences of biological coding identical (complementary) to the coding of this specific RNA. The DNA is entrapped in agar gel in the single-strand form, and the total RNA mixture is slowly filtered through the gel. The specifically adsorbed RNA is a small fraction of the total RNA, perhaps 1 or 2 per cent, and it can subsequently be released from the DNA by suitable elution, thus yielding the coded "messenger" RNA as a separated constituent. Our experiments have demonstrated that DNA and RNA from closely related bacteria show appropriately related degrees of specific adsorption, while unrelated bacteria exhibit no such adsorption between the DNA of one and the RNA of the other.

Another research result of considerable consequence which was confirmed and convincingly illustrated during the year relates to the study of the earth's crust by sound waves from explosions. At increasing distances from an explosion the velocity of the waves which first arrive appears to increase, and this effect has been widely interpreted as showing the existence of various "layers" of rock with intermediate velocities interposed between the low-velocity rocks near the surface and the upper boundary of the earth's mantle at a depth of 40 or 50 kilometers under most land areas. Detailed study of the results of an intensive program of sea explosions in the Gulf of

Maine during 1961, arranged by our Department with the Navy, the Coast Guard, and a group of scientific collaborators (especially the University of Wisconsin), led to the emphatic recognition, again, of a mathematical result known for several decades. Changes in the rate of increase of velocity with depth give rise to "cusps" (multiple-valued regions) on the travel-time curve, and these cusps are both frequent and superposed on one another when the travel-time curve is plotted for any ordinary sample of the real earth. Not only this, but, as a result of it, when efforts are made to determine the velocity structure of regions in the crust below 16 or 18 kilometers' depth, the observed travel-time curves can be reproduced by any one of a considerable variety of assumed structures of velocity as a function of depth, owing to the importance of the rates of change of velocity, superposed on the actual values of velocity at stated depths. This unequivocal demonstration, again, of the very real limitations imposed on our ability to learn about crustal "layers" by the customary methods of explosion seismology came at a time of special pertinence, in view of the greatly increased activities brought about by the Geneva proposals for detection of bomb explosions in relation to arms control.

The cooperative expedition to Maine has been only one aspect of a general procedure of intensive cooperation with selected individual colleagues which is followed in most of our work. Other examples this year relate to studies of local earthquakes in the Andes jointly with three groups (now four) in different western Latin American countries, and to initial preparations here, jointly with other colleagues from eastern South America, for radio astronomy studies of the southern sky, with cooperative instrumentation we are constructing for use in Argentina and Brazil. Similar emphasis on colleague-to-colleague arrangements have been effective in our biophysics program and in our work on the isotope dating of ancient rocks. A program of studies of deep electrical conductivity (80 km) by magnetic variations in Peru and Bolivia is also now becoming a reality.

In a quite different area and a highly specialized technical field, the Department is guiding another cooperative program, largely based to date on our close contacts with the electronic tube industry, which endeavors to put into the service of optical astronomy the best efforts of electronic technicians relating to the intensification of faint optical images. This image tube work has now reached a stage where a real gain in research use ("figure of merit") of 6 to 15 over the best photographic plates is just beginning to be realized by means of convenient (and permanent) sealed-off image tubes. "Cooperation" thus has many very different aspects in the current work of the Department, but in each case it represents a situation where there is special value in our freedom of initiative and a recognition of the infectious characteristic of personal enthusiasm. The image tube work is now primarily supported by the National Science Foundation, and we find that our relationships with the NSF form a conspicuously satisfying aspect of our entire Department's cooperative ventures, including the current emphasis on Latin American projects involving radio astronomy studies of the southern sky and geophysical studies of the Andes. NSF funds are now applied to defray the Latin American expenditures incurred in connection with our collaboration and also to provide some modest stipends for our South American colleagues, especially students. The various items are discussed under the individual sections.

Except for human problems, rooted in conflicting idea-structures and in man's traditional will to dominate over other men—problems of which the research man is painfully and rather helplessly aware—the world we observe around us is an intensely beautiful and interesting

place. It is true that powerful men in different nations, both as individuals and in groups, may express unalterable convictions for a time, and oppose each other even with violence concerning various relationships in the society of human beings. But these social differences have a way of melting into one another, and even reversing, in the course of roughly one lifetime, so that the visible patterns of human group relationships, to the eye of the physicist, seem largely transient, mostly lacking in beauty, and not visibly governed by perceptible regularities or natural laws. Contrast this immediate and unavoidable confusion of his daily environment with the beautiful regularity and systematic relatedness he observes in every aspect of the natural phenomena he studies, from distant stars to living bacteria, and you can sense his deep satisfaction in scientific studies. Every question he frames and every reaction he observes reveals in striking measure the immanence of natural law, a universally patterned relatedness of the kind that men have always recognized as transcendent. Yet these relationships are more and more seen and understood, bit by bit adding to man's stature and his awe of the stupendous and beautifully intricate universe in which he finds himself. This is the continuing miracle of human awareness, as it is observed and experienced in the natural sciences. Based on the demonstrations of repeatable experiments and on the definitions and formal logic of mathematics the satisfying activities of the research scientist, especially in the "exact" sciences, comprise and exhibit a kind of dedicated vocation long familiar in other fields of activity.

The stars are as remote as they ever were, yet we now are fully confident of our identification in them of the familiar atoms that comprise the earth and even our own bodies. The biologists, as geneticists, have exposed to view during the last few decades the beautifully regular and intricate mechanisms of inheritance. The physicist, now as a biophysicist, but still in his traditional role as a "natural philosopher" who studies dynamic and experimental relationships (as contrasted with "natural history" and classification), has found here also a deeply satisfying exhibit of the laws of nature. An additional element is undoubtedly and ineffably present when he moves toward studies of such factors as memory, idea, or self-will in a living organism. There are clear and obvious limits to the fields of inquiry amenable to mathematics and experiment, and each individual human awareness is a demonstration of these limits. The course of history and the usual current distress of political adjustments everywhere may be another demonstration of the same limits, but the great good fortune of being enabled to devote our energies and talents, modest as they may be, to further illuminating the intricate and orderly patterns of the physical world around us, including the material interactions and patterns in living cells and creatures, is the "princely gift" of our time and circumstance.

This search for understanding and for heightened awareness is an ancient use of leisure in a society, usually limited to a perceptive few, and we can all rejoice that it is honored so widely in our own time. The Carnegie Institution and even this Department have indeed witnessed a most remarkable half century of expansion in the history of the human search for knowledge and awareness. Recognition of a problem is at least a necessary step toward its active solution, and so perhaps we may hope that some of the patterns of sustained and critical effort which are the tradition in the natural sciences may ultimately prove of value in resolving some of the conflicts and irregularities in social relationships. Even if these human problems continue to defy solution, today's towering structure of scientific knowledge, as a human expression of the laws of nature, will long stand as a monument to the efforts and satisfactions of our own epoch.

EXPERIMENTAL GEOPHYSICS

RADIO ASTRONOMY

B. F. Burke and M. A. Tuve

Radio Hydrogen Observations

The galactic center. During the past year much of the observing time on the 60-foot dish and multichannel 21-cm receiver has been spent on a program to study the motions of the interstellar hydrogen line in the inner parts of the galaxy. Previous observations at Leiden and Sydney have given ample evidence that the motions close to the galactic center are complex, with clear indication that the hydrogen gas is not only rotating about the center of mass of the galaxy but is also expanding. Since our observing station at Derwood, Maryland, is at a more southern latitude than Dwingeloo (the Dutch station) we were able not only to confirm the observations of the Dutch but also to extend the observations nearly 20° farther south along the galactic plane. The Sydney observations were made with a small dish, using a receiver of relatively wide bandwidth, which also showed the existence of the expansion but with little detail on its structure.

Initially, the program has been to obtain hydrogen-line profiles along the galactic plane at intervals of 1° in longitude from $l^{I} = 301°$ to $l^{I} = 355°$, and to take cross sections every 2° in longitude running from 2° above the plane to 2° below the plane in 0.5° increments in latitude. As the new conversion tables for (l^{II}, b^{II}) were not available, all measurements were made in the (l^{I}, b^{I}) system, using latitude $b^{I} = -1.5°$ as the approximate galactic equator on which the grid of observation points was centered.

Within about 4° of the galactic center, the Dutch observed high-velocity wings on the hydrogen-emission profiles, extending nearly 300 km/sec both to the red and to the blue. These they have interpreted, in part, as a double structure: an inner disk of hydrogen, about 300 parsecs in radius, rotating rapidly (but not expanding); and an outer ring, extending from $R = 500$ to 590 pc, likewise in rapid rotation.

We have confirmed the existence of such high-velocity wings, which show quite clearly on scans taken near the center. Scans taken at latitudes a few degrees above and below the plane are used to give a "cold sky" reference, to check on the zero base line of the receiver. Our receiver can only cover a band about 200 km/sec wide, and hence it is necessary to take several scans, centered on different frequencies, to obtain a complete

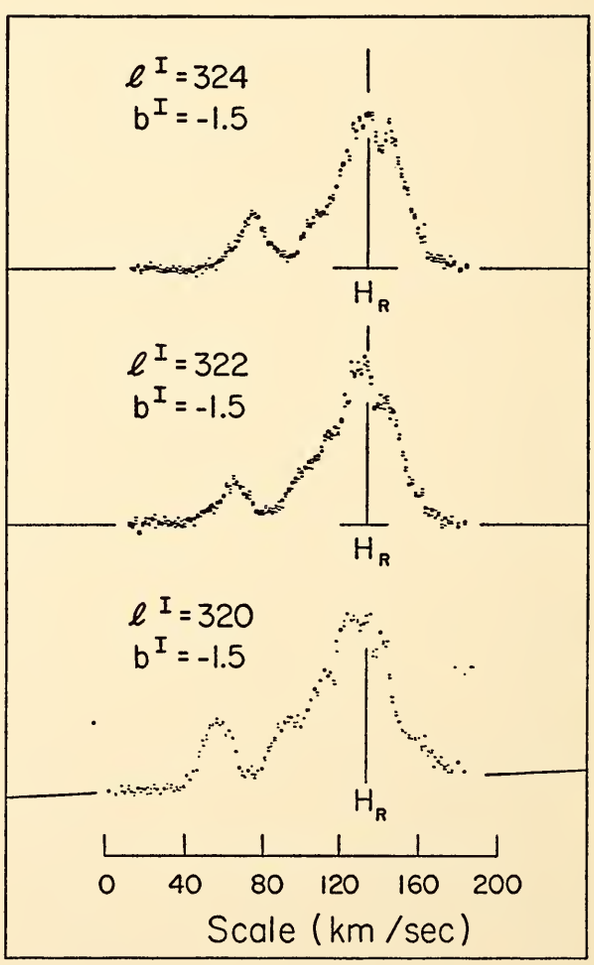

Fig. 1. Sample records taken along the plane of the Milky Way in the vicinity of the galactic center. Each curve is a superposition of four to six individual tracings from the 54-channel spectrograph.

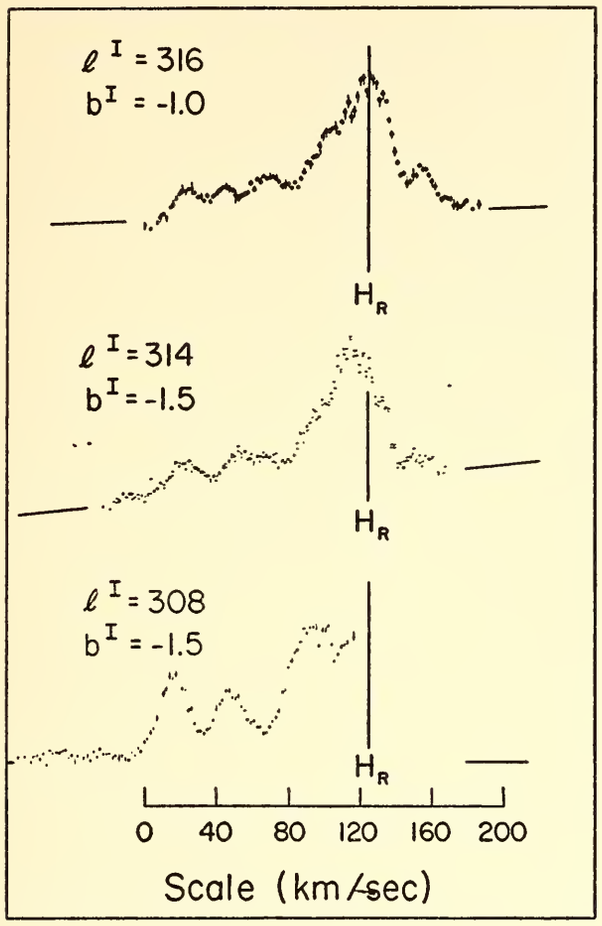

Figure 2. Sample records taken along the plane of the Milky Way in the vicinity of the galactic center. Each curve is a superposition of four to six individual tracings from the 54-channel spectrograph.

line profile. Detailed reduction and interpretation of our curves are not yet complete, but preliminary reductions show good agreement with Leiden.

Most of our analysis so far has been concentrated on features lying farther from the galactic center and in particular on the southern side. Typical sets of records are shown in figures 1 and 2, which are representative of the profiles obtained in the southern sector of the survey. The three profiles in figure 1, centered on the "blue" side of the main emission peak, show the striking feature known as the "3.5-kpc expanding arm" from which Rougoor and Oort demonstrated that noncircular motions exist in the inner parts of the galaxy.

Figure 2 shows representative profiles of more southern longitudes, invisible from Leiden. The profiles are not as simple, since not one but several peaks can be clearly seen. Figure 3 shows the apparent peak velocities for all features, and also the velocity, with respect to the local standard of rest, as a function of galactic longitude. If all the gas were in circular motion, the radial velocity should go to zero at $l^I = 327.8°$, and the actual value of this intercept is a direct measurement of expansion velocity. The "3.5-kpc expanding arm" is represented by the set of points labeled *A*. The variation in velocity with longitude is remarkably linear for this feature as far south as $l^I = 320°$. The apparent expansion velocity derived from our data is 52

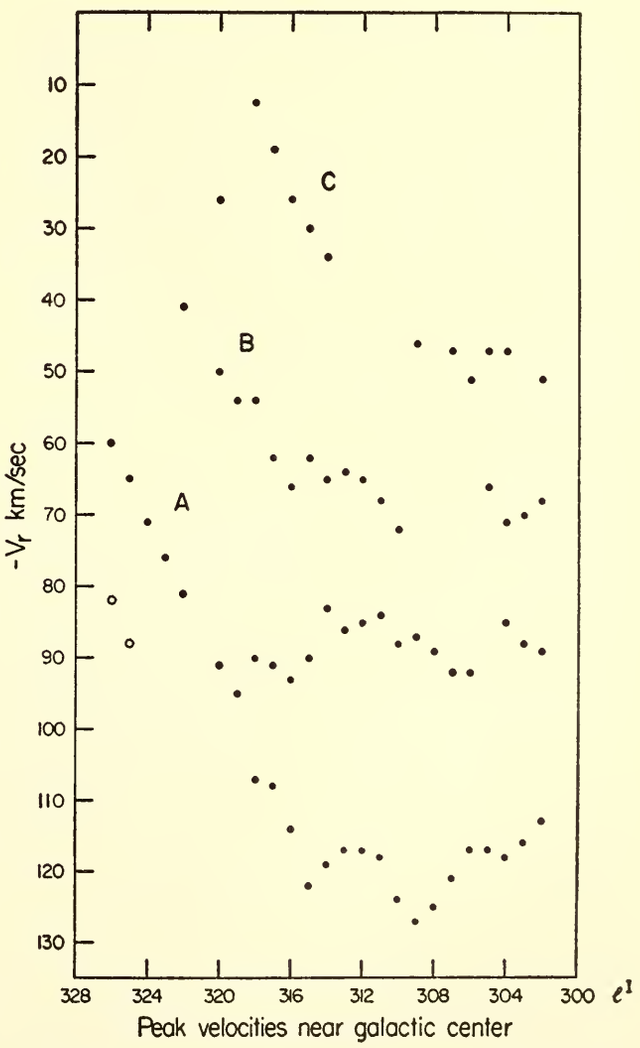

Fig. 3. Variation of peak velocities observed on the southern side of the galactic center in the plane.

km/sec, in very good agreement with the 53 km/sec found by Rougoor and Oort. From the slope of the curve (assuming uniform rotation and expansion) the difference in angular velocity, $\omega(R) - \omega(R_0)$, can be derived, where the distance of the sun from the galactic center is R_0. If we adopt $R_0 = 8.2$ kpc, which for convenience has been used in the following discussion as a scale factor for all galactic distances, $\omega(R) - \omega(R_0) = 35.6$ km/sec/kpc. Unless R is known, the corresponding circular velocity at R cannot be determined; if, following Leiden, we place this feature at 3.5 kpc, and assume $\theta_c(R_0) = 216$ km/sec, then $\theta_c(R) = 216$ km/sec also. If R is smaller than 3.5 kpc, $\theta_c(R)$ is also reduced. For any reasonable guess at R, $\theta_c(R)$ does not fall far from the Leiden circular velocity.

At approximately $l^{\mathrm{I}} = 319°$, the regularity of feature A disappears. The feature divides into two peaks, which can be clearly traced as far as $l^{\mathrm{I}} = 302°$ but with much more scatter to the points. The slope of the highest-velocity feature becomes slightly steeper, as far as $l^{\mathrm{I}} = 315°$, when it flattens out and maintains nearly constant radial velocity as far as the peak can be traced. The lower-velocity part, which may well be a different feature altogether, exhibits nearly constant radial velocity over its entire range.

The two lower-velocity features, labeled B and C, exhibit similar characteristics. The slope of C near the longitude of the galactic center is very nearly the same as for A, although the intercept at the longitude of the center is about +35 km/sec, implying that, if this is an expanding feature, it is on the far side of the galactic center. The feature B has a less negative slope, implying $\omega(R) - \omega(R_0) = 28$ km/sec/kpc. From the apparent velocity at the galactic center, an expansion velocity of 18 km/sec is obtained. Both these values are consistent with this feature's lying farther from the center than the "3.5-kpc arm."

A different presentation of the data is given in figure 4, which shows the intensity of hydrogen emission as a function of longitude and velocity for latitude $b^{\mathrm{I}} = -1.5°$, the approximate galactic plane. The most intense hydrogen emission, at low velocities, is not shown, since it refers primarily to local structures. The dotted lines give the run of the peaks as shown in figure 3. The ridge of the "3.5-kpc expanding arm" can be clearly seen, and also what at first

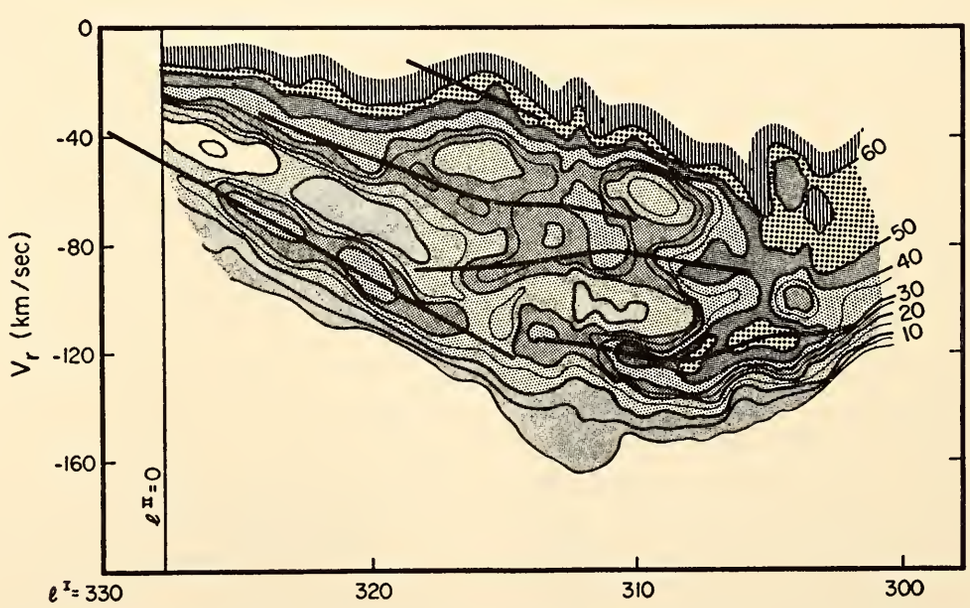

Fig. 4. Brightness of hydrogen emission as a function of velocity and longitude, along galactic plane ($b^{\mathrm{I}} = -1.5°$). Solid lines show the peak velocities from figure 3.

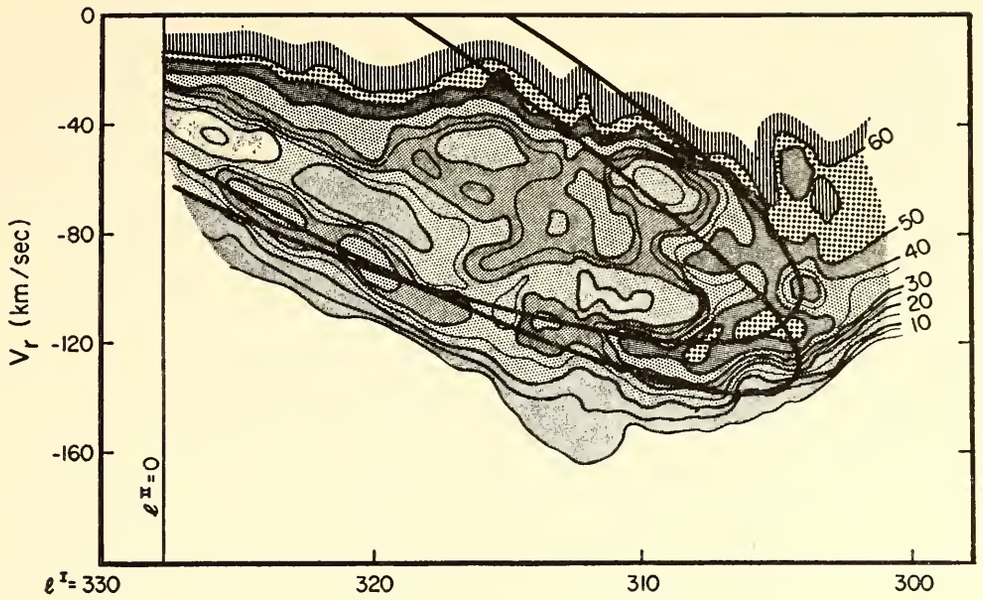

Fig. 5. Same relief map as in figure 4 but with solid lines showing various possible models of circular, uniformly rotating, and expanding arms.

sight looks like its extension to longitude 304°, where the contours suggest that we are looking tangentially to the arm. If we are looking tangentially at this longitude, the feature is 3.8 kpc from the center. Closer examination shows that the two parts do not join very smoothly across the gap around $l^{I} = 316°$. Indeed, there is a faint extension between $l^{I} = 310°$ and 315°, which may well be the remnants of the "3.5-kpc expanding arm." This small discrepancy, though not conclusive, led us to other tests, represented in figure 5. On the same contour map are superimposed the expected velocities for uniformly rotating, expanding, circular arms. One curve has been chosen to fit the straight part of feature *A*, and the other to approximate the "turn around" at $l^{I} = 304°$. It can easily be seen that either fitting fails for the other half.

The "3.5-kpc expanding arm," therefore, is not a uniform circular feature, and in fact may not be at 3.5 kpc. The feature certainly extends as far as $l^{I} = 316°$ and possibly to $l^{I} = 310°$. These longitudes correspond to lower limits of 1.7 kpc and 2.6 kpc, respectively, for the center-to-arm distance. It appears that the problem of the space distribution of the expanding gas in the central region of the galaxy is still far from solved. Work is proceeding on the reduction of the remainder of the observations, in the expectation that the angular extent in latitude will provide further clues to the nature of these gas complexes.

Extragalactic observations. Among the members of our local galactic group the Andromeda nebula, M 31, and the prominent galaxy in Triangulum, M 33, are particularly interesting since they can be partly resolved by instruments like our 60-foot dish. Both these galaxies have been studied by the slightly larger Leiden antenna, but it was thought that a repeat survey would be of some value. A more compelling incentive was to prepare for our projected observing program at Green Bank (West Virginia) on the 300-foot telescope now in construction there. During the spring and early summer an intensive program has revealed the necessity of understanding the factors involving base-line stability, since the antenna temperatures observed on these two objects are never greater than 4°K. To improve the statistical fluctuation level, the receiving bandwidth was broadened by averaging the outputs of

four adjacent channels, and the integrating time was lengthened to 5 minutes. Ordinarily, a series of three observations was made at each point on the galaxy and observations at standard reference points in the sky were interleaved to provide a check on the stability of the instrument base line. Each averaging has an rms of about 0.2°K, and with such sensitivity small perturbations in the base line were frequently observed. It proved to be extremely tedious to track these down, since a complete set of three averages takes about 15 minutes, and initially it is not known whether the fluctuations are caused by external interference (L-band radars, for example), self-generated interference ("birdies" in oscillators and amplifiers), mechanical difficulties such as loose connections, or temperature effects in the front end of the receiver. We have experienced trouble from all these causes, but temporary summer temperature problems give the most difficulty, and we feel confident that observations of the necessary accuracy can now be performed by our receiver. There are still slow drifts during the day, but by careful checking at reference spots they can be allowed for.

A survey on M 31 has been completed; when allowance is made for our poorer angular resolution and our better resolution in frequency (Doppler velocity), we are in substantial agreement with the Leiden survey reported in B.A.N. 482. A similar survey on M 33 is still in progress.

Hydrogen-line equipment and operations. The 60-foot radio telescope at Derwood was operated throughout the year, only minor repairs being required. Observations were made exclusively at 21 cm with the 54-channel spectrograph. Tests on the pointing accuracy have been repeated, by both radio and optical methods. The linear scale correction to the declination indicators was determined by photographing stars at various declinations with the 6 inch Dallmeier camera mounted on the dish and was checked by radio observations of the brighter radio sources. By applying the new declination correction the dish can be pointed with an accuracy of better than 0.02°.

The 54-channel spectrograph performed well throughout the year. The channel stability has been greatly improved by converting all phase-sensitive detectors from hot-tube diodes to matched silicon diodes. Daily adjustments of all diode balances are no longer required for ordinary galactic observations.

A new amplifier, using WE 437A's, was installed after the crystal mixer, with resultant improvement in noise figure. Direct measurements of the excess noise were made by measuring the increase in noise when a resistor was substituted for the antenna feed. In the six months since the preamplifier was installed, the double-sideband excess noise has increased slightly, from 440°K at the outset to 550°K in late spring, with no changes in mixer crystal or 437A tubes.

A great deal of effort has been expended in understanding causes of base-line variation from hour to hour. When one looks at the regions of sky known to have very little hydrogen radiation, the base line is usually displaced from the meter zero and has a slope. Changes in antenna tuning and in the matching transformer between antenna and mixer affect the zero displacement, but finding of the cause of the slope (and of an adjustment to remove it) proved to be an elusive problem. It appears that the only adjustment that affects the slope significantly is the cascade input tuning. Apparently the noise generated in the cascade input circuit at 27 Mc/s is converted up to 1420 Mc/s, is reflected by the antenna, is converted down again, and returns to the cascade, but in frequency-dependent quantities. Removing the zero slope by adjusting the cascade input tuning affects the zero displacement as well, but this can be corrected by other adjustments.

In addition to the existing 54 channels, a single extra-narrow filter, only 2 kc/s in bandwidth, was added. Narrow absorption features suspected of being too sharp

to be resolved with our present bandwidth have been investigated by means of this filter.

Narrow-band observations. During their visit with us, Drs. Vieira and Schwachheim installed the above-mentioned narrow-band (2-kc/s) filter as an extra channel on the 54-channel receiver and with it observed the two narrowest absorption features in the direction of Cygnus X and the galactic center. In both cases the absorption profile seemed slightly deeper, but it appears that the difference between these narrow-band observations and those made with our normal bandwidth (about 10 kc/s to half power) is in general negligible.

South American Cooperation

The Carnegie radio astronomy station, to be located between Buenos Aires and La Plata in Argentina, is well under way. The major instrument, a 30-meter-diameter equatorially mounted parabolic antenna, will be built during the coming year. Several large parts, including the declination and polar axis assemblies and drive assemblies, are now being fabricated in Baltimore and will be shipped this fall. A prototype aluminum rib has been built to test fabrication procedures, and sufficient aluminum stock and other materials for the entire dish are now in storage at Derwood, also to be shipped this fall. A preliminary site in Argentina has been agreed upon, and construction can start when final on-the-spot evaluation has been made. The Instituto Nacional de Radio Astronomía has been created by the Consejo Nacional de Investigaciones Científicas y Técnicas and by the Research Council of the State of Buenos Aires to provide an Argentine organization to participate in this joint venture, which will involve cooperation between the Carnegie Institution of Washington, the University of Buenos Aires, and the University of La Plata, with invitations to colleagues in other institutions to participate.

To extend the base of our cooperative venture we had two visitors from Brazil join in our work here at Derwood: Dr. A. H. G. Vieira, Escola Politécnica, São Paulo, and Dr. G. Schwachheim, Centro Brasileiro de Pesquisas Físicas, Rio de Janeiro. During their four-month visit they participated in hydrogen-line observations and in planning for a new interferometer experiment. Dr. R. A. R. Palmeira, also of the CBPF, who was here five months last year, is a partner in the Brazil activities as well.

Continuum Observations

Precise position array. In *Year Book 60* a list of derived right ascensions of radio sources was given. The small area of the array has been a handicap, and only the brighter sources could be measured with the desirable precision. Hercules A, which is among the ten brightest radio sources, has had a somewhat questionable optical identification. Originally it was suspected that the radio source was associated with a peculiar galaxy, of about the same apparent magnitude as Cygnus A, and, like Cygnus A, showing strong emission lines. Roberts, Bolton, and Harris at Cal Tech derived an improved radio position that appeared to rule out that possibility, and they suggested instead that an even fainter galaxy of about 19^m was associated with the radio source. A position derived at the Cavendish Laboratory by Elsmore and others specified still another spot in the sky, and Dewhirst noted that there was also a peculiar galaxy, with optical brightness of about 18^m, at this position. These last two possible identifications are separated in the sky by little more than a minute of arc, and only by the most careful measurements could it be hoped to distinguish between them.

Figure 6 is reproduced from Dewhirst's discussion, galaxies *a*, *b*, and *c* being the three successive identifications. Although our "PPA" (the 400-Mc/s arrays described in previous reports) measures only right ascension at present, it was considered worth while to make a new series of right-ascension measurements on Her-

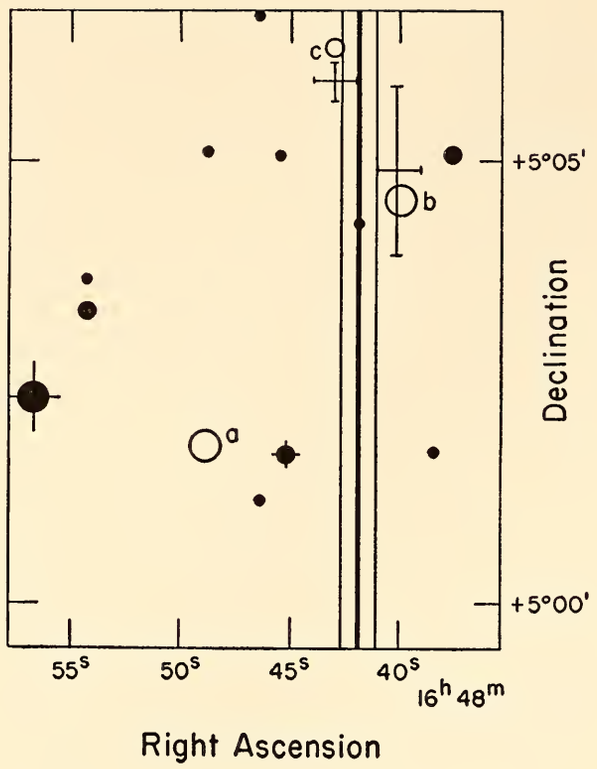

Fig. 6. Field in vicinity of Hercules A. Solid circles are stars. Open circles show possible galaxies associated with radio source. Cross near galaxy *c* is Cal Tech position. Cross near galaxy *b* is Dewhirst position. DTM right ascension shown as vertical line with ±1 second error.

cules A to try to discriminate between the two possibilities. These measurements were made during the winter and early spring of 1962 with the collaboration of Dr. J. W. Hollinger of George Washington University.

To determine the collimation error of the antenna, 17 transit measurements of the Cygnus A source were made during the period January 6–30, 1962. Comparison of the measured radio right ascension with the right ascension of the optical source gave a collimation error of +1.′8, which was applied to the Hercules A observations. Earlier measurements indicated that level and azimuth errors of less than 5 seconds of arc were to be expected, and therefore the data were not corrected for these sources of error.

A total of 30 transit measurements of the Hercules A source were made during the period February 3 to May 7, 1962. The transit times as observed are shown in figure 7. These results lead to a right ascension (1950.0) of $16^h48^m42^s$ for the Hercules A source. This value can be compared with the right ascension determined from a much smaller number of transits in last year's report $\alpha(1950) = 16^h48^m43^s$. The new right ascension is indicated in figure 6, with the estimated error of $\pm 1^s$ shown. The error shown was derived solely from the statistics of the observations and does not include systematic effects such as changes in collimation correction after January 30. The source *c* is closer to the observed right ascension, but source *b* is sufficiently close to be a possibility. Source *a*, however, appears to be clearly ruled out by our observations.

Angular size interferometer. The need for precise positions of radio sources has continued to occupy an important place in our planning, but it is becoming evident that measurements will also be needed of source angular sizes. To get both sufficient angular resolution and enough antenna collecting area, it is necessary to build rather large antenna structures. During the past year we have studied the possibility of using cylindrical paraboloids and large paraboloidal dishes as an interferometer designed to resolve sources only a few seconds of arc in size. Surprisingly enough, a dish of 30-meter diameter, closely following our La Plata design but only mounted as a transit instrument, compares favorably in expense with a cylindrical paraboloid of comparable area and has the very important simplicity of a single antenna

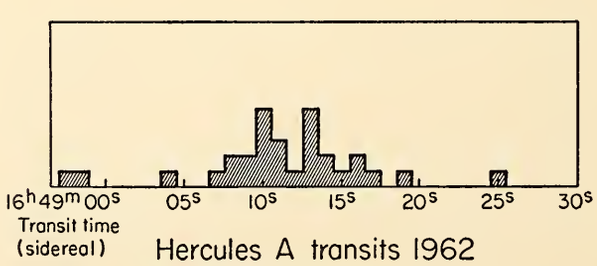

Fig. 7. Observed transit times of radio source Hercules A.

"feed." It was finally decided, in view of the several practical advantages of dishes over cylindrical paraboloids, to start construction of a large base-line interferometer having two or three such dishes as elements. The first dish is now being constructed at Derwood; it will be used with our present 60-foot dish as a two-element interferometer to evaluate the potentialities of the system.

THE EARTH'S CRUST

Seismic Studies

J. S. Steinhart, R. Green,[1] *T. Asada,*[2] *A. Rodríguez B.,*[3] *L. T. Aldrich, and M. A. Tuve*

For more than 50 years the structure of the crust and upper mantle has been represented, chiefly for mathematical convenience, as one or more horizontal layers of constant wave velocity bounded by discontinuities in velocity. During the last 10 years or more this simple picture has appeared inadequate, for several reasons: (1) Efforts to find the near-vertical reflections from the supposed discontinuities have been unsuccessful. (2) Laboratory measurements of seismic velocities in various rock types at high pressures and temperatures, coupled with observed geological complexities, make it extremely unlikely that the upper 20 km of the crust is a constant-velocity layer. (3) Seismograms obtained in the field are more complex than would be expected for a simply layered crust of one or two layers. (4) The concept of horizontal layers may be approximately true, but geographical variation in physical properties has been clearly demonstrated on a large scale and fairly small lateral changes over the horizontal dimensions of an experimental region may introduce large errors into the calculated result. These objections and others detailed in reports of previous years have led us to more and more critical evaluation of the results obtained in explosion seismology and of the reality of the physical structures deduced from the observations.

The questions may then fairly be asked: What real physical entities or structures in the earth can we hope to determine from explosion seismology? What criteria are sufficient to establish these results as definite rather than merely plausible? In the subsequent paragraphs, some modest successes from the Maine experiments will be presented, together with some demonstrations of the very great difficulties and uncertainties encountered with some procedures that have been used by us and by others in the past.

The Maine Seismic Experiment

In July 1961 an intensive study of the earth's crust in Maine was made from 61 explosions detonated in the Gulf of Maine. This was a truly cooperative project, in which the Department of Terrestrial Magnetism was joined by colleagues from the University of Wisconsin, Princeton, Penn State, University of Michigan, and Woods Hole Oceanographic Institution. Some other groups, largely under government research contracts, were able to utilize the explosions for their own special objectives. This kind of cooperative experiment enabled us to obtain far more detailed information from the explosions than ever before (more than 900 seismograms were obtained from 18 stations). Such a large amount of detailed information was obtained that analysis of the results is far from complete, although certain general results are now clear.

Travel times and crustal structure. The usual representation of the results of a seismic profile studying the earth's crust is in terms of a plot of the times of arrival of wave groups as a function of range. Figure 8 shows such a plot, for one station's records, of all the shots on the Gulf of Maine profile. In evaluating the crustal velocity structure from such a

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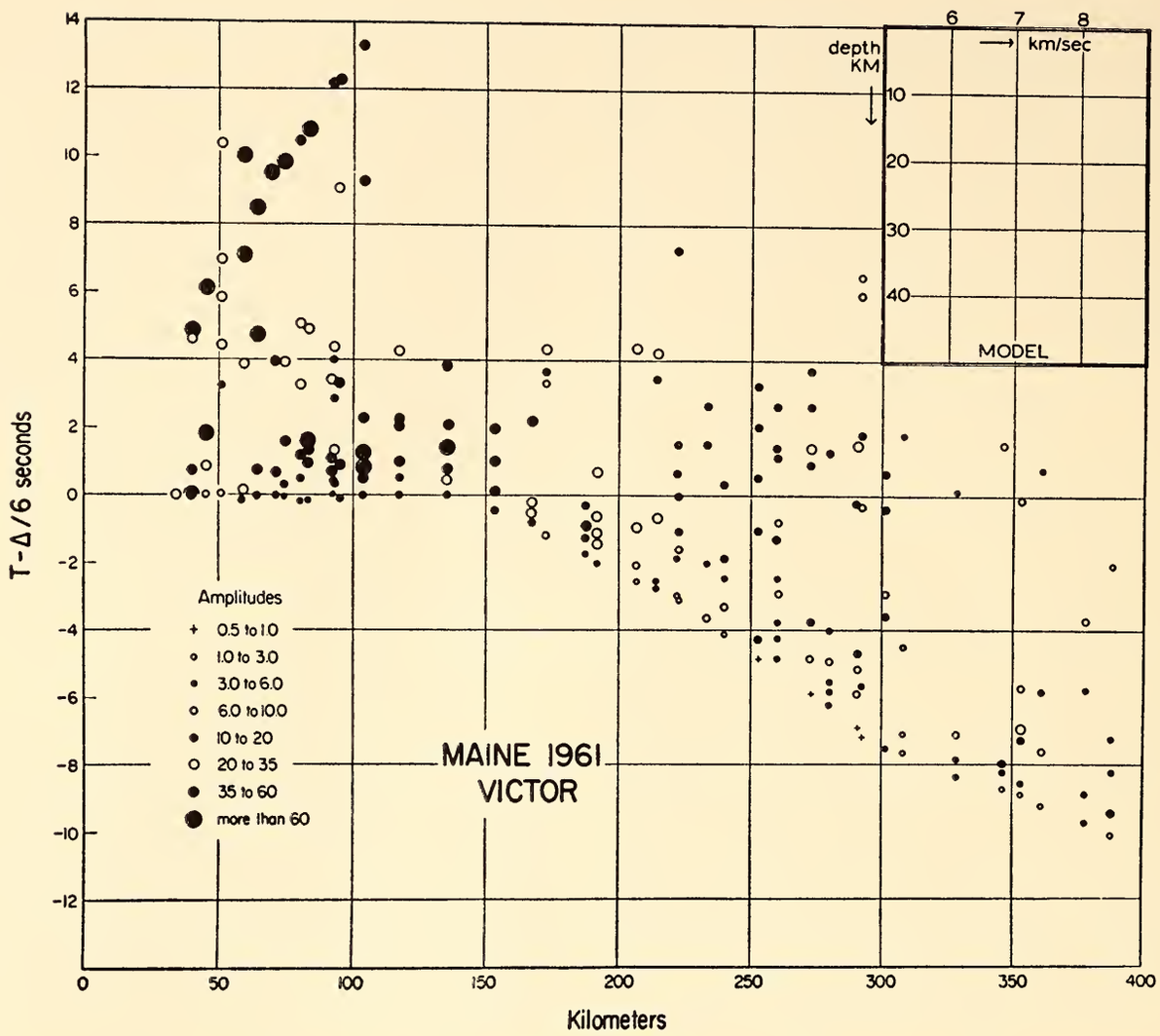

Fig. 8. Plot of the times of arrival of wave groups, for records of one station, of all the shots on the Gulf of Maine profile.

plot it is necessary to find a velocity structure whose travel times agree with the observations. To obtain the traditional solution in terms of flat, homogeneous layers, it is only necessary to fit straight-line segments to the travel times and compute the depths. For Maine, a two-layered crust with velocities of 6.05 and 6.8 km/sec would fit quite well, giving an overall crustal thickness of about 34 km. But we are led to inquire whether this is the only solution appropriate to the observations, especially in view of the objections raised above to homogeneous layers. Tuve, Tatel, and Hart showed, for Maryland–Virginia, a whole range of possibilities that would satisfy the observations.

Figure 9 presents a model in which the homogeneous layers have been replaced by layers having internal velocity gradients, and the discontinuities in velocity have been replaced by rapid gradients. There is no a priori reason for assuming this particular model to be more or less physically real than the homogeneous layer model, except that the increase in velocity in the upper part of the crust approaches more nearly to some measurements given below. The important change is that there is no longer any velocity discontinuity whatever, although there are discontinuous changes in gradient. It will be noted that this model provides a good fit to the travel-time data and that the curvature in the travel times implied

by the velocity gradients is quite small in the region of first arrivals. This matter of measurement of velocity gradients from curvature in the travel times is discussed in more detail below.

Figure 10 shows a further change in the model in which anything that could be called an intermediate layer has disappeared. Again it is noteworthy that the travel times fit the observations well and that the curvature of the travel-time curve in the first-arrival region is small.

Finally, in figure 11 a model is shown with a continuous velocity depth function that has nothing "layerlike" about it. This model was obtained from an objective statistical program for the IBM 7090 computer, in which the first-arrival observations are fed in and the machine fits the best model to them. There is a restriction on the models produced by this program to single-valued travel-time curves (that is, no reversed cusps) which makes it far from ideal for producing a "best-fit" model in any general sense. The valuable lesson to be learned here is that the first-arrival observations may be satisfied by a model like that in figure 11 which is very different from our customary ideas of crustal structure. This is somewhat disappointing, because the most definite travel times that can be measured are those of the first arrival of energy. The relative reliability of measurement of first-arrival time has caused much weight to be placed on this infor-

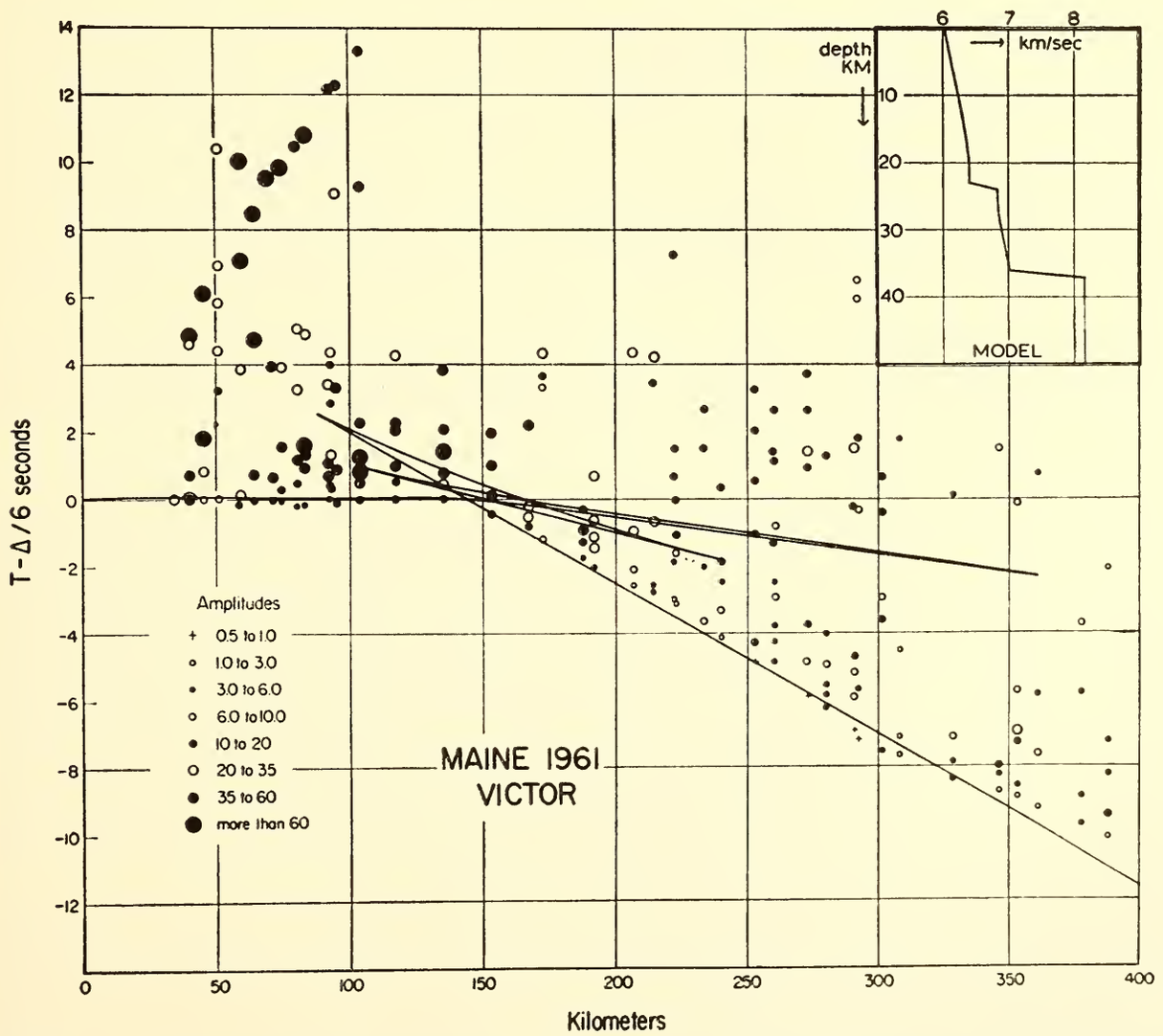

Fig. 9. A model in which the homogeneous layers have been replaced by layers having internal velocity gradients, and the discontinuities in velocity have been replaced by rapid gradients.

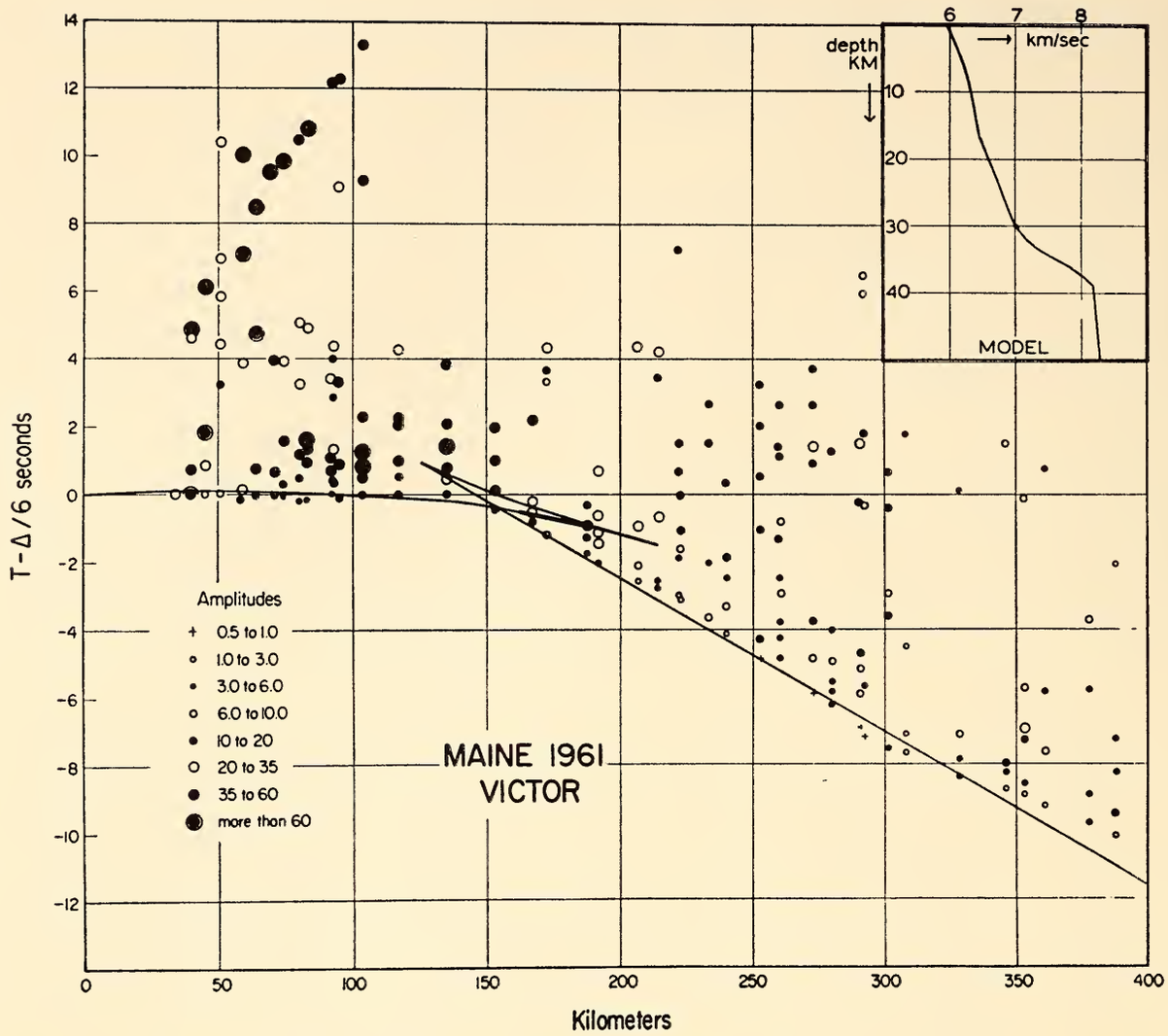

Fig. 10. A further change in the model in which anything that could be called an intermediate layer has disappeared. Note that the travel times fit the observations well, and that the curvature of the travel-time curve in the first-arrival region is small.

mation in the past. Figures 8 through 11 show clearly that first arrivals do not furnish enough information to define a velocity depth function.

The employment of arrival times of later phases is necessary for a more detailed conclusion, but there is a danger here. Choice of the specific later arrivals to be used is unavoidably conditioned by their local amplitudes, because the only phases that can be noticed are those whose amplitudes at this point are substantially above the general confused seismic signal that persists for some seconds after the first arrival. Thus, while a complete travel-time curve would give an unambiguous velocity depth profile, some parts of it cannot be identified because of small amplitudes of the arrivals or because of interference between coincident arrivals. Given these restrictions, we may conclude that travel-time curves, as they are usually thought of, are not enough. We must look at apparent velocities (which are really the first derivative of the travel-time curve, but can be measured separately) and amplitudes.

One positive result does become clear from the above. The layered model in Maine gives a crustal thickness of about 34 km. The two models in figures 9 and 10 give a crustal thickness of 36 and 38 km. For the continuous model (fig. 11), the *M*

is more difficult to define, but taking the definition from *Carnegie Publication 622*, the crustal thickness is about 39 or 40 km. We have then placed bounds on the crustal thickness for all reasonable models. The depth to the Mohorovičić discontinuity in Maine is 36 ± 3 km for any reasonable velocity structure. If we consider the uncertainty in this calculated depth implied by the scatter of points, and assume that the layered model is exactly correct, we find, from the statistical procedures outlined in *Carnegie Publication 622*, that the scatter of points about the fitted model means at least ±1 km uncertainty in the depth. If all we wish to know is the crustal thickness, we know it to ±3 km, and, with the present data, we cannot expect to know it better than ±1 km, even if we know the form of the velocity depth function. But to understand the mechanics of the earth we wish to know the velocity depth structure, and by specialized methods of observation, such as were used in Maine, we may be able to decrease the scatter of the travel-time points through averaging.

The use of apparent velocities. Over the years the results of many seismic refraction profiles have been used to find the thickness of the crust in terms of "virtual" depths (Tatel and Tuve) rather than obtaining the velocity depth function for the crust and hence its true thickness. The virtual depths indicate distinct structural difference between one

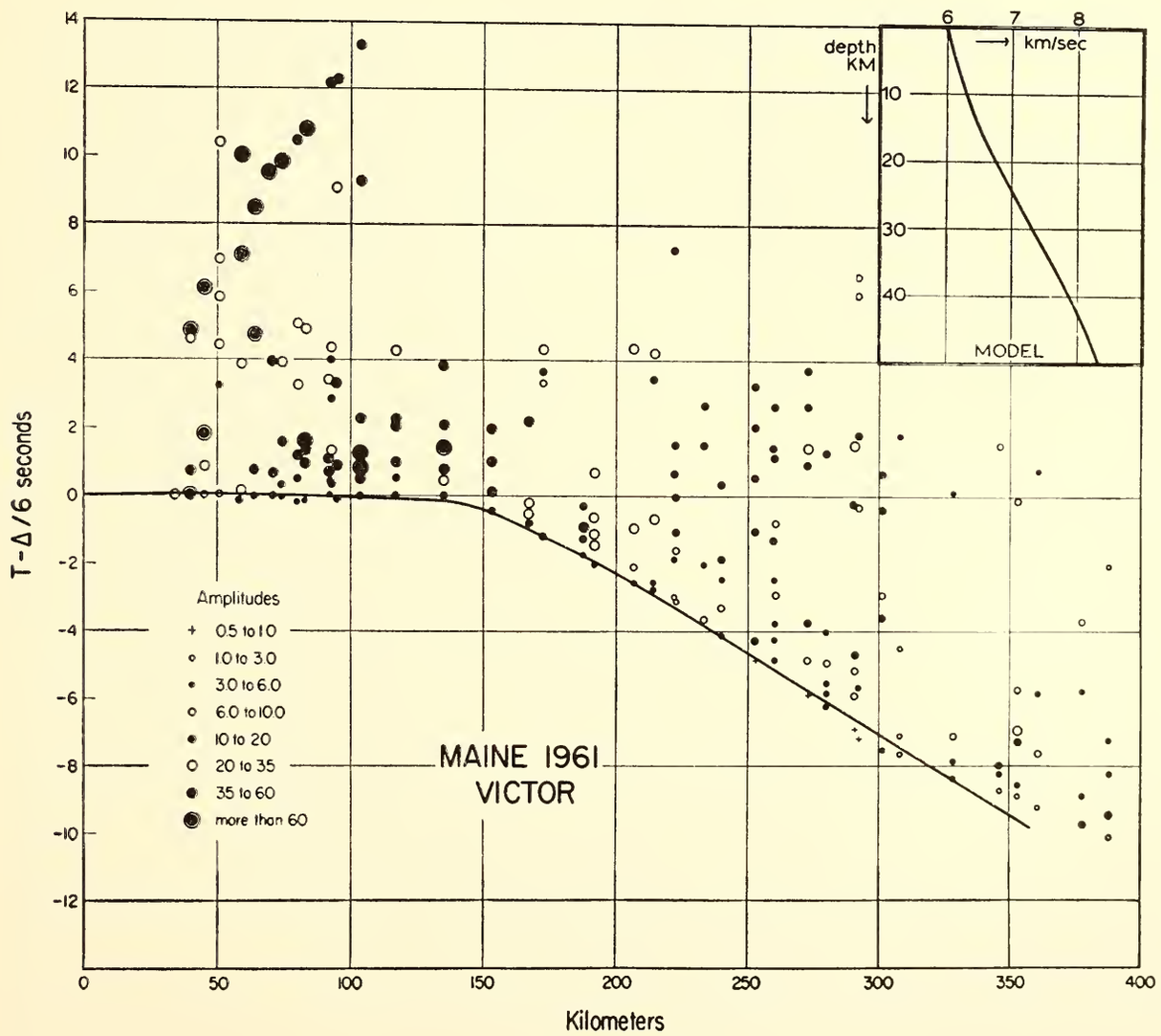

Fig. 11. A model with a continuous velocity depth function that has nothing "layerlike" about it. This model was obtained from an objective statistical program for the IBM 7090 computer.

area and another, and this in itself is valuable geophysical information. On the other hand, most questions about the detailed structure of the crust are difficult to answer with any certainty. The important question considered above is whether the crust is horizontally layered or whether, as one would expect, a better accord with reality can be afforded by considering the rocks at increasing depths in the crust to be so affected by increasing temperature and pressure with depth that a continuous, but nonuniform, velocity gradient exists from the surface downward.

To demonstrate the presence of velocity gradients in the crust, however, it is necessary to show that the time-distance curve has not only slope but also curvature, and furthermore it is necessary to show that this curvature (of the order of $1/500\ \text{km}^{-1}$) is significant. In other words, for a linear velocity gradient it is necessary to demonstrate the significant difference between the curves given by

$$t = \Delta/V \tag{1}$$

and

$$t = 2/a \operatorname{arcsinh} a\Delta/2V_0 = 2/a\{a\Delta/2V_0 - (a\Delta/2V_0)^3/6 + \cdots\} \tag{2}$$

where

t = the travel time.
Δ = the shot-point distance.
V = the average velocity for the constant-velocity layer model.
V_0 = the surface velocity for the model.
a = the velocity gradient.

For small values (0.01–0.04 km/sec/km) of gradient, which are possible in the crust, the difference in arrival times given by (1) and (2) is usually less than 0.1 sec. Unfortunately, the recognition of the first arrival on a seismic record is not unequivocal. Tatel and Tuve demonstrated with model experiments and Gamburtsev et al. showed that the time of the "preferred pick" for the apparent first arrival is dependent on the gain setting of the amplifier of the recording system. The laboratory and field work showed that it is only when the signal-to-noise ratio is high that a consistent pick of a P event can be made.

This emphasis on curvature in the travel-time curve led us to an examination, in terms of information theory, of the accuracy with which arrivals can be determined from a seismic record. Consider a seismic record that is the result of the transmission and reception of information through a ground geophone system, and that has a frequency bandwidth of W c/s and a signal-to-noise ratio of P/N. The maximum rate of transmission of information through such a system in terms of bits of information per second is given (by Shannon and Weaver) as

$$C = \int_0^W \log\left(1 + \frac{P}{N}\right) dW \tag{3}$$

An "arrival" cannot be identified with the uncertainty in arrival time less than the time taken to transmit one bit of information through the channel. To evaluate (3), a knowledge of the spectral distribution of noise over the frequency band of the recorder is required.

If, to obtain an indication of the magnitude of the expected uncertainty, the assumption is made that the noise is random over this band of bandwidth 40 c/s, and for a poor site the signal-to-noise power ratio is 2, the channel capacity is 44 bits/sec. Thus the minimum uncertainty in the pick of an identifiable arrival is 22.8 milliseconds. For the case of an "ideal site" where the signal-to-noise ratio S/N is 100, the channel capacity[4] is less than 185 and the uncertainty in the arrival time of an event is reduced to 6 milliseconds. In practice the optimal value will never be attained, because the sufficient and necessary information required to identify the

[4] For large S/N ratios the channel capacity is $C = W \log (2\pi eS/N + 1)(1 + \epsilon)$, where ϵ is arbitrarily small. Thus the asymptotic approximation in equation 3 will yield too large a figure for channel capacity.

arrival of an event has never been stated in precise language.

With the above discussion of the uncertainty in identifying an arrival as a guide, the expected accuracy of the determination of the velocity V of a wave front over the interval between two points a distance l apart will be evaluated.

$$V = l/t$$
$$\Delta V = (V^2/l)\Delta t + (V/l)\Delta l \qquad (4)$$

Inserting numerical values of 0.02 sec for Δt, 2 km for l, 5 per cent for $\Delta l/l$, and 6 km/sec for V leads to errors of 1 km/sec in velocity. It might appear that improved accuracy can be obtained by increasing l, but this may introduce other problems due to local geological variations. For example, whenever the second derivative of the velocity depth function becomes positive, a triplication occurs in the travel-time curve, leading to the loss of coherence of the wave form over distances greater than a few kilometers. In such circumstances an observer has no assurance that he is basing his velocity determination on the successive arrivals of the same phase (Green and Steinhart). However, using radial seismometer arrays 2 km long, the same event can usually be traced with confidence across the whole array, and the apparent velocities can be determined with the accuracy stated above.

The measurement of apparent velocity has been carried out for nine stations having shot-point ranges of 40 to 140 km for Maine (fig. 12). This range was selected because the time-distance curve is apparently fitted by a straight-line showing of a velocity of 6.05 km/sec over this range and also the first arrivals here are rays that have not been refracted from below the Moho as head waves.

For every station a plot of apparent velocity as a function of shot-point distance has been made and the slope of the straight line of best fit (least squares), together with the estimated standard deviation, $S\lambda$, has been determined. The statistics show that for every station the best estimate of slope is positive (i.e., apparent velocity increasing with distance), and zero slope is statistically

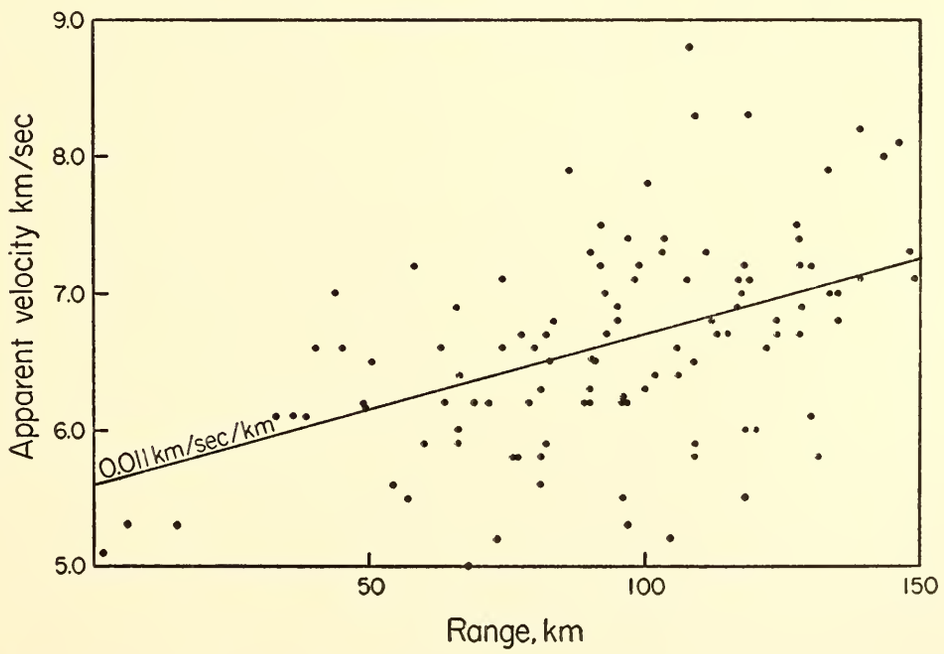

Fig. 12. Measurement of apparent velocity carried out for nine stations having shot-point ranges of 40 to 140 km for Maine. This range was selected because the time-distance curve is apparently fitted by a straight-line showing of a velocity of 6.05 km/sec over this range and also the first arrivals here are rays that have not been refracted from below the Moho as head waves.

unacceptable to four stations. However, a slope of 0.01 km/sec/km range is acceptable to all stations with a confidence of 95 per cent. This would suggest velocity gradients in the crust. Before discussing a smooth velocity gradient in the crust, it is appropriate to examine the suitability of a number of discrete layers there. This is simple, because discrete layers in the crust would be expected to appear as distinct steps in the data for apparent velocity as a function of distance. Figure 12 shows that, irrespective of what may be the true velocity depth function, discrete layers of constant velocity are distinctly not indicated.

If, as a first-order approximation, the apparent velocity is considered to increase linearly with distance at less than $\lambda = 0.01$ km/sec/km range, the corresponding increase in velocity with depth is not linear but is given implicitly by

$$Z_p = (V_p/\lambda\pi)[(V_0/V_p)(\operatorname{arccosh} V_p/V_0) - (\arccos V_0/V_p)] \quad (5)$$

where Z_p is the depth, V_p is the corresponding velocity, and V_0 is the surface velocity. Equation 5 shows that initially the velocity V_p increases very rapidly with depth and that with increasing depth the rate of increase becomes gradual.

Before discussing a velocity gradient in the crust in terms of petrological composition and likely temperatures, it should be pointed out that the superimposition of minor fluctuations upon a general velocity gradient can be both important and observationally confusing.

Every positive increase in the velocity gradient will lead to the recording of (1) large amplitudes, (2) triplication in the time-distance curve (by a cusp), and (3) a rapid increase in the apparent velocities, whereas every decrease in the velocity gradient leads to the recording of (1) vanishingly small amplitudes, (2) a single arrival, or, in an extreme case, a discontinuity in the time-distance curve, and (3) an effectively constant value for the apparent velocity. Because the recorded time of arrival of a phase and its amplitude are inexorably associated, measurements tend statistically to be confined to signals enhanced by the various slight increases in the velocity gradient, those from zones having a decrease in velocity gradient being ignored, and the records will display chiefly the features associated with the larger amplitudes. Hence the velocity gradients as measured by apparent velocities over short spreads (over which coherence is maintained) will tend to be too high.

In figure 13 the temperature is plotted as the abscissa and pressure in kilobars as the ordinate, and on this field are drawn curves of equal velocity for a rock of granitic composition. Superposed on this temperature-pressure field is shown a heavy curve indicating an extreme velocity depth gradient of 0.05 km/sec/km depth. It can be seen that at depths greater than 3 km this gradient cannot be maintained for a rock of granitic composition without a temperature inversion. But it is necessary that the temperature increase with depth, because the heat flow is outward. Consequently, to maintain the gradient and to avoid the inversion, we must admit a change in composition toward more basic rock types. This is in contrast to the situation where the gradient is small (<0.01 sec^{-1}), and can be maintained down to depths of over 20 km. In this case, however, if the temperature at about 20 km appears to exceed 300° the gradient will further decrease, and ultimately a velocity reversal may result. In other words, it is difficult to conceive of a granite layer thicker than 20 km that does not have a velocity reversal, especially when the high heat production rates in granites are considered. The situation is markedly different if compositional changes in the crust are assumed. Birch has suggested that velocity depth gradients can be altered by increasing the amount of gabbroic material present. In figure 14, a set of curves for a gabbro (Hughes and Maurette) is given. The falloff in velocity with temperature is even more remarkable at temperatures above 300°C than

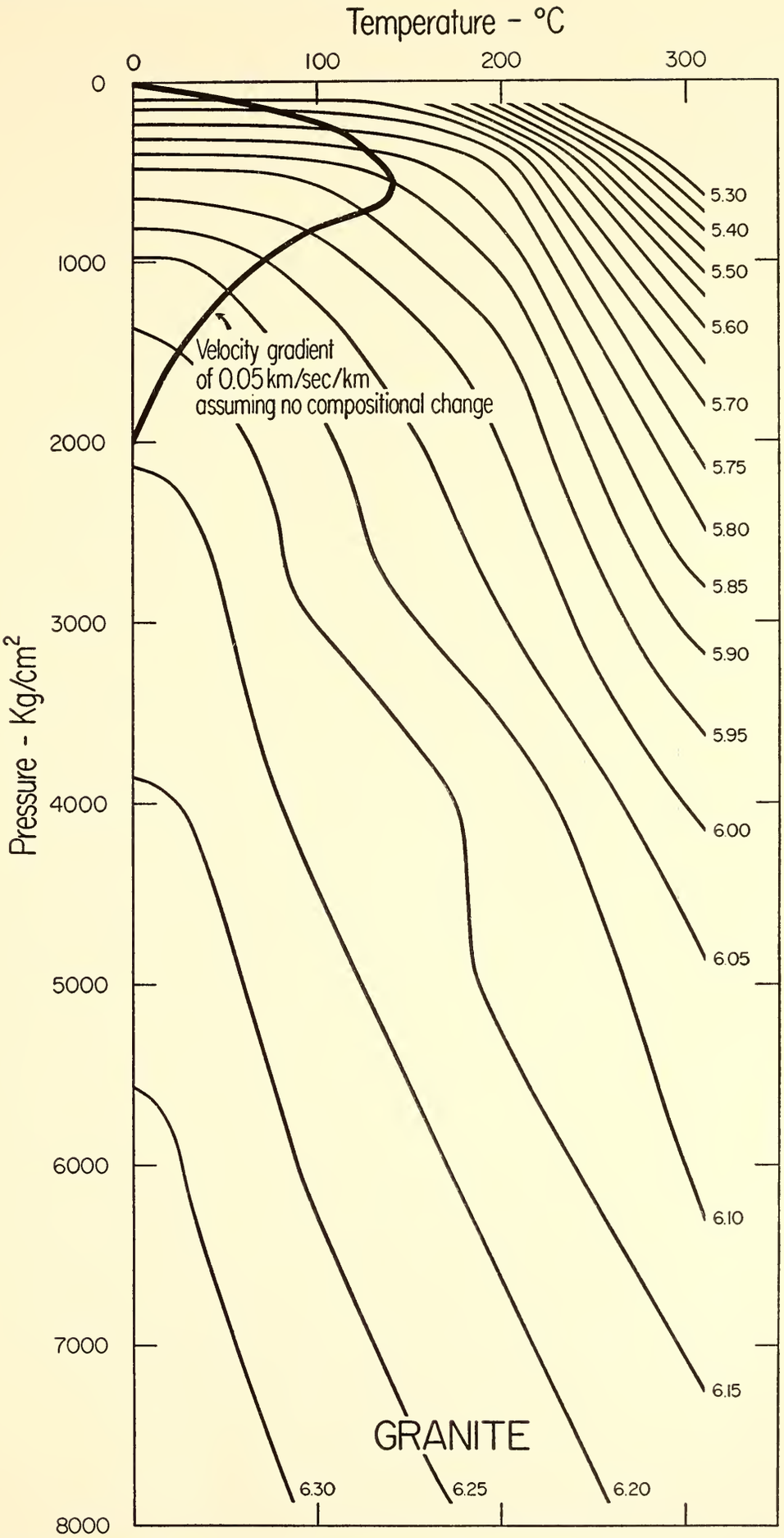

Fig. 13. Temperature-pressure field showing curves of equal velocity for a rock of granitic composition. Heavy curve indicates an extreme velocity depth gradient of 0.05 km/sec/km depth, assuming no compositional change.

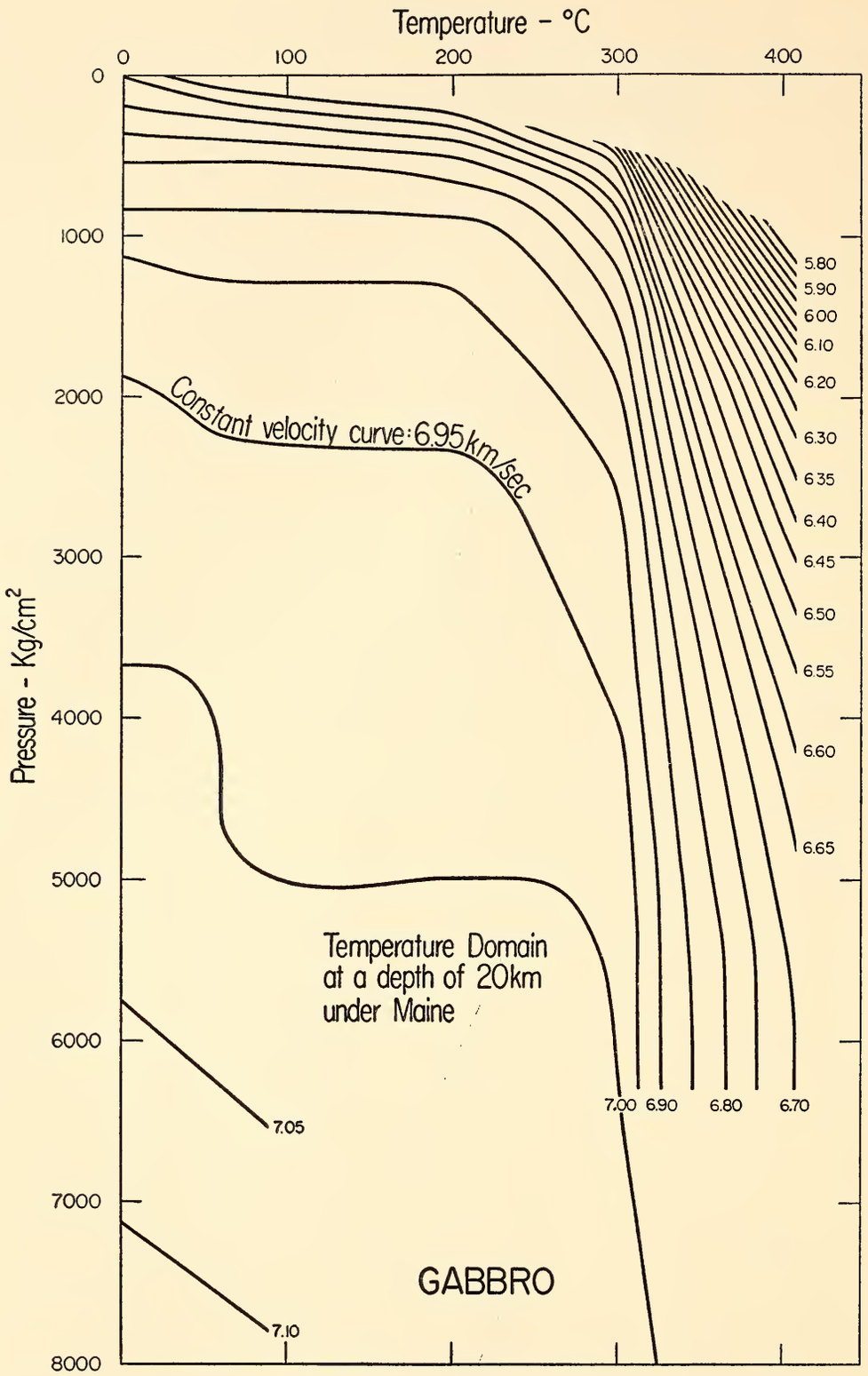

Fig. 14. Temperature-pressure field showing curves for a rock of gabbroic composition. The fall-off in velocity with temperature is even more remarkable at temperatures above 300°C than for granite; consequently, if no low-velocity layer is present in the crust, the temperature is below 300°C.

for granite; consequently, if no low-velocity layer is present in the crust, the temperature is below 300°C.

The situation in Maine appears to be that roughly the upper 3 km of the crust is granitic and below this the percentage of gabbro increases so as to maintain a generally steady gradient with but minor

fluctuations. At a depth of 20 km the material is almost entirely gabbroic. At depths greater than 20 km there can be very little increase in velocity with depth (see fig. 14). Indeed, it is quite possible that there is actually a slight velocity decrease for some distance below 20 km. Furthermore, such a decrease in the velocity gradient below 20 km is in agreement with the travel-time curve.

The velocity depth function as deduced from the observations is in very close agreement with the curve given by equation 5, which shows a rapid increase in velocity with depth close to the surface and a decreasing rate at greater depths.

It is appropriate to mention that the proposed model crust has appreciably less granitic material than is often assigned to a continental crust (Clark, *Year Book 59*).

South American Local Earthquake Network

Operations have been continued during the year in Peru, Bolivia, and northern Chile by our academic colleagues there, using the array of about 19 relatively temporary stations where our simple vertical seismographs have been installed in the Andes. Difficulties with precision timekeeping and with local seismic noise have limited the value of some of these efforts, but a profusion of interesting data exists in the voluminous records obtained. Some of the records have been interpreted by Fr. G. Saa at Antofagasta, Chile, and by our staff members with Professor A. Rodríguez B. at Arequipa, Peru. The data obtained in Bolivia under the guidance of Dr. R. Salgueiro and Fr. R. Cabre have not been intensively studied. The U. S. National Science Foundation (grant G-14593) has contributed generously to the expenses in South America of this cooperative project, which was initiated and equipped with the help of special Carnegie grants.

Studies of attenuation. The anomalous high attenuation first reported in *Year Book 57* has been examined again in the light of records from the South American earthquake network. These studies have been progressing slowly, because of operating difficulties and equipment troubles at the various sites, but it is clear that the local earthquakes show the same marked attenuation of seismic energy as the 1957 explosions.

Recent studies further indicate that the high attenuation occurs in the upper tens of kilometers of the crust and that attenuation is most extreme for higher frequencies. As our equipment is improved and additional stations begin to operate on a continuing basis it will be possible to work more quantitatively with this problem.

The San Agustín Fault System. The discovery of a near vertical fault zone 300 or more kilometers deep, parallel to the margin of the Pacific in southern Peru, was first reported in *Year Book 60*. More complete and detailed information was gathered in 1961 to confirm this finding and delineate the details of the fault zone. The system was named the San Agustín Fault System in recognition of the University in Arequipa, Peru, where the work was done. A paper by Rodríguez, Steinhart, and Asada, giving these results, is in press. Further investigations of the vertical and horizontal dimensions of the fault zone are in progress.

Crustal structure in southern Peru. From the small, four-station earthquake network used to study the San Agustín Fault System, a new method of studying crustal structure has been evolved. It depends on the frequent occurrence of earthquakes in appropriate positions and so is not suitable for universal application. Nevertheless, it offers a new look at crustal structure and may be of wide applicability in the Andes and perhaps in other active earthquake areas.

If the observed travel time for some station is T, the distance to the earthquake hypocenter is kT, where k has the dimensions of a velocity and can be identified physically as the average velocity from hypocenter to station. Let us assume that the earth is a constant-

velocity half-space. Then if we have observations of the travel time at four stations we can set up four simultaneous equations to solve for the three position coordinates of the hypocenter and the value of k. Most graphical methods of locating earthquakes are, in fact, exactly this procedure done graphically.

If our naïve assumption that the earth is a constant-velocity half-space were correct, we could plot the depth of focus against the calculated value of k for the various earthquakes and obtain a straight line, since k would be the same independent of depth. But this assumption is known to be not true, for velocity is known to vary with depth. The values of the depth of focus, H, and k obtained by this simple procedure may then be called apparent depth and apparent k.

It can be shown analytically that the geographical coordinates of the epicenter directly above the quake are very insensitive to the simple assumption above. That is, we will get the correct epicenter but the wrong values of depth and k. Now, if we restrict ourselves to consideration of earthquakes occurring in some small epicentral region and apply our simple procedure we will be dealing with earthquakes that have, in fact, occurred beneath that region, and for each earthquake we will have values for apparent depth and k.

Figure 15 shows the Arequipa, Peru, network with two regions encircled and the earthquake epicenters shown. Figures 16 and 17 show the values of apparent depth plotted against apparent k for these two regions. The observed points are not in a straight vertical line, as they would be for constant velocity (k), and

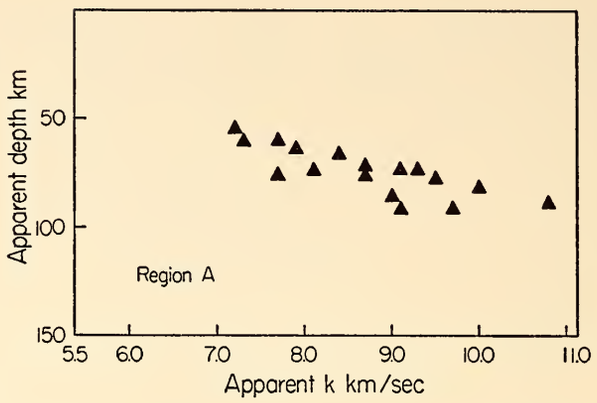

Fig. 16. Values of apparent depth plotted against apparent constant velocity for region A encircled in figure 15.

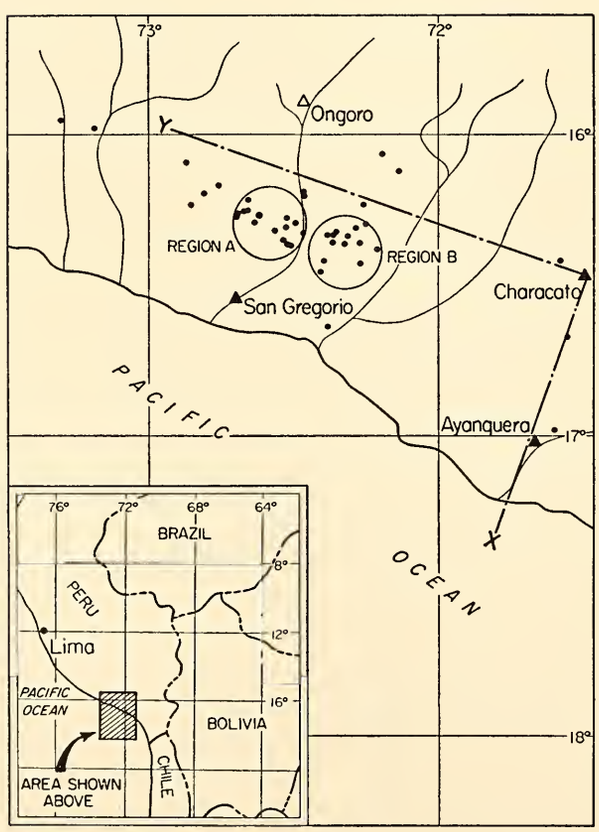

Fig. 15. Arequipa, Peru, seismic network. Regions A and B (encircled) designate where numerous local earthquakes have occurred, and the earthquake epicenters are shown.

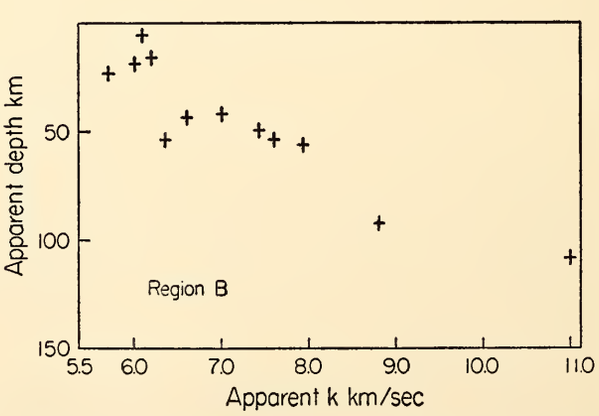

Fig. 17. Values of apparent depth plotted against apparent constant velocity for region B encircled in figure 15.

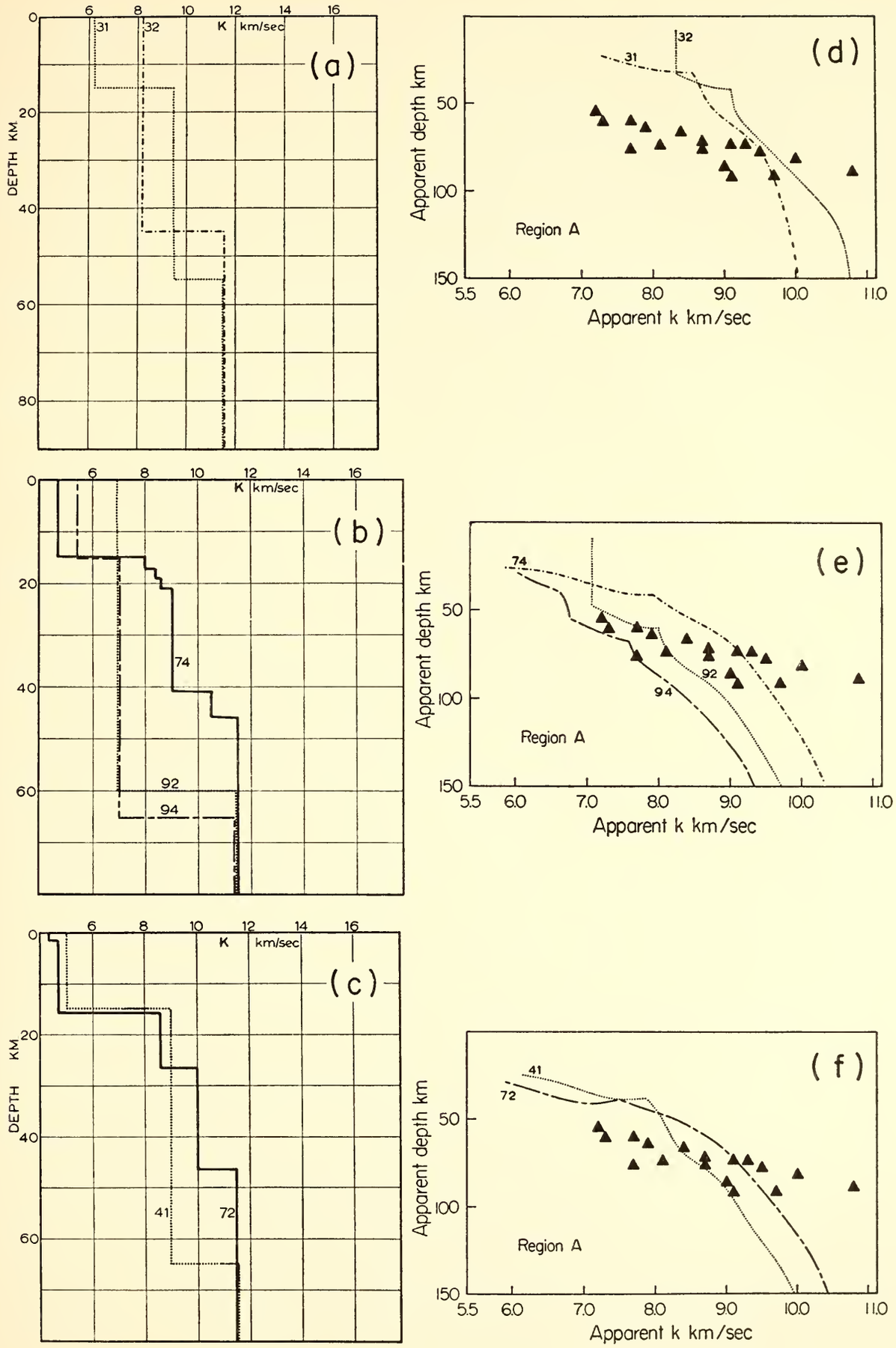

Fig. 18. Possible models for velocity versus depth, and the corresponding theoretical curves superimposed on the observations from region *A* of figure 15.

the amount by which they deviate is a reflection of the velocity structure of the crust and upper mantle. Hence, figures like 16 and 17 contain quantitative information about crustal structure, and we should find a way to extract it.

At this point we must digress to point out one experimental problem that exists with these data. Because of small irregularities in the speed of the recording drums and various local difficulties in receiving time signals, it was not possible to determine absolute time accurately enough to use the travel time of the first arrival, the compressional wave, directly. Recourse was then had to the measurement of the time difference between arrival of the compressional and shear waves at each site. This P to S time is also proportional to the distance traveled, provided that the compressional and shear waves travel essentially the same paths. We can proceed with the analysis as before with this additional assumption, which is equivalent to requiring the ratio of these two velocities to be a constant. As will be seen below, it is not entirely justified.

Having the observational data of figures 16 and 17 we may then propose a typical crustal structure and compute the true S-P travel times for that specific structure. Treating these times as though they were observational data, we may apply the same procedure as was applied to the actual earthquake data and produce a theoretical curve of apparent k versus apparent depth. If the model we assumed is correct, the theoretical curve should pass through the observed points. This theoretical analysis was programmed for the IBM 7090 and a number of models were calculated. In figure 18, a, b, and c show the models, and d, e, and f show the theoretical curves superimposed on the observations from region A. It will be noted that none of the theoretical curves fit the observations especially well, despite the wide range of models investigated.

The only conclusion that seems reasonable is that for the lower part of the crust the compressional and shear waves do not travel the same paths. This, in itself, may be a demonstration that Poisson's ratio and some of the elastic constants change with depth in a way different from that usually assumed. Recent work has been directed toward improving the timing of the instruments. When the compressional- and shear-wave travel times can be treated separately, the above procedure can be followed for each and the crustal structure can be examined in more detail.

Radioactive Ages of Rocks

L. T. Aldrich, S. R. Hart, I. Hayase,[5] *G. L. Davis,*[6] *G. R. Tilton,*[6] *B. R. Doe,*[7] *and H. Baadsgaard*[8]

The work of the group this year strikes a balance between application and methodology. In the course of international cooperation, preliminary investigations have been carried out on rocks from Japan, Brazil, and Thailand. Though furnishing much helpful information, the results support the general finding that ages from areas of complex geological history have complex interpretations and that careful work by various methods on several minerals is necessary for real understanding.

Studies of minerals low in potassium and of the effects of contact metamorphism on mineral ages have been continued as a means for understanding the complex age patterns frequently encountered. The results of these studies are summarized below.

Kyoto University–Carnegie exchange. During this report year the Department activated an exchange program with the Geological and Mineralogical Institute of the University of Kyoto which had been

[5] Guest Investigator; from University of Kyoto.

[6] Geophysical Laboratory, Carnegie Institution of Washington.

[7] U. S. Geological Survey.

[8] Guest Investigator; from University of Alberta.

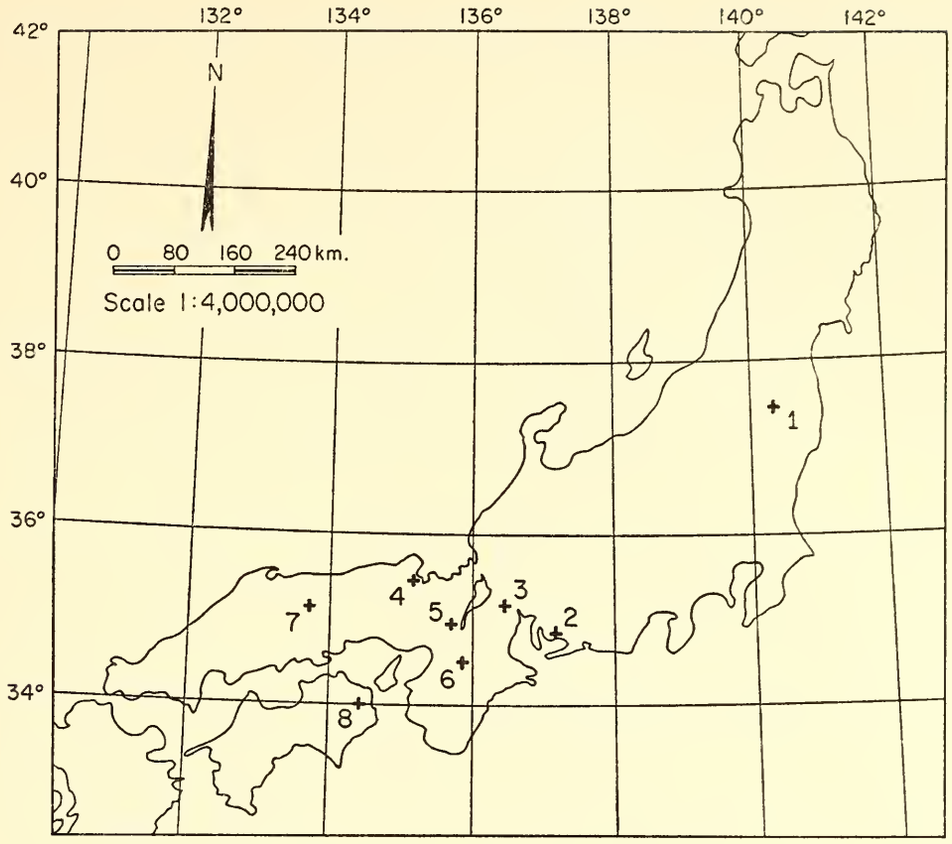

Fig. 19. Locations of rock samples from Japan on which radioactive age determinations were made by Professor I. Hayase.

in the discussion stages for the last two years. As a result, Dr. I. Hayase came to DTM as a Carnegie Guest Investigator. While at the Department he became familiar with the techniques of measuring mineral ages by analyzing several samples collected in Japan. Modern geochronological methods are of major importance in Japan, where a combination of overlapping regional metamorphic and volcanic phenomena and numerous faults, both vertical and thrust, make the geological structure difficult to understand at best. In addition, there are numerous isolated igneous bodies whose age relationships can be found only by age measurements. The geological history is so complex that too simple an interpretation of these measurements could result from using a single mineral and a single decay system.

Figure 19 shows the locations from which the samples were collected. Table 1 gives the ages measured by Dr. Hayase while at the Department. Several observations may be made about the data. First, there are no contradictions between the ages and known geological information about them. Second, discordancies of Rb-Sr and K-Ar ages are common enough to indicate the complexity of the geological history, already suggested. Third, the samples at Miyazaki show a difference between biotite and muscovite ages very similar to differences found on older pegmatites in other parts of the world. Finally, the measurements have only increased the need for further analyses on different minerals by various decay systems before they themselves can be completely understood.

The second part of the exchange between the two departments is now in progress. A Carnegie staff member, L. T. Aldrich, is now in Kyoto as a visiting professor in Dr. Hayase's Institute, advising in the establishment of a complete laboratory for the measurement of

TABLE 1. Ages Measured on Micas Collected in Japan

Sample No.	Location	Rock	Mineral	Ages, millions of years		Geological Information
				Rb-Sr	K-Ar	
1	Ishikawayama	Pegmatite	Biotite	115	80	?
2	Miyazaki-Mikawa	Pegmatite	Muscovite	100	70	?
			Biotite	40	40	
3	Unoyama	Pegmatite	Muscovite	80	60	Post Permo-Carboniferous
4	Miyazu	Granite	Biotite	65	45	Post Permo-Carboniferous
5	Kyoto	Granite	Biotite	100	90	Post Permo-Carboniferous
6	Nara	Pegmatite	Muscovite	70		Post Permo-Carboniferous
7	Ningyo Pass	Granite	Biotite	130	50	Pre-Miocene
8	Tokushima	Schist	Muscovite	265 ± 50		Paleozoic?

mineral ages. To expedite this phase of the work the Department constructed and shipped to Japan mass-spectrometric equipment which is now in operation in Kyoto. This apparatus will serve two purposes: to enable the measurement of ages to begin at once; and to serve as a model for similar equipment to be built in Japan. The laboratory in Kyoto has been and will be a meeting place for those in Japan interested in geochronology, and other laboratories now about to make similar measurements will also be visited. The Department looks forward to many years of collaboration with Japanese scientists in this field, primarily because of the personal contacts possible only on an extended visit such as this.

Thailand. Another group of Asian samples measured this year resulted from the collaboration with Dr. Saman Buravas of the Royal Department of Mines of Thailand. Two samples of particular interest to that Department, which were also of some general interest because of their correlation with Paleozoic sedimentary formations, were separated, and the appropriate minerals were analyzed. The results are given in table 2. The Phuket granite was thought on very loose geological grounds to be Cretaceous, and the age measurements confirm the assignment. The Tak granite is known to have metamorphosed Permian limestone in the area from which it was collected, and this fact places a lower limit on the age of the formation. The granite had been assigned a post-Permian, pre-Triassic age, and the data agree with that of the Holmes' "B" time scale for that boundary. It is seen, even for these young samples, that the ages of the different minerals of the same rock exhibit considerable discrepancy.

Brazil. Further international ties were developed this year through the visit to the Department of Dr. Lenz Cesar from the Universidad do Ceara, Brazil. While becoming acquainted with the techniques of age determination, Dr. Cesar measured several mineral ages on a sample of granite from near Sera da Moeda, Quadrilatero Ferrifero, Brazil. On the basis of K-Ar ages of biotite, this granite

TABLE 2. Data on Minerals from Granites from Thailand

Sample	Lat.-Long.	Mineral	Ages, millions of years	
			K-Ar	Rb-Sr
Tak granite	17°5′–99°5′	Muscovite	60 ± 5	85 ± 5
		Feldspar	....	150 ± 25
Phuket (tin) granite	8°10′–98°20′	Biotite	215 ± 10	225 ± 10

was assigned by Herz, Hurley, Pinson, and Fairbairn[9] to a group of "late" granites having intrusive ages of about 500 m.y. Table 3 shows the mineral ages determined here by several methods for this granite. It is clear that this granite is at least 1050 m.y. old; the younger ages

TABLE 3. Mineral Ages from Granite from Moeda Road, Quadrilatero Ferrifero, Brazil

Mineral	Ages, millions of years	
	K-Ar	Rb-Sr
Biotite	640	630
Feldspar	470	1050

undoubtedly represent the effect of a later metamorphic event. The actual occurrence of 500-m.y. intrusive rocks in this area as given by Herz et al. must be considered unproved until other ages than the biotite K-Ar ones become available.

Excess Argon in Pyroxenes

In *Year Book 60* we reported on the promising results obtained in the use of amphiboles and pyroxenes for K-Ar dating. Subsequent work supports the value of amphiboles such as hornblende. However, several pyroxenes have been found that contain large excesses of radiogenic argon, thus severely limiting their general usefulness for K-Ar dating.

Damon and Kulp[10] demonstrated the existence of excess or initial radiogenic argon and helium in the minerals beryl, cordierite, and tourmaline. These minerals have a ring structure with large channels, which appears to account for the incorporation of the initial radiogenic gas. In the same paper Damon and Kulp postulated that amphiboles might also contain excess gas due to the partial vacancy in the alkali-cation position. Hart,[11] investigating amphiboles and pyroxenes for use for K-Ar dating, found no evidence for excess argon in hornblendes. At that time pyroxenes were considered, a priori, to be unlikely hosts for excess argon, as they do not have structural vacancies or holes of the size found in the amphiboles and ring silicates.

Recent data, shown in table 4, clearly demonstrate the presence of excess radiogenic argon in pyroxenes. These purified mineral samples were generously made available by R. T. Dodd of Princeton University. The Bear Mountain locality is well dated at about 1000–1100 m.y. by concordant U-Pb ages on zircons from basement gneisses and intrusive granites (Tilton, Wetherill, Davis, and Bass[12]). The two hornblende ages are somewhat lower but in essential agreement with the zircon ages. Both pyroxene ages are clearly too high. The only reasonable explanation for these ages is incorporation of radiogenic argon into the pyroxene

TABLE 4. K-Ar Ages of Hornblendes and Pyroxenes from Gneisses at Bear Mountain, New York

Mineral	K, %	Ar^{40*} in 10^{-5} cc STP/g	Ages, millions of years
			K-Ar
Hornblende	1.40	7.39	1,000
Hornblende	1.30	5.96	900
Hypersthene	0.102	0.937	1,500
Diopside	0.00863	0.969	10,400

[9] N. Herz, P. M. Hurley, W. H. Pinson, and H. W. Fairbairn, Age measurements from part of the Brazilian Shield, *Bull. Geol. Soc. Am., 72,* 1111–1115, 1961.

[10] P. E. Damon and J. L. Kulp, Excess helium and argon in beryl and other minerals, *Am. Mineralogist, 43,* 433–459, 1958.

[11] S. R. Hart, The use of hornblendes and pyroxenes for K-Ar dating, *J. Geophys. Res., 66,* 2995–3001, 1961.

[12] G. R. Tilton, G. W. Wetherill, G. L. Davis, and M. N. Bass, 1000-million-year-old minerals from the eastern United States and Canada, *J. Geophys. Res., 65,* 4173–4179, 1960.

during either initial crystallization or a later recrystallization. As the structural sites in pyroxene are not large enough to admit argon, it is suggested that the excess argon is held in crystal imperfections such as dislocations and defects. If this explanation is correct, excess gases might be expected in many other minerals, as most natural minerals contain abundant defect structures. As yet there have been no recognized occurrences of excess argon in micas, feldspars, or hornblende. This should be considered a possibility in very young samples, especially those from deep-seated environments where appreciable argon pressures can exist.

Study of Mineral Ages in a Contact Metamorphic Zone

Work has been continuing on the contact zone study described in *Year Book 60*. Additional samples have been collected at distances from 2400 to 22,000 feet. As shown in figure 20, the samples at the greatest distances have been virtually unaffected by the contact heating. A complete transition has been traced from severely affected ages adjacent to the contact to unaffected ages at 22,000 feet. A study of the mineral assemblages in this zone shows only one change that can be related to the contact heating—a change in the structure of the K-feldspar from monoclinic near the contact to triclinic at distances greater than 1000 feet. It occurs in the same interval where the biotite ages are changing most rapidly. Interestingly, the feldspars retain appreciable argon in this zone where the structure has changed. This fact probably reflects the rather minor nature of the disordering that accompanies the feldspar transition from triclinic to monoclinic. On the basis of laboratory studies, this transition is believed to occur at about 500°C. It is difficult to reconcile this temperature with that obtained from consideration of any reasonable model of heat flow from this intrusive, which suggests temperatures at least 100°C lower.

The observed biotite age curves can be

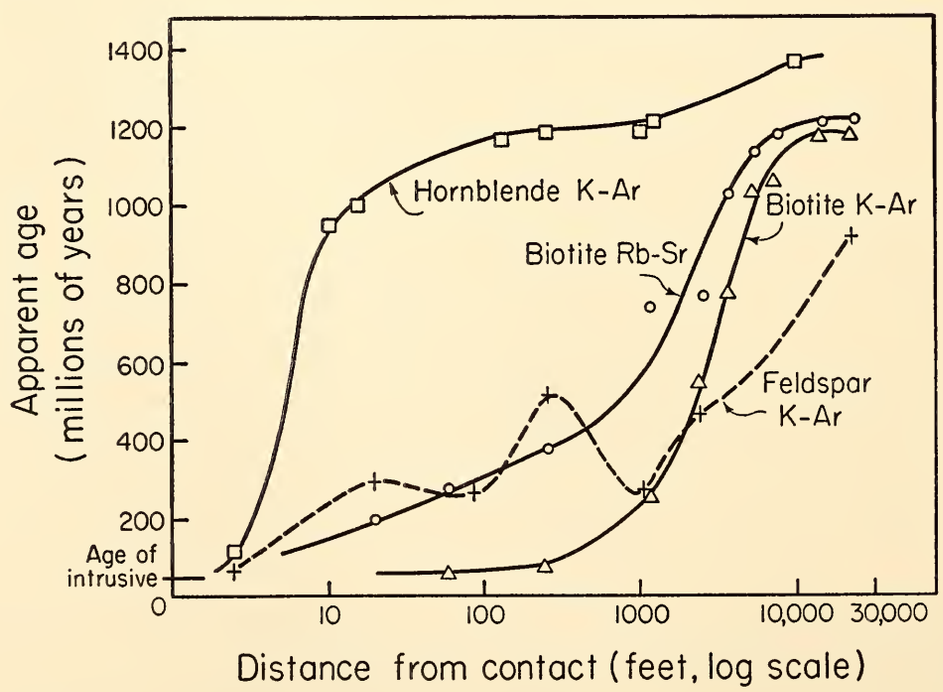

Fig. 20. Variation of ages of minerals as a function of distance from an intrusive contact. Note the variability of response to the intrusive event between minerals and between the methods of measuring the age.

closely fitted by a model in which volume diffusion of argon and strontium is assumed to be dependent on a temperature-time function obtained from simple heat-flow calculations. The model shows that the temperature dependence of the diffusion constants of argon and strontium is essentially the same and that the difference in the K-Ar and Rb-Sr age curves is due to a constant difference of about a factor of 4 in the diffusion constants of argon and strontium.

THEORETICAL AND STATISTICAL GEOPHYSICS

S. E. Forbush

Morphology and Temporal Variations of the Intensity of Charged Particles in the Van Allen Trapped-Radiation Belt[13]

The results of a preliminary investigation of the temporal and spatial variations of intensity in the Van Allen trapped-radiation belt were described in *Year Book 60*. This investigation was continued, utilizing all the data available for transits of Explorer VII through the radiation belt from October 1959 through December 1960.

The purpose of the investigation was to determine how the temporal changes of intensity and morphology in the trapped-radiation belt are related to geomagnetic variations. An objective of particular interest was to determine whether temporal variations of intensity in the belt might be responsible for variations in the geomagnetic field of the equatorial ring current responsible for geomagnetic storm-time changes. Trapped charged particles spiral around geomagnetic lines of force and oscillate between mirror points in the two hemispheres. In addition, such particles undergo a longitudinal drift around the earth: westward for positively and eastward for negatively charged particles. This longitudinal drift current seems the likely source of the geomagnetic storm-time changes that have previously been ascribed to an equatorial ring current. The present investigation is based on counting rates from a Geiger counter which are most likely due to electrons with energies above about 1.5 Mev.

From observations the intensity in the belt is determined as a function of position and time. An investigation of temporal variations of intensity as a function of three positional coordinates would be practically impossible. Fortunately, it has been shown[14] that, for the time-equilibrium state, a parameter L may be defined as a function of the integral invariant I and scalar magnetic field B such that, everywhere on the shell defined by the motion of a trapped particle in the earth's actual field, L closely approximates the equatorial radius of the shell. This means that, along a given shell, L = constant, the intensity depends on the scalar magnetic field B and the distribution of mirror points, which in turn depends on the distribution of pitch angles at the equator. L is measured from the earth's center in units of the earth's radius.

The orbit of Explorer VII was such that, for a given L, B varied systematically with period of about 100 days and opposite phase in the northern and southern hemispheres. Utilizing the observed counting rates, for a given value of L, for consecutive passes in the two hemispheres, and the corresponding values of B, it was possible to determine empirically the dependence of intensity on B. This dependence was then used to

[13] An investigation carried out jointly with D. Venkatesan and G. Pizzella of the Department of Physics and Astronomy of the State University of Iowa.

[14] C. E. McIlwain, *J. Geophys. Res.*, *66*, 3681–3691, 1961.

"correct" the observed intensities to a constant B for each of several values of L between 2.5 and 4.7. The corrected intensities were then used to investigate temporal variations and morphology to determine how changes in them were related to changes in the equatorial geomagnetic field of the ring current U, which were derived from geomagnetic data from four equatorial magnetic observatories about 6 hours apart in longitude.

The results of the investigation showed that, for a given L, the correlation between the "corrected" counting rates from consecutive passes (50 minutes apart) in the northern and southern hemispheres was decidedly higher than that for the observed counting rates. This indicated not only the necessity for but also the validity of the correction for B.

The value of L at which the maximum intensity occurred (L_{max}) showed a marked tendency to decrease with increasing U; that is, the maximum intensity in the belt tended to occur closer to the earth during magnetic storms. On the average, L, in units of the earth's radius measured from the earth's center, decreased from 4.0 to 3.0 for an increase of about 100 gammas in the southward geomagnetic field of the equatorial ring current.

For all the transits through the belt over Australia the values of B were at least as great as those that (for corresponding values of L) occur at sea level south of Africa. Thus all the trapped particles measured over Australia have mirror points that would not be above sea level over the region south of Africa where they would consequently be completely absorbed. This means that the intensity observed over Australia is maintained by replenishment of the particles absorbed in the region south of Africa. Moreover, the replenishment must take place within the time taken for electrons to drift longitudinally eastward from the longitude of south Africa to that of Australia, that is, within a period of the order of an hour or less. This replenishment may be due to some mechanism causing a migration of mirror points to lower values of B.

For values of $L > L_{max}$ the intensity shows a weak tendency to decrease during magnetic storms, whereas for $L < L_{max}$ the opposite tendency occurs. No evidence was found to indicate that any important contribution to the ring current could come from the longitudinal drift of the measured electron intensity (above 1.5 Mev). Thus it seems likely that the major contribution to the ring current must arise either from electrons of energy less than 1.5 Mev (the threshold value for the counters used) or from protons of energy less than 18 Mev, the threshold value of the counter for protons.

For values of $L \geqslant 4.1$ earth radii, variations of a factor of 10 or more in counting rate occurred on the average every 15 or 20 days. For $L \leqslant 2.5$ these shorter-period fluctuations were practically absent, although there were at $L = 2.5$ two or three increases of intensity by a factor of about 100 which appeared to decay slowly over a period of two or three months. These longer-period variations diminished rapidly with decreasing L until at $L = 1.5$ they were barely discernible.

Geomagnetic Equatorial Ring Current Measures and Latitude of Auroral Currents

For the investigation described in the preceding section 3-hour values of the geomagnetic equatorial ring-current field (ERC or U) were derived for the period from October 1959 through December 1960. In addition, 3-hour ERC values were maintained as near to date as receipt of the necessary observatory magnetic data permits, which now is through December 1961. These values were derived to facilitate cooperation in the interpretation of magnetic measurements, which ultimately will locate the equatorial ring current, now being obtained by others from satellite-borne magnetometers. The ERC values were derived after

the method of Kertz,[15] using data from magnetic observatories about equally spaced in longitude and nearest the equator.

The strong auroral zone current system and its southward shift during magnetic storms are likely to be closely associated with particles coming from the outer Van Allen belt. To permit closer investigation of this connection the latitude of the auroral currents has been derived for a number of magnetic storms using data from several magnetic observatories in Scandinavia, which is about the only longitude sector where observatories near the same longitude are close enough together to make such a determination feasible. For these determinations corrections were made for the ERC field at the auroral zone by means of the ERC values that had been derived. These values will be applied in studies involving measurements in the outer Van Allen belt and also in connection with an investigation of storm-time variations in cosmic-ray intensity begun in collaboration with Professor Alfvén of the Royal Institute of Technology, Stockholm, Sweden.

Conductivity Anomaly Program for Peru

An observational program was outlined to provide magnetic data for determining whether crustal conductivity anomalies (Bartels-Schmucker anomalies) may exist in Peru. The program will be carried out by the Instituto Geofísico del Peru with about eight Askania variographs loaned by the U. S. Coast and Geodetic Survey. The existence or absence of such anomalies provides one means of exploring the earth's crust at depths of the order of 100 km.

A preliminary model to provide time marks at 10-minute intervals on the film was constructed and tested. One prototype is to be constructed and tested. If it proves satisfactory, timers will be provided for use with Mercer battery-operated chronometers. Thus, reliable time marks would be available at the field stations—a matter of importance, since the interpretation of any anomalies may depend to considerable degree upon the relative phases of the observed components of the night-time sudden commencements which will provide the uniform external inducting field. Moreover, the timers will provide automatic control of daily calibrations.

In view of troubles experienced with the *Z* system of the Askania variograph during the International Geophysical Year the equations for the system were derived and practical experiments outlined for determining the cause for any anomalous drift in *Z* base lines after temperature compensation in Peru. For some of the variometers previously used in Peru during the International Geophysical Year the analysis showed that most of the troubles were probably due to a mechanical unbalance in the *Z* system. Tests were outlined for determining all the important parameters required to specify the state of the system.

Cosmic-Ray Program

Observations and reductions of data. Cosmic-ray ionization chambers were operated throughout the report year at Huancayo, Peru, and at Fredericksburg, Virginia. Scalings and reduction of records have been maintained current for Fredericksburg and Huancayo.

Cooperation in operation of cosmic-ray meters. Grateful appreciation is expressed to the U. S. Coast and Geodetic Survey and the staff of its magnetic observatory at Fredericksburg, Virginia, for efficient operation of the meters during the past report year, and to the Government of Peru and the Director and staff of the Instituto Geofísico del Peru for making cosmic-ray records from Huancayo available.

[15] Walter Kertz, Ein neues Mass für die Feldstärke des erdmagnetischen äquatorialen Ringstroms, *Abhandl. Akad. Wiss. Göttingen, Math.-Physik. Kl., Beiträge zum internationalen geophysikalischen Jahr,* Heft 2, Göttingen Vandenhoeck 8, Ruprecht, 1958.

LABORATORY PHYSICS

NUCLEAR PHYSICS

L. Brown,[16] *N. P. Heydenburg, H. Rudin,*[17] *and G. M. Temmer*

Polarized Ion Source

The desirability of having available polarized beams of protons and deuterons for the study of nuclear interactions was discussed in some detail in *Year Book 57*. It was pointed out at that time that the Department's pressurized Van de Graaff generator, with its large high-voltage terminal equipped with 6 kw of a-c power, was ideally suited for the installation of a complex atomic beam apparatus for the production of polarized particles. It was announced in last year's report that arrangements had been made for a cooperative endeavor between the Department and the Basel group for the installation of their atomic beam apparatus in the DTM accelerator. This was to be a copy of their first model, which successfully demonstrated the production of polarized deuterons by the atomic beam method for the first time.

The Basel polarized ion source was completed by the summer of 1961 and was shipped to our laboratory in August. The installation of the source in the DTM accelerator was begun in September and was essentially completed and ready for testing by the end of January 1962. The Basel polarized source has been described elsewhere.[18] Basically it consists of (1) a discharge tube for the production of atomic hydrogen, (2) a set of diaphragms and fast pumps for defining the atomic beam, (3) a quadrupole magnet for the selection and focusing of atoms having the desired orientation in space, (4) an ionizer for the atomic beam, and (5) an arrangement for preaccelerating and focusing the ionized atoms. After the source had been assembled in the accelerator terminal, a system was constructed to control from outside the steel pressure tank the various vacuum pumps, vacuum valves, and electronic equipment associated with the source inside the high-voltage terminal. A cooling arrangement was also necessary for cooling the diffusion pumps, baffles, and ionizer in the high-voltage terminal. An external heat exchanger, and a circulating system for Freon 11, were installed for this purpose.

After a number of unpleasant failures of the cooling system, a deuteron beam of about 10^{-9} ampere, after acceleration, was obtained. Although somewhat weaker than the beam obtained during tests at Basel, it was sufficient to proceed with a test for polarization. The polarization was determined from the angular asymmetry of the disintegration protons in the reaction $He^3(d, p)He^4$, measured somewhat above the broad resonance occurring at 470 kev. A small reaction chamber was constructed for this test consisting of a gas cell for the He^3 and two CsI particle detectors placed at 20° and 90° with respect to the direction of the incident beam. The polarization for a deuteron with spin 1 cannot be characterized by vector polarization alone, as it can for a proton with spin ½, but the polarization tensor must be considered as well. For the above reaction, the ratio of the cross sections at 20° and 90° is given by

$$A = \frac{\sigma(20°)}{\sigma(90°)} = \frac{1 - \frac{1}{4}P_{zz}(3\cos^2 20° - 1)}{1 - \frac{1}{4}P_{zz}(3\cos^2 90° - 1)}$$

where P_{zz} is a component of the polarization tensor and is predicted to have the value $P_{zz} = -\frac{1}{3}$ for our polarized beam. The experimental value of A was found to be 1.216 ± 0.048. Using this equation, P_{zz} was found to be -0.301 ± 0.063, in good agreement with the expected value.

[16] Carnegie Institution Fellow.

[17] Carnegie Institution Fellow; from University of Basel.

[18] H. Rudin, H. R. Striebel, E. Baumgartner, L. Brown, and P. Huber, *Helv. Phys. Acta, 34*, 58, 1961.

We plan to utilize the polarized deuteron beam in the study of a number of nuclear reactions, among them the following: $d(d, p)t$, $He^4(d, d)He^4$, and $Li^6(d, \alpha)\alpha$.

Summary of Cooperative Program with Florida State University

The arrangement for the participation by Heydenburg and Temmer in the nuclear research program at Florida State University has continued during the past year, Heydenburg spending part time at DTM on the polarized ion source project. The experimental work on the $Li^7(p, \alpha)\alpha$ reaction (Han and Heydenburg), reported in *Year Book 60*, has been completed, and the angular distributions have been analyzed in terms of the Legendre polynomials. In addition to the resonances at 3.0 Mev and 5.6 Mev previously reported, there is evidence for another at 6.5 Mev and a weak one at 9.0 Mev. These four resonances correspond to energy levels in the compound nucleus Be^8 at 19.9, 22.1, 23.0, and 25.0 Mev.

The $Li^6(d, \alpha)\alpha$ reaction can be employed to study the excitation region of Be^8 above 22.3 Mev. The Be^8 levels at 22.1 and 23.0 Mev cause a sharp rise in the yield of alphas and an apparent resonance peak at a bombarding energy of 0.4 Mev, as was shown in earlier work at DTM. We have extended this early work to bombarding energies up to 12 Mev. The Be^8 level at 25.0 Mev causes a prominent resonance peak in the excitation curve at a deuteron energy of 3.85 Mev (as compared with the very weak resonance in the $Li^7(p, \alpha)\alpha$ reaction occurring at the same excitation in Be^8). From this evidence and other considerations it is believed that these four relatively narrow states in Be^8 can be described in terms of $Li^6 + d$ configurations rather than $\alpha + \alpha$ configurations. M. Nomoto has been able to make spin and parity assignments for these levels from an analysis of our data. These are as follows: 19.9 Mev level 2+, 22.1 Mev level 2+, 23.0 Mev level 4+, and the 25.0 Mev level 2+.

Angular distributions and an excitation curve were observed for the $Li^6(p, \alpha)He^3$ reaction (Han and Heydenburg). No resonance structure is seen in the excitation curve for proton bombarding energies from 3 to 12 Mev. A complete analysis of the angular distribution curves has not yet been made, but strong forward and backward peaking of the He^3 cross section suggests that the reaction mechanism involves both a deuteron pickup by the incident proton and a direct interaction of the proton with a triton in the nucleus. This reaction has special interest for cluster-model considerations, since Li^6 is one of the best-understood nuclei from that point of view.

The $Be^9(p, \alpha)Li^6$ reaction (Blieden and Temmer) is a striking example of a direct interaction process. The yield curve varies smoothly with proton bombarding energy, showing no resonance structure. The angular distributions have identical shapes between 6 and 11.5 Mev proton energy for alphas to the ground state of Li^6, and between 9 and 11.5 Mev for alphas to the first-excited state. The curves are being analyzed in terms of Satchler's distorted wave analysis, and preliminary results show good agreement.

In contrast to the above reaction the $F^{19}(p, \alpha)O^{16}$ reaction (Warsh and Temmer) shows a rather anomalous behavior, having angular distribution curves that cannot be fitted with the Satchler analysis. It appears that the heavy-particle stripping process may also have to be considered in analyzing these data, as indicated by the large alpha yields in the backward direction.

Two new energy levels in Ne^{20} were found from a study of the alpha groups from the $Na^{23}(p, \alpha)Ne^{20}$ reaction; they are particularly interesting in that they lend further support to the surprisingly regular rotational band structure of Ne^{20}. The excitation curves and angular distributions for this reaction and the

$Al^{27}(p, \alpha)Mg^{24}$ reaction are very complex.

A yield curve at a 90° angle has been obtained for the reaction $N^{15}(p, \alpha)C^{12}$ (Roy, Adams, and Temmer) showing considerable resonance structure. This reaction has special interest in connection with the level structure of O^{16}, which has closed proton and neutron shells and for which several detailed theoretical predictions have been made. Angular distributions will be measured for this reaction.

Temmer has considered the possibility of a so-called *resonant transfer* process, involving no energy loss, of one or more nucleons between the target and projectile in a nuclear encounter. This is based on an analogy to a recently discovered atomic process demonstrating the repeated resonant transfer of an electron between two identical nuclei. A nuclear example would be the transfer of a proton from O^{16} to N^{15}. Experiments attempting to demonstrate the existence of this effect, which contributes to elastic scattering, are contemplated.

BIOPHYSICS

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Introduction

The report of the Biophysics Section in *Year Book 56* was the first in which we mentioned the particles (now called ribosomes) found in cells and their possible role in the synthesis of nucleic acids and proteins. Before that time our attention had been directed toward the biosynthesis of amino acids and nucleotides and later toward the mechanisms by which those small molecules were concentrated and held in the cells. Five years ago our knowledge of the particles was barely adequate to appreciate that the synthesis of ribosomes and their role in protein synthesis was a promising field for investigation.

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[20] Carnegie Institution Fellow.

After five years of concentration on these problems the broad outlines and many of the details have been worked out. *Year Book 57* reported the first separation of ribosome precursors by chromatography and by sedimentation. These techniques were markedly improved during the years to provide the detailed flow diagrams in *Year Book 60*. *Year Book 58* reported the identification of newly synthesized protein still attached to ribosomes which pointed to their role in protein synthesis. We consider ourselves privileged to have been able to participate in the unusually rapid developments that have occurred during these five years.

At the present moment it is possible to account quantitatively for the flow of material as it passes from the external medium through pools of low-molecular-weight compounds through two sequential macromolecular precursors and finally into the stable end products. In addition, it appears that one of these precursors, while attached to a ribosome, can act as the template for protein synthesis. A more complete discussion of our present knowledge and the questions remaining to be answered will be found under "Conclusions," at the end of this section. The details of the experimental work are given in the body of the report.

Incorporation of RNA Bases into the Metabolic Pool and RNA of E. coli

Studies of the incorporation of C^{14}-uracil reported last year showed direct entry of radioactivity into the RNA without delay by the large pool of nucleotides. The scheme shown in diagram 1 was proposed to describe the kinetics of uracil incorporation. *P* represents a very small pool or sequence of reaction steps leading from uracil to a chemical form suitable for incorporation into RNA. *S* represents a large pool of compounds that can exchange with some uracil compound in *P*. The rate of exchange between *S* and *P* is not fast, and equilibrium between

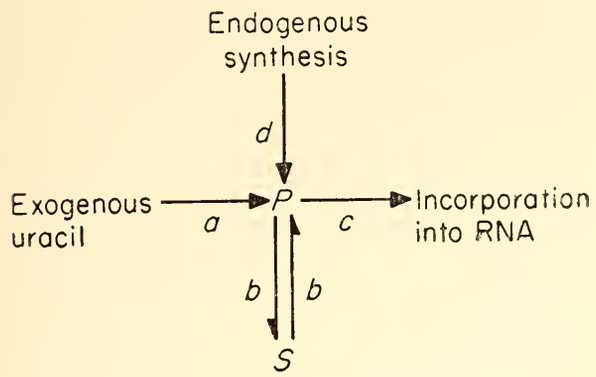

Diagram 1

the specific radioactivity of P and S requires several minutes, at least. P then effectively forms a bypass, around the large pool, for the entry of exogenous uracil into RNA.

Since an alternative explanation of the undelayed entry of radioactivity into RNA has been proposed by Gros et al. it seemed worth while to examine the kinetics of incorporation of the three other RNA bases. The additional information reported here, particularly the fact that the time constants and bypass fractions vary widely among the four bases, clarifies the situation. The existence of a bypass mechanism is amply demonstrated.

Incorporation of cytosine. The results of an experiment in which 10^{-6} M cytosine-2-C-14 was supplied to exponentially growing cells are shown in figure 21. It is clear that the kinetics of cytosine incorporation are qualitatively similar to the kinetics of uracil incorporation reported last year. There is an initial rapid incorporation into RNA, and this rate is maintained while exogenous cytosine remains. At the end of this first phase the rate of incorporation into RNA abruptly falls by a large factor. During the second phase (after the exogenous cytosine is exhausted) the radioactivity of the pool falls slowly. The semilog plot (fig. 22) shows that the radioactivity of the pool decreases in an approximately exponential fashion. The time constant (decay to $1/e$) is about 21 minutes—more than twice that observed for uracil.

The results of an experiment at a higher concentration of cytosine (10^{-4} M) are shown in figure 23. Here again two phases are observed. Initially C^{14} from cytosine enters the RNA at less than half

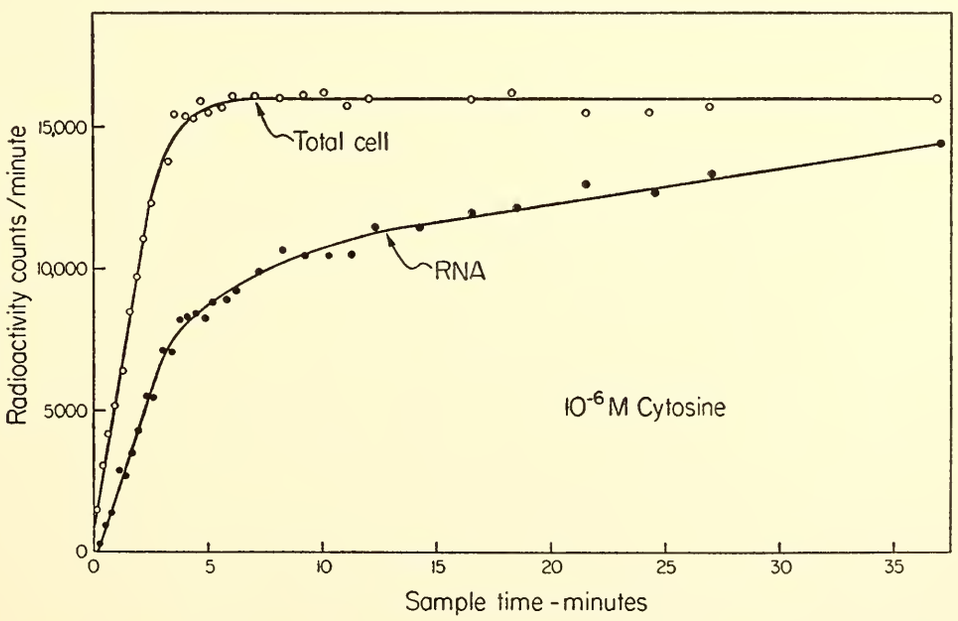

Fig. 21. Incorporation of 10^6 M C^{14}-cytosine by *E. coli* ML 30 growing at 37°C with a generation time of 51 minutes. Cell density 0.5 mg (wet) per ml. Open circles represent radioactivity of total cell samples collected by membrane filtration. Solid circles represent RNA radioactivity, samples collected by membrane filtration after treatment with 5 per cent TCA.

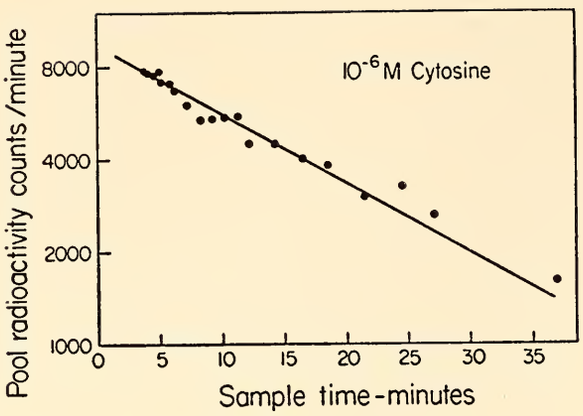

Fig. 22. Decay of the radioactivity of the C^{14}-cytosine labeled pool. Data obtained from experiment of figure 21 by subtracting the RNA radioactivity from the whole cell radioactivity.

the final rate. Only after a relatively long period does it achieve its final rate.

The curves shown in figures 21, 22, and 23 are precisely those to be expected on the basis of the schematic diagram if it is assumed that the time constant is a measure of the quantity of compounds in the pool S in relationship to the flow b.

The fraction of the total flow bypassing the pool S may be estimated from the fraction of the radioactivity (fig. 21) that has entered the RNA at the time the exogenous cytosine is exhausted. This appears to be about 45 per cent. The bypass fraction of the flow may also be estimated from the ratio of the initial to ultimate slopes in figure 23. The result is consistent with the experiment at low concentration. Although the shape of the curve in figure 23 suggests the presence of components in the pool of intermediate time constant, their effect is not apparent in figure 22. If such additional components are present, the time constant quoted is a weighted average of many and the accuracy of the estimate of the bypass is reduced. The calculation of the bypass flow and time constants is discussed in more detail below.

The time constant estimated here is probably influenced by the appearance of C^{14} from cytosine in uracil compounds in the pool and RNA. Table 5 shows the results of several measurements of the ratio of the amount of radioactivity in

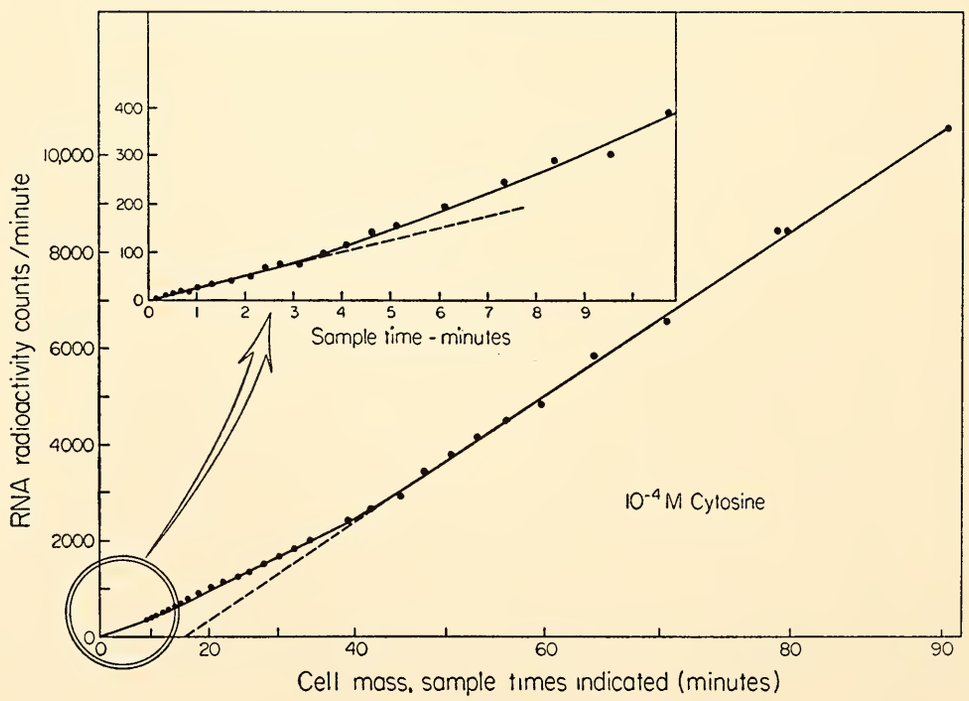

Fig. 23. Incorporation of 10^{-4} M C^{14}-cytosine by *E. coli*. Initial cell density 1/3 mg (wet) per ml. RNA radioactivity (TCA-precipitable) plotted against cell mass with sample times indicated. The upper curve represents the data at early times with both scales expanded by a factor of 10. Thus the slopes on the two curves may be directly compared.

TABLE 5. Interconversion of Cytosine and Uracil Compounds

Labeled Supplement	Competitor	Ratio* of Radioactivity of RNA Uridylic to RNA Cytidylic
C^{14}-cytosine 10^{-4} *M*	----	1.4
C^{14}-cytosine 10^{-4} *M*	----	1.6†
C^{14}-cytosine 10^{-6} *M*	----	1.7
C^{14}-cytosine 5×10^{-5} *M*	----	1.6
C^{14}-cytosine 5×10^{-5} *M*	C^{12}-uracil 5×10^{-5} *M*	0.6
C^{14}-uracil 5×10^{-5} *M*	----	1.0
C^{14}-uracil 5×10^{-5} *M*	C^{12}-cytosine 5×10^{-5} *M*	1.0

* After the radioactivity was completely incorporated into RNA the cells were treated with 5 per cent TCA, washed, and hydrolyzed with alkali, and the nucleotide residues were separated by electrophoresis.

† In this experiment samples were taken at 10-minute intervals. By 10 minutes the ratio had already reached 1.3, and at 20 minutes it had reached essentially its final value.

the uridylic acid to that in the cytidylic acid of the RNA. In view of the very considerable interconversion between cytosine and uracil compounds it is surprising that the effective time constants of pool *S*, measured with the C^{14}-uracil and C^{14}-cytosine, are so different. By comparing lines 4 and 5 with lines 6 and 7 of table 5, however, it appears that C^{12}-uracil has an effect on the conversion of C^{14}-cytosine whereas C^{12}-cytosine does not affect the conversion of C^{14}-uracil. This lack of symmetry in the competition experiments and the relatively greater conversion of cytosine compounds to uracil compounds indicates the complexity of the interconversion processes.

Guanine. Figure 24 shows the results of an experiment in which 10^{-6} *M* guanine was supplied to exponentially growing cells. Here again there is a rapid incorporation into RNA during the first phase when guanine is present externally. During the second phase, after the external guanine has been exhausted, the radioactivity of the pool is relatively slowly transferred to RNA. No logarithmic plot of the decay of the guanine pool is presented, since the small amount of radioactivity in the pool and the scatter

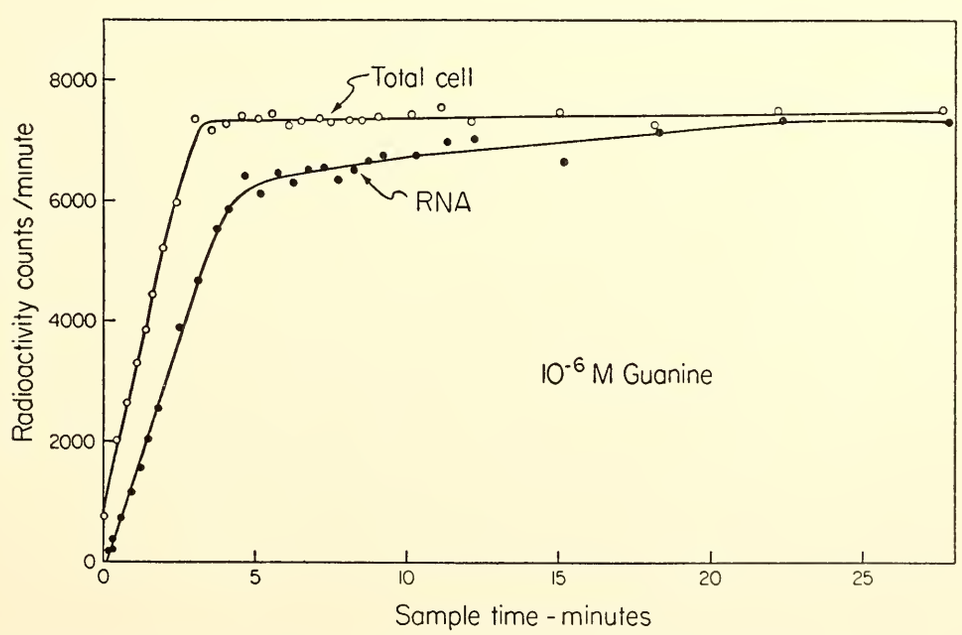

Fig. 24. Incorporation of 10^{-6} *M* C^{14}-guanine by *E. coli*. Cell density 0.7 mg (wet) per ml.

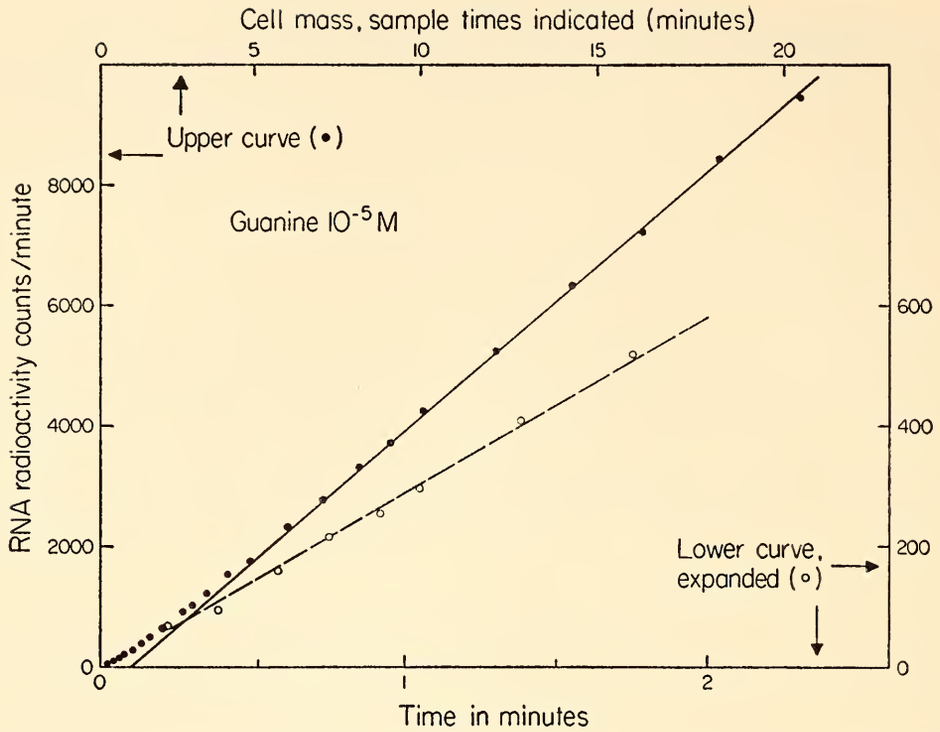

Fig. 25. Incorporation of 10^{-5} *M* C^{14}-guanine by *E. coli*. Cell density initially 0.3 mg (wet) per ml. RNA radioactivity plotted against cell mass with sample times indicated. Solid circles refer to upper and left scales. Open circles refer to lower and right scales, both of which are expanded by a factor of 10 so that the initial slope may be directly compared with the final slope.

in the points lead to great inaccuracy. Various experiments have given mean time constants between 3 and 6 minutes for the decay of the pool radioactivity after the external guanine is exhausted.

Figure 25 shows the results of an experiment in which a higher concentration of guanine (10^{-5} *M*) was supplied. Here a comparatively small amount of curvature is observed, and a straight line through the points taken after 5 minutes extrapolates to about 1 minute.

The results with guanine are qualitatively similar to those with uracil and cytosine. Two phases in the incorporation curves are observed at both high and low concentrations. The quantitative aspects, however, are quite different. The flow through the pool *S* is small, and the time constant is not long (about 3 minutes). The pool of guanine nucleotides may be calculated from its time constant and flow. The total pool of guanine compounds may also be estimated directly from the extrapolated time in the experiment of figure 25 to be sufficient to supply the guanine required for 1 minute's growth of the cellular nucleic acid. This estimate is valid if there is little exchange between pool guanine compounds and external guanine and if the conversion to adenine compounds is not too large. This is equivalent to 7 μM per gram dry cells.

C^{14}-guanine does, in fact, appear to only a slight extent in the adenylic acid of RNA. In three experiments C^{14}-guanine at concentrations of 2×10^{-6} *M*, 10^{-5} *M*, and 5×10^{-5} *M* was allowed to be entirely incorporated into RNA. The ratio of the radioactivity of the adenylic acid of the RNA to that of the guanylic acid ranged between 0.1 and 0.2.

Figure 26 shows the results of a "chase" experiment in which C^{14}-guanine (3.6×10^{-7} *M*) was initially supplied the cells and was followed 15 seconds later by 10^{-5} *M* C^{12}-guanine. This result differs from that obtained with uracil (McCarthy, 1962), in which the specific activity of the

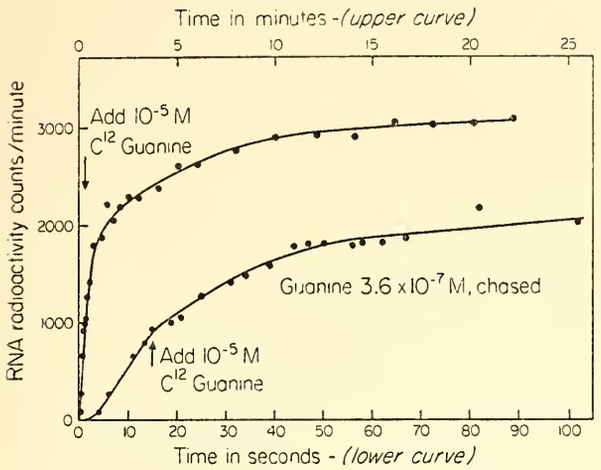

Fig. 26. Guanine chase experiment. Exponentially growing *E. coli*. Cell density 1.8 mg (wet) per ml. Initially C^{14}-guanine (3.6×10^{-7} *M*) was added. Fifteen seconds later C^{12}-guanine was added to bring the concentration to 10^{-5} *M*. Lower curve represents the same data as the upper curve but with time scale (alone) expanded by a factor of 15.

uracil passing through the bypass appeared to be diluted almost instantly. With guanine there appears to be a delay of 20 to 30 seconds before the tracer passing through the bypass is completely diluted. There is an instantaneous change in slope to about half that reached during the initial 15 seconds. This implies the existence, in the pool of guanine compounds, of a small component with a short time constant. Such a complexity is also suggested by the shape of the RNA curve in figure 24, just after the external guanine has been exhausted at 3 minutes, and by the uncertainty in the determination of the decay time constant for the pool of guanine compounds.

Adenine. Figures 27 and 28 show the incorporation of C^{14}-adenine at 10^{-7} and 10^{-5} *M*. Here again the qualitative

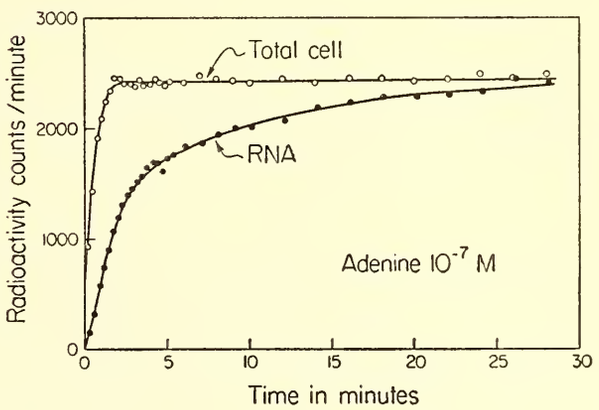

Fig. 27. Incorporation of 10^{-7} *M* C^{14}-adenine by growing *E. coli*. Cell concentration 1/3 mg (wet) per ml. Open circles represent total cell radioactivity. Solid circles represent TCA-precipitable (RNA) radioactivity.

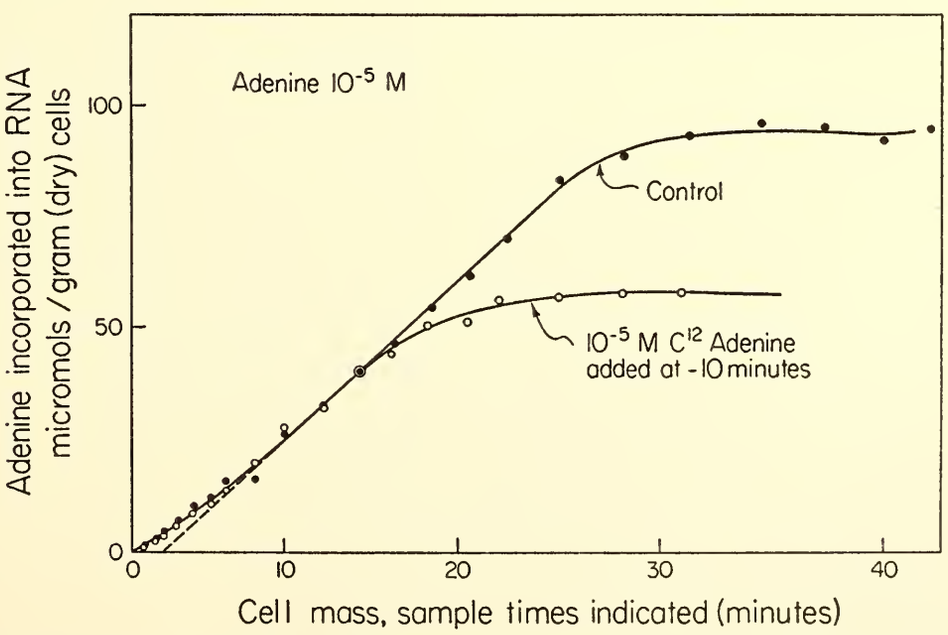

Fig. 28. Incorporation of 10^{-5} *M* C^{14}-adenine by growing *E. coli*. Solid circles, control. Open circles 10^{-5} *M*. C^{12}-adenine added 10 minutes before carrier-free C^{14}-adenine.

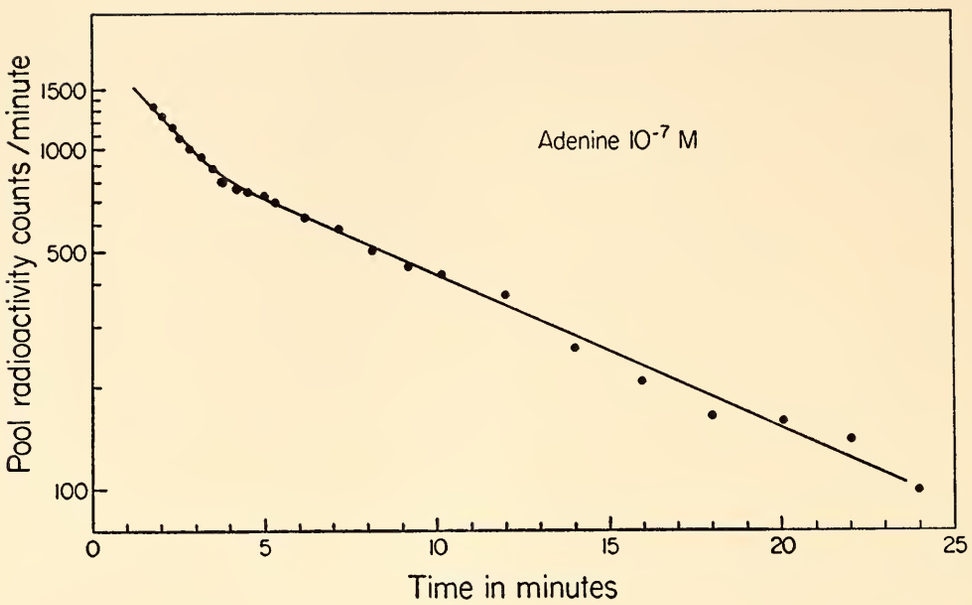

Fig. 29. Semilogarithmic plot of radioactivity of pool after external adenine was exhausted. Data from figure 27.

features are similar to those of the other three bases. Figure 29 (derived from the experiment of figure 27) shows a semilogarithmic plot of the radioactivity of the pool after the external adenine was exhausted. It is immediately apparent that the decay of the pool radioactivity cannot be represented as a single exponential.

It might be suggested that the shape of the curve in figure 28 is influenced by a change in the pool size. The open circles in figure 28 represent the results of an experiment in which 10^{-5} *M* C^{12}-adenine was added 10 minutes before the tracer. There is no indication of any difference between the two curves except that to be expected from the utilization of a certain fraction of the carrier adenine before the tracer was added. There was, therefore, no measurable expansion of the pool of adenine compounds even at this relatively high concentration of external adenine.

The appearance of radioactivity from C^{14}-adenine in the guanylic acid residues of RNA was measured at two concentrations (10^{-7} *M* and 10^{-5} *M*). At both, the radioactivity of the adenylic acid residues was about three times that of the guanylic acid residues.

Calculation of the bypass flows and time constants. To adequately compare the experimental results with the predictions of the schematic diagram, the bypass flows and time constants of the pool *S* have been calculated for each of the four bases from experiments at both high and low concentration.

An experiment is considered to be at a low concentration if the external supply of labeled base is exhausted before the specific activity of the pool *S* has become comparable to the specific activity of the tracer. In other words, the external supply is exhausted before the rate of incorporation of radioactivity has risen significantly above the initial rate.

If the external supply lasts well beyond the time when the final rate of incorporation into RNA has been achieved the experiment is at a high concentration, and little further change occurs as the concentration is increased. Experiments at intermediate concentrations, where neither of these conditions is met, are more difficult to interpret.

For experiments at low concentration the bypass flow can be estimated directly from the fraction of the total radioactivity entering the cell that enters the RNA.

Thus $(c - b)/c$ listed in the first row in table 6 was calculated from the ratio of the slopes of the RNA incorporation curve (TCA-precipitable radioactivity) and the total curve. Where experiments were available at both 10^{-6} and 10^{-7} M the results agreed.

For experiments at high concentrations the bypass flow was calculated from the ratio of the initial slope of the RNA incorporation curve to its slope after the final rate had been achieved (i.e., after S had reached its maximum specific radioactivity). Since there was significant cell growth, all the experiments at high concentration have been plotted against the increase in cell mass. Straight lines on such a plot correspond to constant rates of incorporation per cell. By marking the sample time on the abscissa it becomes possible to estimate the time constant directly by extrapolation.

Row 3 (table 6) lists the time constant of the pool S estimated on semilogarithmic plots of the pool radioactivity as a function of time from experiments at low concentrations. The time constant estimated in this way is a measure of the ratio of the flow through the pool to the size of the pool if the pool is constant in size. There is no evidence that supplementation with RNA bases expands the nucleotide pools. In cases that have been tested by "preload" experiments (adenine and uracil) very little, if any, expansion occurs. The agreement between the data in row 3 and row 4, table 6, supports this conclusion.

Row 4, table 6, lists the time constant for the increase in the rate of entry of radioactivity into RNA as it rises from its initial rate to its final rate. For this purpose the linear part of the curve (e.g., fig. 23) at late times is extrapolated until it strikes the time axis, giving the effective delay time T'. To a sufficiently close approximation the desired time constant is given by $T = R_2T'/(R_2 - R_1)$, where R_1 is the initial slope and R_2 the final slope.

TABLE 6. Bypass Flows and Time Constants

Base Supplied	Uracil*	Cytosine	Guanine	Adenine
Fraction of flow in bypass				
Low-concentration experiments†	0.40	0.45	0.74	0.4
High-concentration experiments‡	0.37	0.37	0.68	0.46
Pool time constant (minutes)				
Low-concentration experiments§	10	21	2–6	2–12
High-concentration experiments‖	11	24	3.1	4

* Data from *Year Book 60.*
† From ratio of RNA incorporation rate to total cell incorporation rate.
‡ From ratio of initial RNA incorporation rate to final RNA incorporation rate.
§ Time constant of exponential decay of pool radioactivity.
‖ Extrapolated delay time (corrected).

Discussion. The evidence presented here and in last year's report shows that the incorporation of the four RNA bases may be represented by diagram 1. The relative bypass flow $(c - b)/c$, the size of the pool S, and the time constant of the pool (proportional to S/b) vary widely among the four bases. The nucleotide pool appears to contain more than one component, and the time course of the decay of the radioactivity of the pool is not always represented by a single exponential. Further, uracil compounds and cytosine compounds are rapidly interconverted. Studies of cytosine (table 5) and uracil show that some of this conversion occurs before entering the pool S.

The schematic diagram would obviously grow in complexity if these features were explicitly indicated. It is clear that a very large number of consequential steps are ignored or briefly symbolized. As a result, the question must be raised whether the central feature of the diagram—the existence of a bypass around the large pool—is indeed supported by the evidence.

In the first place it is clear that the evidence rules out models of the type

$$\text{External tracer} \rightleftarrows S \rightarrow \text{RNA}$$

where the flow into RNA is precisely that required for growth. Such models are not consistent with an undelayed entry into RNA of a given fraction of the tracer that enters the cell (e.g., fig. 21), nor do they give any explanation of the second phase rise in rate in experiments at high concentration (e.g., fig. 23).

The only alternative that has been proposed that will explain the qualitative features may be represented by diagram 2.

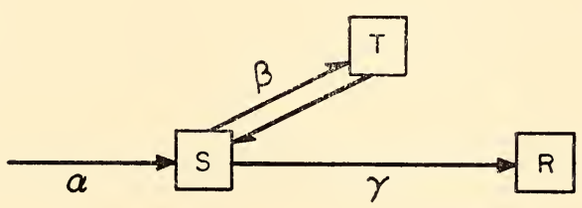

Diagram 2

S represents the nucleotide pool. R represents stable RNA, and the flow to R is exactly that required for growth. T represents an unstable (but TCA-precipitable) RNA. It has been proposed by Jacob and Monod that RNA acting as template for protein synthesis might have the property of rapid synthesis and breakdown. If the flow β due to the turnover of T is very large, T and S will effectively have the same specific radioactivity at any time. Thus a certain fraction (determined by the relative size of T and S) of the radioactivity entering the cell will appear without delay in TCA-precipitable RNA. With the proper choice of S and T, calculations from this model give the experimentally observed curves for any particular base (e.g., guanine). For a different base (e.g., uracil), it is necessary to change the size of both S and T to fit the observations. The sum of S and T determines the time constant listed in table 6, and the ratio $(T/(T + S)$ is the "bypass flow." The size of S may be chosen for each base (in the absence of direct measurements of the nucleotide pool size); the relative quantities of T for the different nucleotides are known from the nucleotide composition of the early-labeled RNA reported below. Further, the time constant for the rise in radioactivity of T, according to this model, is the same as that of the pool S—a particularly useful argument as it is not affected by the interconversion of the nucleotides. Since all other RNA labeling will be delayed (according to this scheme) by the time constant determined by $S + T$, all the observed early-labeled RNA must be considered to be T. With uracil as tracer the time constant of the early-labeled RNA fraction is about 2½ minutes and the time constant listed in table 6 for S is 10 minutes.

Thus, at present there appears to be no alternative to the existence of a bypass around the nucleotide pools. The existence of the bypass does not, of course, rule out the presence, in addition, of a fraction of RNA which turns over by degradation to low-molecular-weight fragments. Equally, the existence of a rapidly labeled fraction of RNA does not prove the existence of turnover by degradation. As described below, it appears that about one-third of the early-labeled RNA fraction does turn over but that the rate of synthesis and degradation is not as rapid as that required for T in schematic diagram 2.

In one sense it is not surprising that the nucleotide pools are bypassed. Their principal function may not lie in their role as nucleic acid precursors. ATP and

GTP presumably function in the energy transport system, and the other compounds presumably play a role in a great variety of reactions.

In last year's discussion of the relationship of the bypass to the mechanism of pool formation it was pointed out that the carrier model for amino acid pools was consistent with the observations of uracil incorporation. Since the incorporation of the other three bases is in essential respects similar to that of uracil, the carrier model gains further support.

RNA Composition

The RNA of bacteria is remarkably invariable in nucleotide composition; the DNA nucleotide composition may vary widely from species to species. There may exist a small fraction of the bacterial RNA having a nucleotide composition like that of the DNA, uracil substituting for thymine. In no case as yet, however, has the composition of such fractions been reported to be identical with that of the DNA. In the present work, five species of bacteria and one of yeast have been examined for any consistent correlation between the RNA and the DNA nucleotide composition in one or more of the RNA fractions that could be isolated. The technique of isotope dilution was used to determine the nucleotide composition of these RNA fractions with the greatest possible accuracy, so that even fairly small differences could be detected among the fractions of a given species.

The bacteria and yeast were grown in the presence of P^{32} orthophosphate for several hours during exponential growth. They were then harvested and were washed three times. The pellet was resuspended, and the cells were broken in the French pressure cell. The cell extract was centrifuged at 105,000g for 2 minutes to remove cell walls and unbroken cells. The supernatant was further centrifuged at 105,000g for 45 minutes to pellet the 70S ribosomes. The pellet was then washed with buffer, resuspended, and repelleted by a further centrifugation for 45 minutes. In this way a purified sample of 70S ribosomes was prepared. *E. coli* ML 30 unlabeled 70S ribosomes were also prepared from one batch of cells by the same method. Using the sucrose density-gradient sedimentation method, 30S and 50S ribosomes derived from the 70S particles were purified (fig. 30).

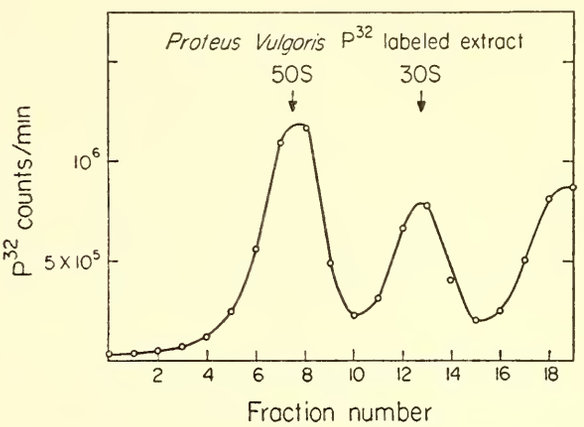

Fig. 30. Sucrose density-gradient sedimentation pattern of P^{32}-labeled 30S and 50S ribosomes of *P. vulgaris*. Sucrose concentration 5 per cent to 20 per cent in TCM/100 buffer. Centrifugation at 37,000 rpm for 160 minutes, 4°C.

S-RNA was purified by further centrifugation of the bacterial extract from which the 70S particles had been removed (240 minutes at 105,000g). After precipitation by 3 volumes of cold 95 per cent ethanol, the S-RNA was dissolved in TCM buffer and was adsorbed on DEAE. S-RNA eluted at 0.5 M NaCl, and any degraded ribosomal RNA not pelleted by centrifugation eluted at 0.8–1.0 M NaCl (fig. 31).

Unfractionated cell RNA was obtained by precipitating labeled cells in cold 5 per cent (w/v) TCA and filtering off the material on Millipore filters.

The composition of the single batch of *E. coli* ML 30 70S RNA used as unlabeled carrier in all subsequent determinations was measured by alkaline hydrolysis of a sample, column chromatography, and summation of the ultraviolet absorbencies. In the digests, approximately 98

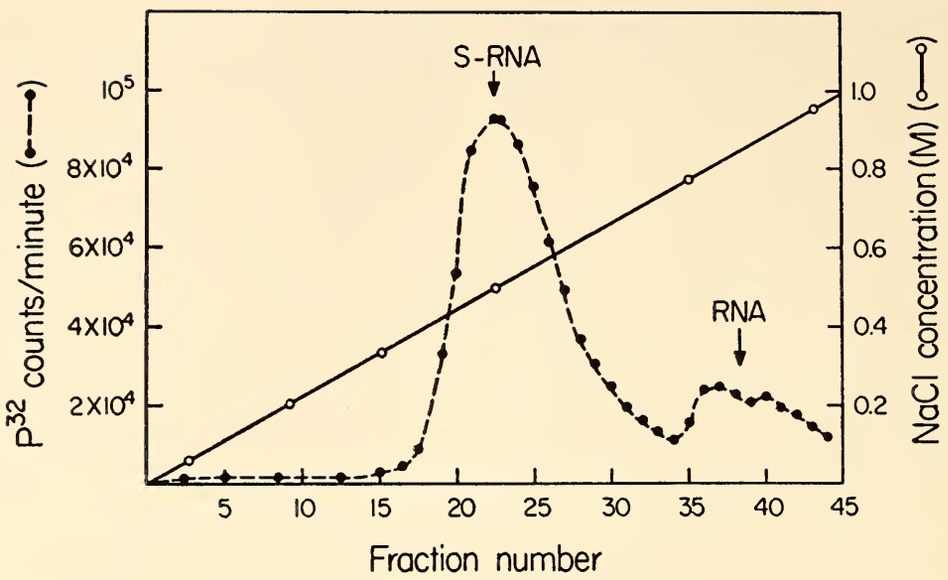

Fig. 31. Elution by NaCl from a DEAE-cellulose column of phenol-treated supernatant of P^{32}-labeled *B. subtilis* cell extract, obtained after centrifugation at 105,000*g* for 240 minutes. Linear gradient of NaCl (0.2 *M* to 1.0 *M*) in TCM buffer.

per cent of the material hydrolyzed was recovered from the column. These measurements were checked against the result obtained by the summation of the P^{32} counts/minute contained in each nucleotide after hydrolysis and column chromatography of a labeled sample of *E. coli* 70S RNA, prepared in the same way.

The possibility of the fractionation of the standard RNA by the phenol procedure was also checked by comparison of the composition determined from phenol-extracted 70S RNA and from TCA-precipitated P^{32}-labeled 70S ribosomes of *E. coli*. No significant differences could be detected.

Table 7 indicates the nucleotide composition of the *E. coli* ML 30 70S RNA as determined by two methods. The results are the mean of several determinations by each method.

Composition of the major fractions. The nucleotide compositions of the unfractionated cell RNA precipitable by cold 5 per cent TCA, the 70S, 50S, and 30S ribosomes, and the S-RNA in the five bacterial species are given in tables 8 to 12. In comparison, the nucleotide composition of the 80S, 60S, and 40S ribosomes of yeast and the S-RNA is given in table 13. The slight differences observed in the 30S and the 50S ribosomes' nucleotide composition in a given species are reproducible to better than 1 per cent. As, in several of the determinations, the compositions of the RNA in the 50S and 30S

TABLE 7. Determinations of the Nucleotide Composition of *Escherichia coli* 70S RNA

Nucleotide	By Summation of P^{32} Counts in Nucleotides, mole %	By Summation of UV Absorbencies of Nucleotides at *p*H 2, mole %
C	21.7	22.0
A	25.2	25.1
G	32.8	32.4
U	20.3	20.5

Several determinations by each of the two methods were carried out. The mean nucleotide composition used in experiments was: C 21.9 mole per cent, A 25.1 mole per cent, G 32.6 mole per cent, U 20.4 mole per cent.

TABLE 8. Compositions of RNA Fractions of *Pseudomonas aeruginosa* ATCC 9027
(DNA composition A = T = 13 mole %, G = C = 32 mole %)

Nucleotide	Total RNA	70S	50S	30S	S-RNA
C	22.2	21.7	21.2	21.6	28.3
A	25.7	25.7	26.3	25.1	20.8
G	31.3	31.6	31.2	32.8	33.8
U	20.8	21.0	21.3	20.5	17.1
$\frac{\text{Purine}}{\text{Pyrimidine}}$	1.33	1.35	1.35	1.36	1.20
$\frac{G + C}{A + U}$	1.15	1.14	1.10	1.19	1.64

TABLE 9. Composition of RNA Fractions of *Aerobacter aerogenes* ATCC 211
(DNA composition A = T = 22 mole %, G = C = 28 mole %)

Nucleotide	Total RNA	70S	50S	30S	S-RNA
C	22.6	21.9	22.0	22.4	29.2
A	25.0	25.5	25.6	25.3	19.7
G	31.7	31.5	31.2	30.8	32.5
U	20.7	21.1	21.2	21.5	18.8
$\frac{\text{Purine}}{\text{Pyrimidine}}$	1.32	1.33	1.32	1.27	1.10
$\frac{G + C}{A + U}$	1.19	1.15	1.14	1.15	1.60

TABLE 10. Composition of RNA Fractions of *Escherichia coli ML* 30
(DNA composition A = T = 24 mole %, G = C = 26 mole %)

Nucleotide	Total RNA	70S	50S	30S	S-RNA
C	22.1	21.9	21.5	22.7	29.5
A	25.2	25.1	25.4	24.8	19.7
G	32.5	32.6	33.5	31.0	33.8
U	20.2	20.4	19.6	21.5	17.0
$\frac{\text{Purine}}{\text{Pyrimidine}}$	1.37	1.36	1.44	1.26	1.17
$\frac{G + C}{A + U}$	1.20	1.20	1.22	1.16	1.71

particles differ in individual nucleotides by as much as 10 to 15 per cent in a single species, the differences are probably real. Neither the unfractionated cell RNA nor the 70S RNA nor the S-RNA was found to have a definite correlation with the DNA for any species. In fact, the compositions of these fractions in the five bacterial species are all invariable within the limits of the experimental error of determination. Yeast has a ribosomal RNA and total cell RNA nucleotide composition basically unlike that of bacteria. If there is in these fractions an RNA with a composition like that of the DNA, the accuracy of measurement by

TABLE 11. Composition of RNA Fractions of *Bacillus subtilis* ATCC 6051
(DNA Composition A = T = 29 mole %, G = C = 21 mole %)

Nucleotide	Total RNA	70S	50S	30S	S-RNA
C	22.1	22.3	22.5	22.3	28.3
A	25.5	25.9	26.5	26.5	20.2
G	31.4	31.0	32.0	29.6	33.9
U	21.0	20.8	19.3	21.6	17.6
$\frac{\text{Purine}}{\text{Pyrimidine}}$	1.32	1.32	1.39	1.28	1.17
$\frac{G + C}{A + U}$	1.17	1.15	1.20	1.08	1.65

TABLE 12. Composition of RNA Fractions of *Proteus vulgaris* ATCC 4669
(DNA composition A = T = 31 mole %, G = C = 19 mole %)

Nucleotide	Total RNA	70S	50S	30S	S-RNA
C	22.6	21.7	21.3	23.0	29.3
A	24.6	26.2	26.5	24.7	19.1
G	32.0	31.4	31.4	31.9	33.3
U	20.8	20.7	20.8	20.4	18.3
$\frac{\text{Purine}}{\text{Pyrimidine}}$	1.30	1.35	1.37	1.30	1.11
$\frac{G + C}{A + U}$	1.21	1.13	1.11	1.22	1.67

TABLE 13. Composition of RNA Fractions of *Saccharomyces cerevisiae*
(DNA composition A = T = 32 mole %, G = C = 18 mole %)

Nucleotide	Total RNA	80S	60S	40S	S-RNA
C	19.4	19.2	19.0	19.1	26.3
A	26.8	27.2	27.9	25.2	19.2
G	28.3	28.2	28.4	28.4	34.3
U	25.5	25.4	24.7	27.3	20.2
$\frac{\text{Purine}}{\text{Pyrimidine}}$	1.23	1.24	1.29	1.15	1.15
$\frac{G + C}{A + U}$	0.91	0.90	0.90	0.91	1.55

the isotope dilution technique cannot permit it to be more than 10 per cent of the RNA.

The composition of the 14S RNA fraction. It has been established that the first detectable labeled polynucleotide material formed during the incorporation of P^{32} or C^{14}-uracil into bacterial RNA has different sedimentational and chromatographic properties from the RNA detectable by ultraviolet absorption. It has also been found that most of the C^{14}-uracil that is incorporated into this fraction is eventually incorporated into the RNA of the ribosomes. This fraction is termed the "eosome" or 14S component. As this

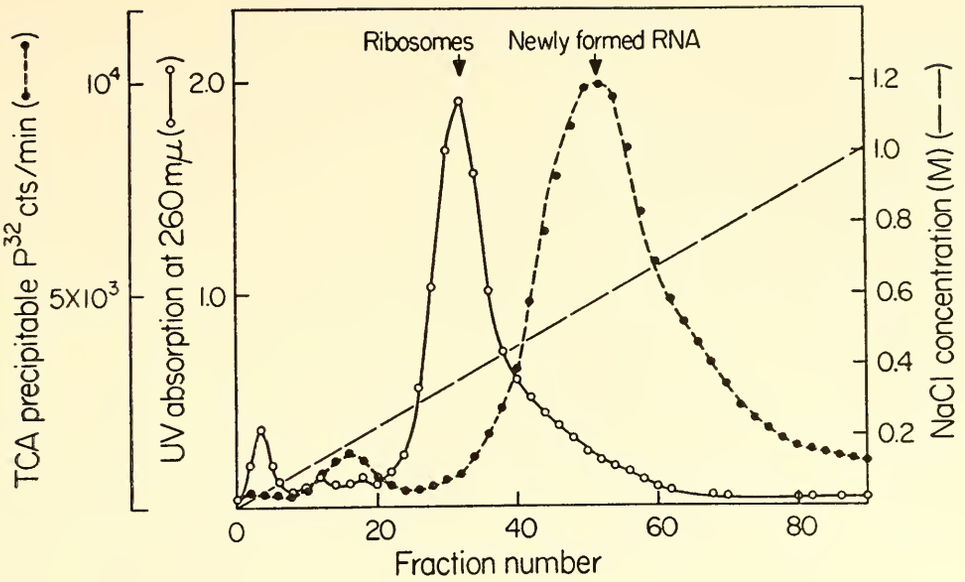

Fig. 32. Elution by NaCl from a DEAE-cellulose column of a cell extract from an *E. coli* culture labeled for 3 minutes by P^{32}. Linear NaCl gradient (0.2 *M* to 1.0 *M*) in TCM buffer.

material accounts for effectively all the P^{32}-labeled RNA present in short periods of isotope incorporation, its nucleotide composition should be similar to that of unfractionated cells at these times.

The five species of bacteria used in the bulk RNA studies above were exposed to short periods of P^{32}-orthophosphate incorporation during exponential growth. The cells were then squirted into 10 per cent cold TCA and filtered on Millipore filters. Many washes of TCA were given to remove most of the 5′-nucleotides on the filter. From an aliquot of cells that had been poured onto crushed ice rather than into TCA, 14S RNA was then isolated. Analyses of the pulse-labeled RNA in the five species are given in table 14.

The extracts from the cells poured onto crushed ice were adsorbed on DEAE and eluted by a linear NaCl gradient of 0.2 *M* to 1.0 *M* NaCl in TCM buffer. Figure 32 shows a typical elution pattern. It can be seen that only one labeled component, not tracking with any of the ultraviolet-absorbing material, elutes at 0.6 *M* NaCl. This material was pooled, TCA-precipitated, and collected by filtration. Analysis of the filters gave the compositions listed in table 15. In each of the species examined, the base composition of the 14S or "eosome" obtained in this way is identical within experimental error to that of the total cell-labeled RNA at this time.

A culture of *B. subtilis* was given a 3-minute labeling period with P^{32} during exponential growth. The nucleotide com-

TABLE 14. Composition of Labeled RNA Formed during Short Exposure of Bacteria to P^{32} Orthophosphate

Species	Time of Labeling with Isotope, minutes	Labeled RNA Composition, TCA-precipitable, mole %				$\frac{G+C}{A+U}$
		C	A	G	U	
Ps. aeruginosa	4	25.4	21.1	31.9	21.6	1.34
A. aerogenes	4	23.4	24.8	30.3	21.5	1.16
E. coli	2	22.9	25.0	29.5	22.6	1.10
B. subtilis	2	23.3	25.6	27.7	23.4	1.04
P. vulgaris	4	22.2	26.7	27.0	24.1	0.97

TABLE 15. Composition of the 14S (Eosome) RNA Component of Bacteria Purified by DEAE Chromatography

Species	Time of Labeling with Isotope, minutes	14S RNA Composition				G + C / A + U
		C	A	G	U	
Ps. aeruginosa	4	25.6	20.8	31.7	21.9	1.31
E. coli	2	22.7	25.1	29.1	23.1	1.07
B. subtilis	2	22.5	25.3	28.0	24.2	1.02
P. vulgaris	4	21.9	27.0	27.6	23.5	0.98

position of the total cell-labeled RNA was measured, and a sample of the cell juice was treated with phenol; after alcohol precipitation, and dissolving the RNA in TCM buffer, it was then adsorbed on a methylated serum albumin-coated kieselguhr column. The RNA was eluted by a linear gradient of NaCl from 0.4 to 1.1 *M* in 0.04 *M* phosphate buffer, *p*H 6.7. The elution pattern is shown in figure 33.

The labeled RNA does not track exactly with the 16S and 23S RNA produced from the bulk of the RNA components of the cell. There are three radioactive peaks, but analysis of each showed that there was no difference in base compositions of any one peak from the composition of the material eluted at 0.6 *M* NaCl from DEAE or from the total cell-labeled RNA at this time. It is evident that under these conditions no further fractionation of the newly formed RNA labeled with P^{32} has been achieved.

The 14S component of *E. coli* labeled for 3 minutes by P^{32} was isolated by sucrose density-gradient centrifugation in the swinging bucket. After centrifugation at 37,000 rpm for 160 minutes a peak sedimenting at about 14S was clearly resolved by its radioactivity (fig. 34). This peak was collected and TCA-

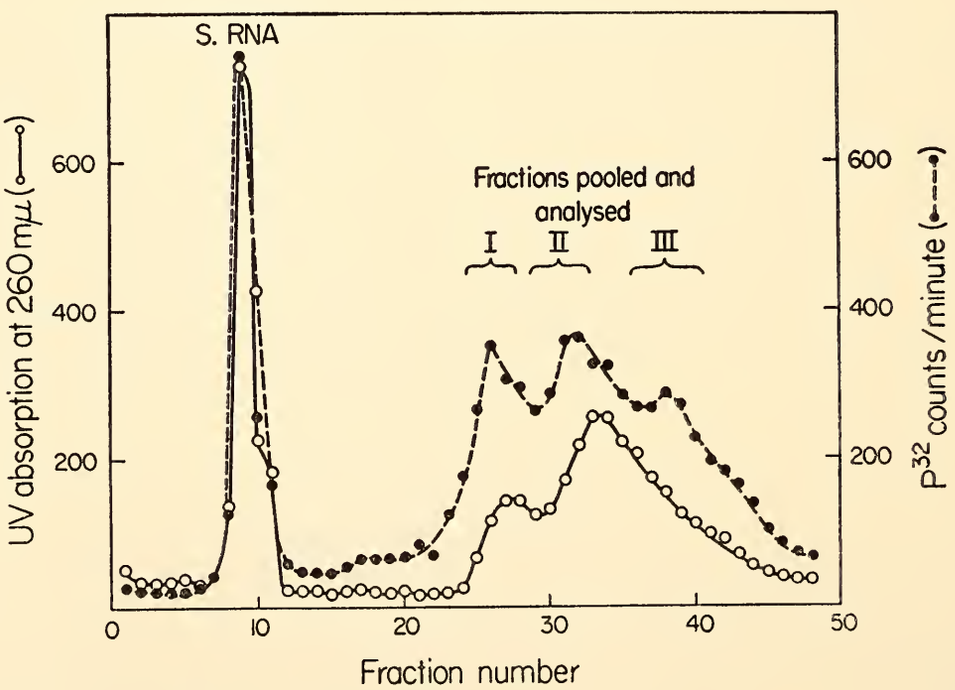

Fig. 33. Elution by NaCl from a methylated serum albumin-coated kieselguhr column of a cell extract from an *E. coli* culture labeled for 3 minutes by P^{32}. The cell extract was treated with phenol to remove protein from the ribosomes before adsorption on the column. Linear gradient of NaCl (0.4 *M* to 1.1 *M*) in 0.04 *M* potassium phosphate buffer, *p*H 6.7.

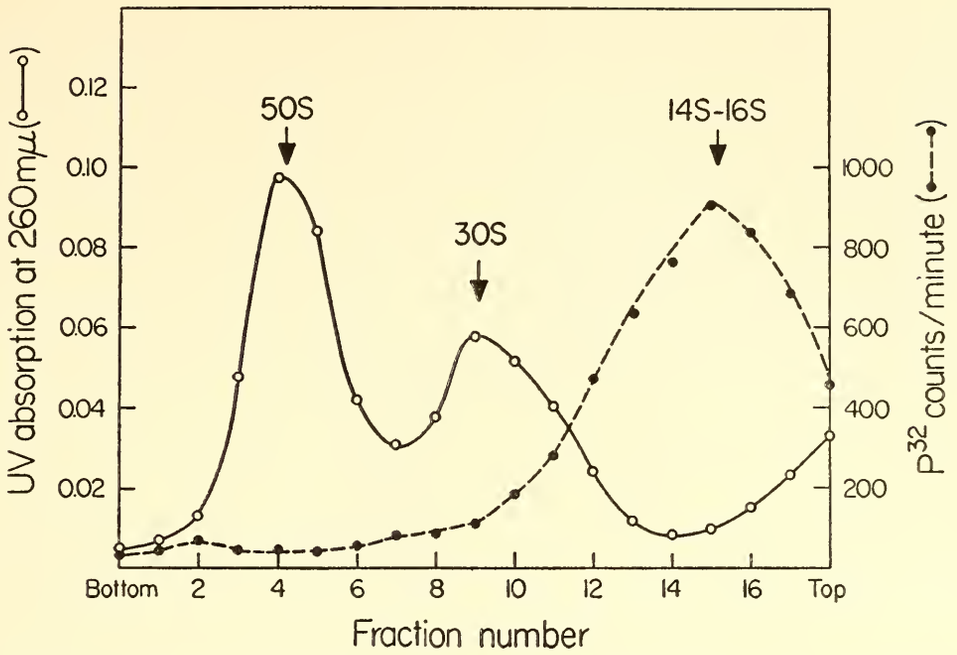

Fig. 34. Sucrose density-gradient sedimentation of a cell extract from *P. vulgaris* labeled for 3 minutes by P^{32}. Sucrose concentration 5 to 20 per cent in TCM/100 buffer. Centrifugation at 37,000 rpm for 160 minutes, 4°C.

precipitated. Its analysis showed that it was identical to the total cell-labeled RNA at this time, and to the material eluted from DEAE at 0.6 *M* NaCl. This would indicate that the eosome or 14S RNA can be isolated as a discrete object without measurable change in nucleotide composition and that column chromatography, either by the Mandell and Hershey column of phenol-treated RNA, or by DEAE of untreated cell extracts, does not result in the isolation of newly formed RNA with a nucleotide composition any different from that obtained by TCA precipitation of unfractionated labeled cells.

The analyses of the bulk RNA components in the five species of bacteria used indicate no obvious relationship in the nucleotide composition of the various purified RNA fractions to the DNA. The composition of the RNA comprising most of this material in the cells, the 70S ribosomes, is remarkably constant from species to species. The S-RNA also appears to be constant in composition (fig. 35).

Subfractionation of the 70S component of bacteria into 50S and 30S or of yeast 80S into 60S and 40S has brought to light some differences in nucleotide composition of the two fractions. In general,

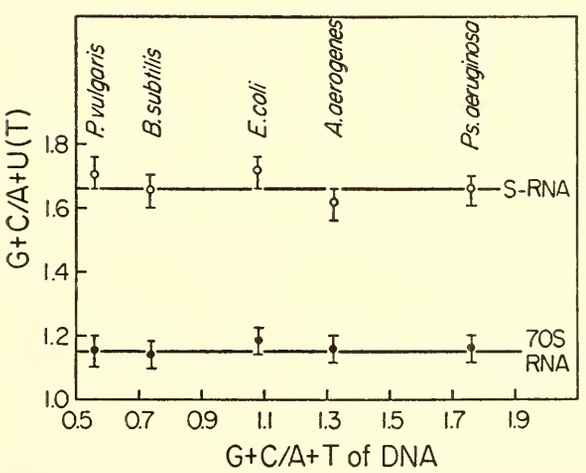

Fig. 35. Comparison of G + C/A + U values for S-RNA and 70S RNA, from bacteria with DNA G + C/A + T values ranging from 0.6 to 1.78.

purine contents are higher, pyrimidines lower, in the larger (50S or 60S) than in the smaller (30S or 40S) ribosomal subunits. There is, however, no uniformly

consistent relationship in composition between the DNA, and either 30S or 50S, in the bacterial species.

The 14S fraction has been found to be very different in nucleotide composition from the normal total TCA-precipitable RNA in the cell or from the ribosomes. Figures 36 and 37 indicate a possible relationship between the DNA composition of the bacteria and the 14S RNA and 70S RNA nucleotide compositions in each of the five species. It can be seen (fig. 36) that, if the 14S fraction were in fact composed of two entities of RNA with different nucleotide compositions corresponding to either the DNA or the ribosomal RNA, then in each case the 14S RNA would be made up of approximately 33 per cent DNA-like and 67 per cent ribosomal RNA-like material.

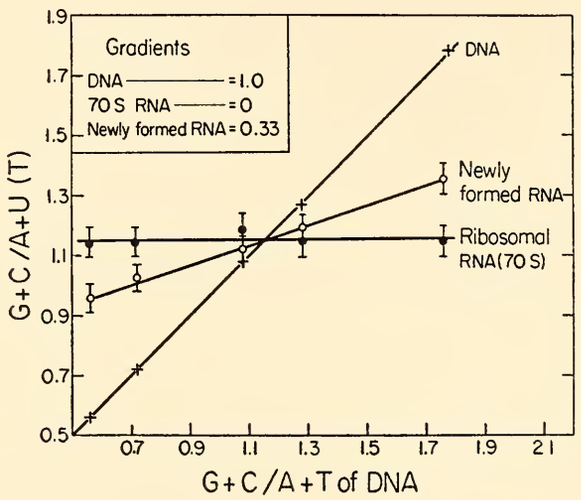

Fig. 36. Comparison of G + C/A + U (T) values for 70S ribosomal RNA, 14S RNA, and DNA from bacteria with DNA G + C/A + T values ranging from 0.6 to 1.78.

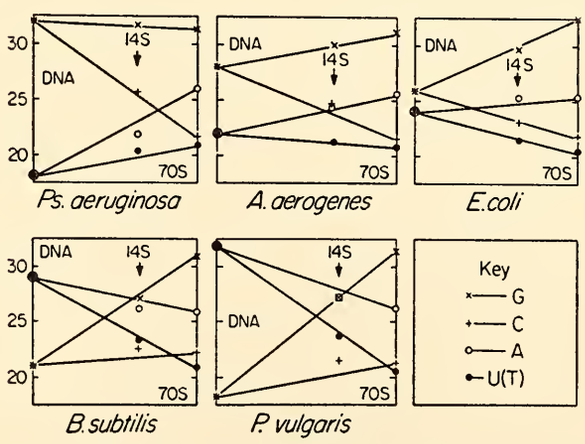

Fig. 37. Graphical representation of nucleotide composition of DNA, 14S RNA, and 70S ribosomal RNA in each of the five bacterial species used. Ordinate, nucleotide composition (mole per cent). On the left ordinate of each graph, DNA base composition; on the right ordinate, 70S RNA base composition. 14S RNA composition is best fit between these two compositions.

Kinetic Studies of RNA Composition

Published studies of rapidly labeled RNA contain two conflicting sets of observations. On the one hand it is evident from kinetic studies of the flow of P^{32}-orthophosphate or C^{14}-uracil in and out of this fraction that it can be considered predominantly as a precursor to the ribosomal RNA. On the other hand, analyses of the pulse-labeled total RNA and the purified 14S fraction indicate an apparent nucleotide composition intermediate between that of ribosomal RNA and the bacterial DNA. Consequently, depending on the type of observations made, the rapidly labeled fraction has been described as mostly ribosome precursor or mostly the "messenger" or informational RNA postulated to be necessary for the genetically directed synthesis of specific proteins in ribosomes.

It therefore seemed possible that the 14S RNA fraction contained molecules of different compositions and functions. The present study was undertaken to correlate kinetic and composition measurements and to determine the rate of synthesis of D-RNA. The term DNA-like RNA or D-RNA has been used when the polynucleotide composition is observed; template RNA designates RNA observed to act as template; and messenger RNA describes RNA shown to have all the properties postulated by Jacob and Monod.

The nucleotide composition of labeled RNA formed after various times of exposure to P^{32} has been measured for each of five species of bacteria. In each

case P^{32} was added to the bacteria growing exponentially in the low-phosphorus-tris medium. Growth and incorporation were terminated in samples taken at intervals by adding them to an equal volume of 10 per cent TCA. Since the method of nucleotide analysis itself represented an adequate purification of the 2′,3′-nucleotides, no attempt was made to remove other macromolecules from the RNA. Likewise, DNA contamination is of no account, since DNA is unaffected by the alkaline hydrolysis conditions. On the other hand, labeled 5′-nucleotides from the TCA-soluble pool are serious contaminants. For all but very brief exposures to P^{32} it was found that 5′-nucleotide contamination could be eliminated by filtration of the TCA precipitate through membrane filters followed by multiple washes with 5 per cent TCA. After washing, the filters were dried and kept frozen until hydrolyzed. In this way nucleotide compositions of the pulse-labeled part of the total RNA could be measured without the possibility of fractionation or selective degradation during purification steps.

For samples taken after exposures to P^{32} for a period shorter than 1 per cent of the generation time this technique proved insufficient. In this time range the specific radioactivity of the 5′-nucleotides may be a thousand times that of the 2′,3′-nucleotides, and small amounts of soluble material can seriously contaminate the nucleotide peaks in the analysis. Therefore, very early samples were chilled rapidly by addition to crushed, frozen medium and washed in cold tris magnesium buffer. The frozen cells were disrupted in the French pressure cell, and the effluent was added directly to phenol at 37°C. The RNA was then purified by means of a second phenol extraction and two alcohol precipitations. The final alcohol precipitate was taken up in buffer and made to 5 per cent with TCA. Filtration and washing on the filter completed the purification. In this way samples of labeled RNA could be analyzed from cells given P^{32} for periods as short as 10 seconds.

Tables 16 to 20 contain the results of these nucleotide analyses of RNA after P^{32} exposures of 10 seconds to several hours. They also compare the G + C/A + T ratio of the DNA (which varies from 0.61 to 1.78 among the five species) with the G + C/A + U ratio of the newly formed RNA. Assuming that the newly formed RNA is a mixture of two types, comparison of the G + C/A + U ratio with that for the bacterial DNA and the ribosomal RNA (G + C/A + U = 1.15) or the total RNA (G + C/A + U = 1.20) gives a measure of the amount of labeled RNA in the sample having a nucleotide composition resembling DNA. Such a comparison is, of course, most useful in

TABLE 16. Nucleotide Composition of Newly Formed RNA in *Proteus vulgaris*, mole %

Time of Labeling with Isotope, minutes	T_e*	C	A	G	U(T)	$\frac{G+C}{A+U}$
0.25	0.0019	23.5	26.4	26.6	23.5	1.00
1	0.0076	23.5	27.3	26.4	22.9	1.00
2	0.915	23.5	27.1	26.7	22.7	1.01
4	0.030	22.7	26.7	28.1	22.5	1.03
8	0.061	22.9	26.3	28.7	22.1	1.07
16	0.106	23.0	25.6	30.6	20.8	1.16
20	0.152	22.5	24.9	31.6	21.0	1.18
40	0.304	22.4	24.8	31.5	21.3	1.17
300	2.72	22.6	24.6	32.0	20.8	1.21
DNA		19.0	31.0	19.0	31.0	0.61

* Fraction of eth time (see text).

TABLE 17. Nucleotide Composition of Newly Formed RNA in *Bacillus subtilis*, mole %

Time of Labeling with Isotope, minutes	T_e	C	A	G	U(T)	$\frac{G+C}{A+U}$
0.17	0.0023	23.5	25.5	27.3	23.7	1.03
0.5	0.0069	24.0	25.1	27.9	23.0	1.08
1	0.014	22.8	25.5	27.2	24.5	1.00
2	0.028	23.3	25.6	27.7	23.4	1.04
4	0.055	23.7	26.2	28.0	22.1	1.07
8	0.100	22.5	26.2	29.3	22.0	1.08
14	0.193	22.1	25.5	31.5	20.9	1.15
28	0.386	21.6	26.0	31.7	20.7	1.14
360	4.96	22.1	25.5	31.4	21.0	1.15
DNA		21.0	29.0	21.0	29.0	0.72

TABLE 18. Nucleotide Composition of Newly Formed RNA in *Escherichia coli*, mole %

Time of Labeling with Isotope, minutes	T_e	C	A	G	U(T)	$\frac{G+C}{A+U}$
0.17	0.0023	22.8	25.1	28.8	23.3	1.11
0.5	0.0069	23.2	25.3	29.8	21.7	1.13
1	0.014	23.4	24.8	30.1	21.7	1.14
2	0.028	22.9	25.0	29.5	22.6	1.10
5	0.069	22.4	26.0	29.9	21.7	1.10
10	0.138	22.0	25.1	30.9	22.0	1.12
20	0.276	20.9	25.6	32.5	21.0	1.14
30	0.414	21.4	25.8	33.3	19.5	1.20
50	0.690	21.6	25.5	32.8	20.1	1.18
360	4.96	21.9	25.1	32.6	20.4	1.08
DNA		26.0	24.0	26.0	24.0	

species in which the compositions of the DNA and the ribosomal RNA are very different, i.e., *P. vulgaris*, *B. subtilis*, and *Ps. aeruginosa*. Even in species in which these differences are small, i.e., *E. coli* and *Aerobacter aerogenes*, the very early-labeled RNA and the total RNA are not identical.

Two features are immediately clear from examination of the data in tables 16 to 20. In each bacterial species, the composition of the RNA at the earliest times is that which would result from a mixture of 30–40 per cent having a DNA-like composition and 60–70 per cent ribosomal. Moreover, the change in base composition or G + C/A + U ratio is not an especially rapid one. In general there is little difference among the first three or four analyses representing times from about 0.3 per cent to about 4 per cent of a generation time. If the nucleotide composition represents the weighted mean between different amounts of two different types of RNA molecules, the relative amounts of radioactivity in the two different molecules present do not change during the first 5 per cent or so of the generation time. There is no indication of an extremely rapidly labeled component. Analyses made during the period between 5 and 20 per cent of a generation time show a change in composition toward that of ribosomal RNA. At times greater than 20 per cent of a generation time, the nucleotide composition of the labeled fraction is indistinguishable from that of the total RNA.

Fractionation of pulse-labeled RNA. The early-labeled RNA can be separated into two fractions of different composition by differential dissociation from ribosomes. The pulse-labeled cells of the four bacterial species shown in table 21 were prepared by exposure for 3 minutes to P^{32}. After chilling and washing, the cells were broken in tris buffer containing 10^{-2} *M* $MgCl_2$. After removal of cell debris by a short centrifugation (2 minutes at 105,000*g*) most of the ribosomal material and about 90 per cent of the labeled RNA were pelleted by means of a 45-minute centrifugation at 40,000 rpm. The ribosome pellet (2–5 mg) and the interior of the centrifuge tube were rinsed with distilled water at 2°C to remove all traces of the buffer. The pellet was then resuspended in distilled water and centrifuged at 40,000 rpm for 120 minutes. The magnesium concentration resulting largely from the bound ribosomal ions can be estimated at $2–4 \times 10^{-4}$ *M*.

The top half of the supernatant was removed, TCA-precipitated, and filtered. The pellet was also resuspended, precipitated, and filtered. Table 21 contains the results of the nucleotide analyses of the various fractions together with the fraction of labeled macromolecules recovered. For *E. coli* the results are the mean of six different experiments; the other results are the mean of two experiments.

The separation technique is most successful for *E. coli* material. Very little of the pulse-labeled RNA was degraded during the procedure, and the fractions obtained had nucleotide compositions very close to those of pure ribosomal

TABLE 19. Nucleotide Composition of Newly Formed RNA in *Aerobacter aerogenes*, mole %

Time of Labeling with Isotope, minutes	T_e	C	A	G	U(T)	$\frac{G+C}{A+U}$
1	0.012	23.5	23.4	32.0*	21.1	1.25
2	0.023	24.1	24.5	30.2	21.2	1.19
4	0.046	23.4	24.8	30.3	21.5	1.17
8	0.092	22.8	24.9	30.7	21.6	1.17
14	0.161	22.1	25.4	31.4	21.1	1.15
20	0.230	21.9	25.7	31.2	21.2	1.16
40	0.460	21.9	25.7	31.5	20.9	1.13
360	4.14	22.0	25.6	31.7	20.7	1.16
DNA		28.0	22.0	28.0	22.0	1.27

* The measurement of G in the sample labeled for 1 minute is inaccurate, owing to contamination by orthophosphate.

TABLE 20. Nucleotide Composition of Newly Formed RNA in *Pseudomonas aeruginosa*, mole %

Time of Labeling with Isotope, minutes	T_e	C	A	G	U(T)	$\frac{G+C}{A+U}$
0.25	0.0015	26.2	21.4	31.9	20.5	1.39
2	0.012	25.3	21.6	32.3	20.8	1.36
4	0.025	25.4	21.1	31.9	21.6	1.34
8	0.050	25.8	20.8	31.6	21.8	1.35
14	0.086	26.1	21.8	31.0	21.1	1.33
20	0.126	24.7	22.0	31.7	21.6	1.29
40	0.252	23.9	23.5	32.0	20.6	1.27
60	0.388	22.6	25.5	31.2	20.7	1.16
360	0.207	22.2	25.7	31.3	20.8	1.15
DNA		32.0	18.0	32.0	18.0	1.78

TABLE 21. Fractionation of 3-Minute P^{32} Pulse-Labeled RNA by Water Treatment

$\frac{G+C}{A+U}$ in DNA	*Ps. aeruginosa* 1.75		*A. aerogenes* 1.27		*E. coli* 1.08		*P. vulgaris* 0.61	
	SN	Ppt.	SN	Ppt.	SN	Ppt.	SN	Ppt.
C	28.4	22.0	---	21.9	25.2	21.8	---	22.1
A	20.8	25.5	---	25.6	24.4	25.0	---	25.5
G	31.2	31.6	---	31.6	26.9	32.6	---	31.8
U	19.6	20.9	---	20.9	23.5	20.6	---	20.6
$\frac{G+C}{A+U}$	1.47	1.15		1.14	1.09	1.19		1.17
Per cent P^{32}	52	40	5	46	30	70	12	34
Per cent P^{32} RNA recovered	92		51		100		46	

RNA and of *E. coli* DNA. Distortions of the real nucleotide composition by a combination of unequal labeling of pool nucleotides and nonrandomness in DNA sequences are not apparent in the measured composition of the D-RNA. Apparently the "water-shock" treatment causes the DNA-like fraction to become dissociated from the ribosomes, leaving behind the labeled RNA which resembles ribosomal RNA. Examination of water-treated 70S ribosomes in the analytical ultracentrifuge shows that the ribosomes have been dissociated into two fractions of approximately 60S and 10S, unlike the usual dissociation into 50S and 30S ribosomes. The supernatant fraction from both *E. coli* and *Ps. aeruginosa* appears to be quite similar to the DNA in composition.

Unfortunately, the treatment results in the degradation of a large proportion of the pulse-labeled RNA in both *P. vulgaris* and *A. aerogenes* presumably due to RNase liberation. Even there, however, it is evident that the fraction remaining with the ribosomes is purely ribosomal in base composition. The degradation, therefore, appears to be selective, the DNA-like fraction being preferentially destroyed. Again the rapidly labeled fractions appear to contain two types of molecule. Sucrose density-gradient sedimentation of both water-shock supernatant and pelleted labeled RNA in the presence of 10^{-4} *M* Mg showed similar broad peaks of radioactivity with a 14–16S maximum.

Kinetic studies of RNA synthesis with P^{32}. The delay in incorporation of P^{32} into RNA brought about by the large pool of TCA-soluble RNA precursors prevents direct correlation of base composition

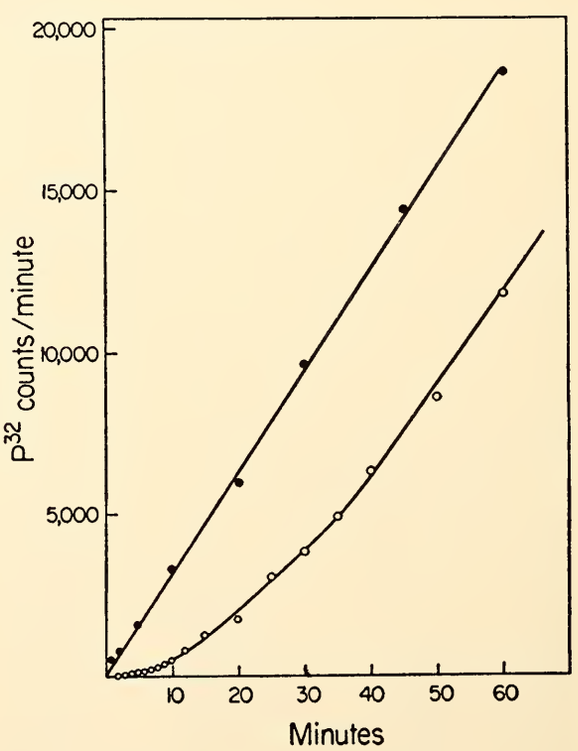

Fig. 38. The incorporation of P^{32} as orthophosphate into exponentially growing *Proteus vulgaris*.

changes (tables 16–20) with studies of the flow of C^{14}-uracil into ribosomes. To make such a comparison, four samples of *P. vulgaris* extracts were prepared after various exposures to P^{32}. Sedimentation analysis on sucrose gradients allowed measurement of the fraction of labeled RNA present in the various species of ribosomes and precursors. In addition, the rate of uptake of P^{32} into the total RNA gave a measure of the TCA-soluble pool.

Figure 38 shows the incorporation curve of P^{32} into *P. vulgaris*. The data were obtained from samples of whole cells and TCA-extracted cells filtered through membrane filters. In addition, some samples at late times were extracted with hot ethanol to make corrections for the lipide phosphorus. The size of the phos-

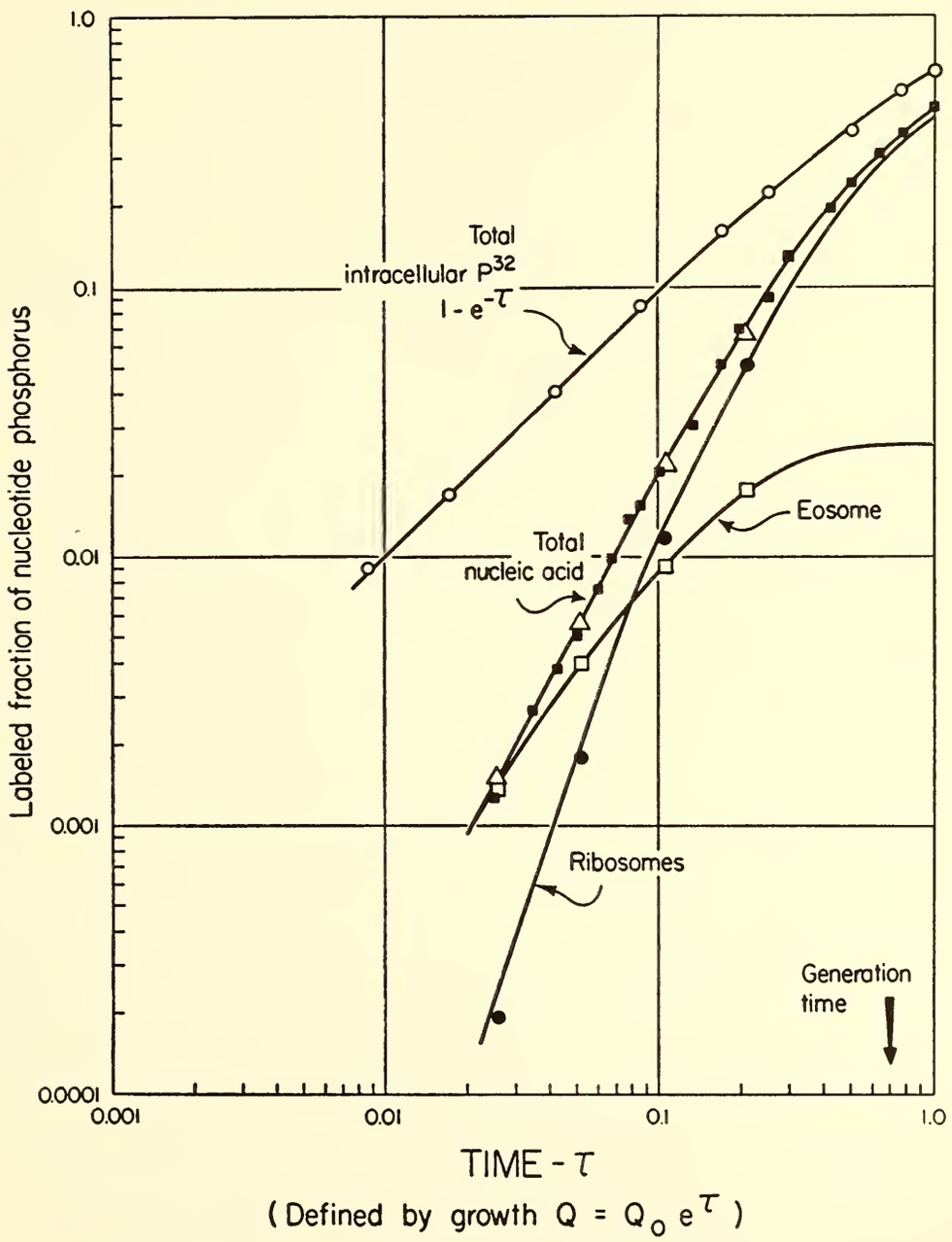

Fig. 39. Log-log plot of the time course of the labeling of the RNA of *Proteus vulgaris*. Labeled fraction of nucleotide phosphorus for the total cell and total nucleic acid, data from figure 38; for the total nucleic acid, 14S eosome fraction, and total ribosomal material, data from figure 40. The lines drawn were calculated from the equations for the case where the phosphorus precursor pool is 16 per cent of the total nucleotide phosphorus and the 14S fraction is 2.7 per cent of the total RNA (see text).

phorus pool was estimated by the method of kinetic analysis described in *Year Book 60*. Times were converted to τ, where $Q = Q_0 e^{\tau}$ gives the growth of the cells or any component. Normalization of the data so that the labeled fraction of nucleotide phosphorus fitted $1 - e^{-\tau}$ gave the top curve in figure 39. Similar treatment of the data for uptake of P^{32} into total RNA gave the second curve, from which the size of the phosphorus pool can be obtained. The phosphorus pool is, of course, complex, containing mono-, di-, and triphosphates of the various nucleotides, and inorganic phosphate. Moreover, there are a great number of reactions

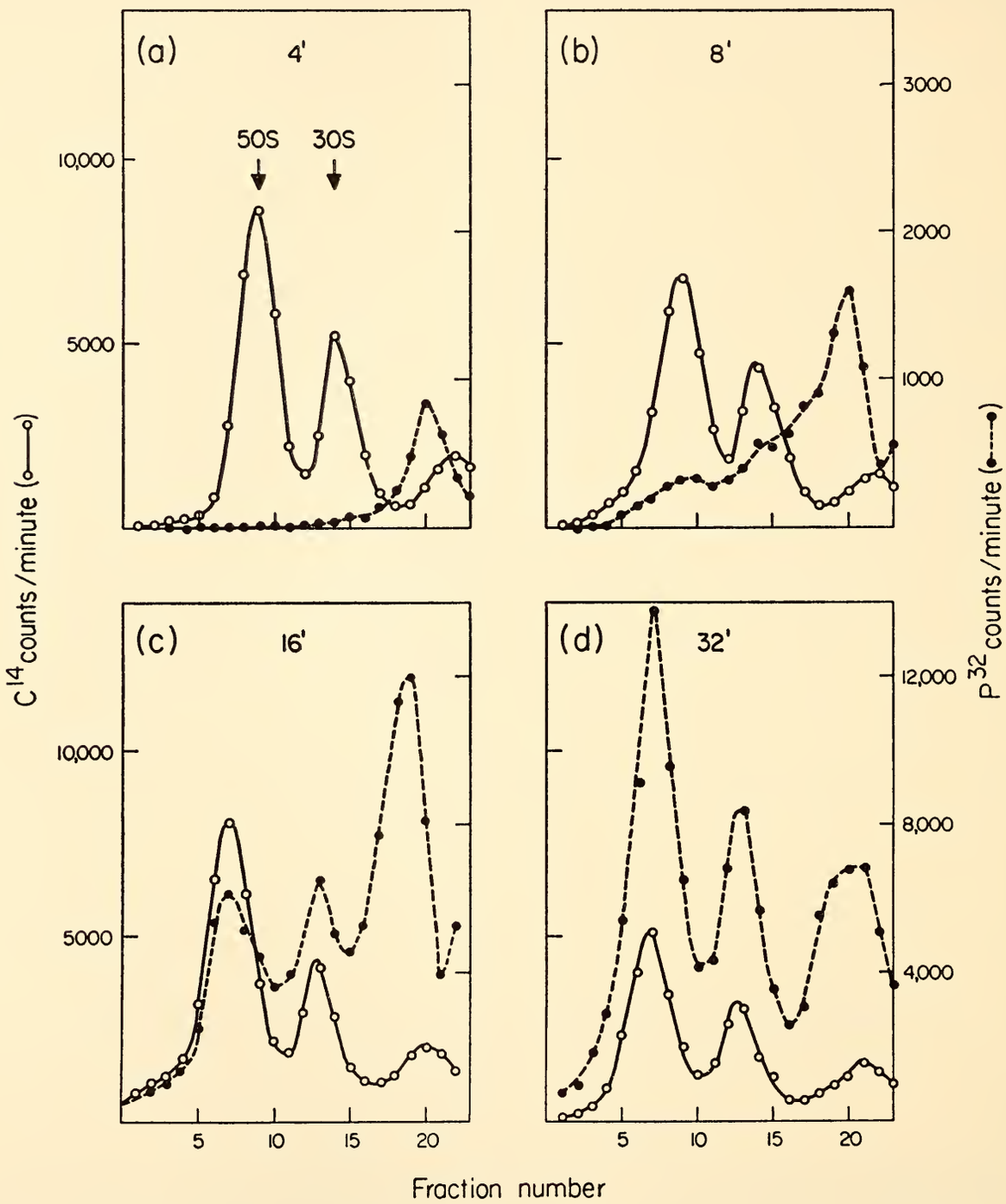

Fig. 40. Sedimentation analysis of four total cell extracts from *Proteus vulgaris* labeled for three generations with C^{14}-uracil and given (*a*) a 4-minute exposure to $P^{32}O_4^{\equiv}$, (*b*) 8 minutes, (*c*) 16 minutes, (*d*) 32 minutes. Extracts prepared from about 0.5 mg dry weight of cells in tris-HCl, 0.01 *M*, *p*H 7.4, $MgCl_2$ 10^{-4} *M*. Centrifugation 150 minutes at 37,000 rpm, 4°C.

among the various components, and a part of the phosphorus is destined for lipide synthesis. Nevertheless, it behaves for kinetic purposes like a single delay pool. The size of the pool thus measured is the ratio of the phosphorus in these small molecules to the total phosphorus in nucleic acid and the precursor molecules. The above procedure gave 0.16 for the size of the phosphorus precursor pool in *P. vulgaris*. (Total phosphorus in nucleic acid and precursors = 1.) The curve drawn through the points in figure 39 was calculated using this value.

Four samples of *P. vulgaris* extracts were prepared after growth for two generations in C^{14}-uracil and 4, 8, 16, and 32 minutes' exposure to P^{32}. The extracts were fractionated by sedimentation, and the fractions were assayed for TCA-precipitable C^{14} and P^{32} (fig. 40). Such pulse-labeling experiments are the inverse of those already described in detail in which P^{32} was used as the steady label and C^{14} as the pulse label. The labeled fraction of the nucleotide phosphorus was computed for the total RNA, the rapidly labeled 14S fraction, and the bulk 30S and 50S ribosomes, and was plotted in figure 39. The points for the total RNA fit the total incorporation curve already described. The other two sets of points fit well to theoretical curves calculated for diagram 3.

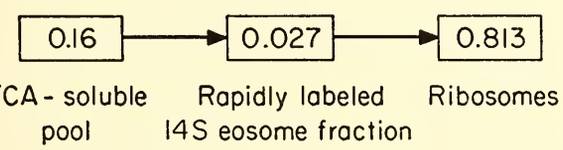

Diagram 3

The quantity of the rapidly labeled fraction has been assumed to be equal to 2.7 per cent, the best estimate from experiments with *E. coli*. It should be pointed out, however, that the curves are relatively insensitive to the magnitude of this number except at late times.

It is apparent then that the kinetics of P^{32} labeling of the rapidly labeled fraction are consistent with a precursor product relationship between the 14S fraction and ribosomes. These kinetic studies with a different organism and a different labeling scheme are in agreement with the *E. coli* studies already published, after the inclusion of the 16 per cent TCA-soluble pool.

Correlation of nucleotide compositions with kinetic data. The sedimentation analyses of figure 40 give a measure of the changing distribution of P^{32} among the various RNA-containing components of the cell. It is therefore possible to reexamine the measurements of the base composition of the total RNA in terms of the relative amounts of ribosomes and 14S component present. A juxtaposition of the data of table 16 and figure 40 is shown in figure 41.

The time course of the G + C/A + U ratio is plotted on a scale running from 1.00 (the average of the earliest determinations) to 1.22, the value for total RNA of *P. vulgaris* after long periods of labeling (table 16). Since the value for DNA or pure D-RNA on this scale is 0.61, the origin, representing the zero time composition, would correspond to 36 per cent D-RNA and 64 per cent R-RNA. The fraction of the total P^{32}-labeled RNA present in ribosomes, i.e., all counts sedimenting at 30S or greater, was computed for each time point in figure 40. These fractions are plotted in figure 41 on a scale running from zero to 0.8, since the remaining 20 per cent of the RNA is S-RNA. Finally, the expected function for the change of the fraction of the label present in ribosomes was calculated from the theoretical curves already plotted in figure 40.

At early times, while all the P^{32} radioactivity is present in the 14S fraction, the nucleotide composition remains essentially constant. Changes in composition toward that of the total RNA begin at the time when label first enters ribosomes. Thus the change in G + C/A + U can be accounted for by the increasing relative

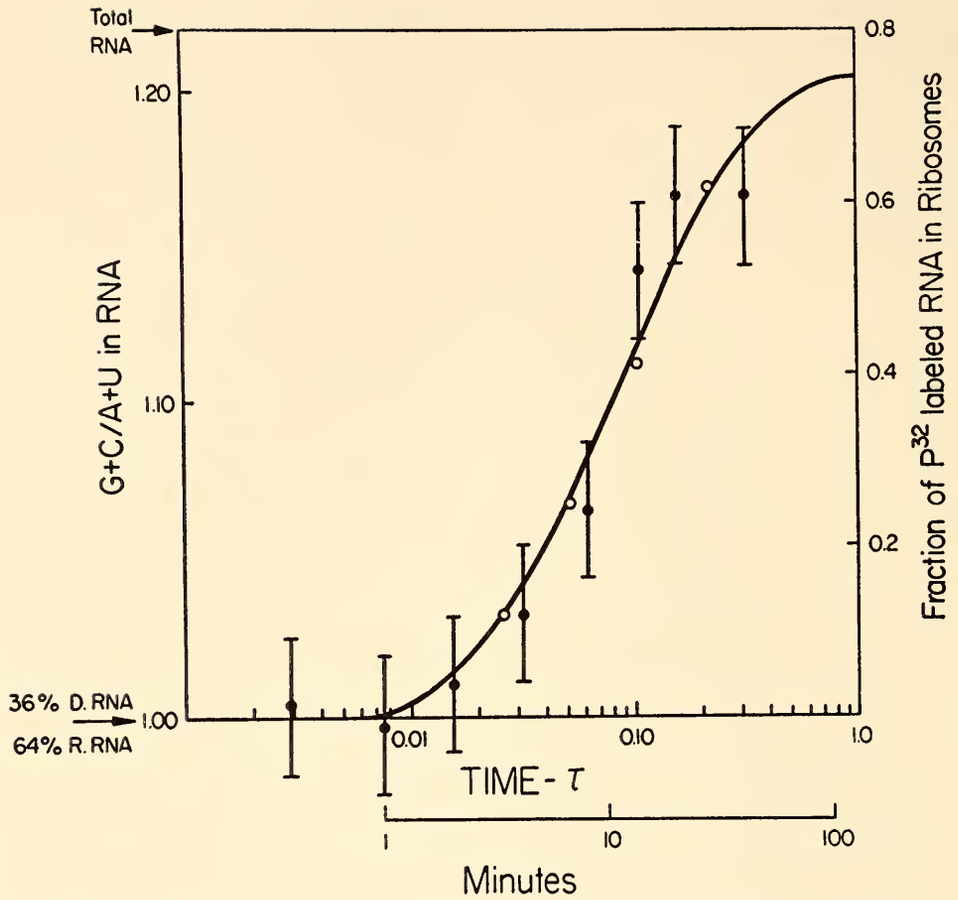

Fig. 41. A juxtaposition of the data of table 16 and figure 40. The nucleotide analyses (vertical markers) are plotted against time in terms of G + C/A + U ratios. The left-hand scale runs from 1.00, the average of the earliest determinations, to 1.22, the value for total RNA. The fraction of the total P^{32} label present in ribosomes (30S or greater) has been plotted on a scale from zero to 0.8. The data are those shown in figure 40. The curve drawn through the points is a theoretical one for the fraction of P^{32}-labeled RNA in ribosomes as a function of time calculated as described in the text.

proportion of labeled ribosomes of pure RNA composition. The data imply a rapidly labeled RNA fraction of 2–3 per cent of the total RNA, with a time constant of 2–3 minutes, one-third of which is D-RNA. Since the composition of the 14S fraction is constant, the lifetime of the two RNA molecules present must be very similar.

Composition of the isolated 14S fraction. It is clear from table 16 and figure 41 that there is no measurable change in the G + C/A + U ratio. More direct evidence for the constancy of the composition of the 14S fraction is shown in table 22. At longer times, when the total composition is beginning to change (table 16) and the 14S fraction no longer contains all the radioactivity, very similar results were obtained, indicating proportions of D-RNA and R-RNA of 1:2. The agreement between these late-time values

TABLE 22. Nucleotide Composition of Purified 14S Fraction of *Proteus vulgaris*, mole %

Time of Labeling with Isotope, minutes	T_e	C	A	G	U(T)	$\frac{G+C}{A+U}$
2	0.0148	23.5	27.1	27.1	23.3	1.02
5	0.0370	22.7	26.7	27.6	23.0	1.01

of the purified 14S fraction and the early-time total compositions indicates that unequal 5′-nucleotide specific radioactivities did not seriously influence the early determinations. For *P. vulgaris*, base compositions of this fraction prove to be constant throughout the range from 15 seconds to 5 minutes.

Resolution of pulse-labeled RNA by chromatography. The technique of chromatography on columns of methylated beef serum albumin-adsorbed on kieselguhr has been used to study the pulse labeling of various RNA fractions. The fractionation of DNA by this column has been shown by Mandell and Hershey to depend on the molecular weight of the nucleic acid. Furthermore, preparations of total bacterial nucleic acid prepared by the phenol method are resolved into S-RNA, DNA, and two peaks of ribosomal RNA resulting from the two sizes of molecules (16S and 23S) found after phenol treatment. This technique has been employed in the fractionation of the rapidly labeled RNA and in following the uptake of C^{14}-uracil and P^{32}-orthophosphate into the various nucleic acid fractions.

RNA labeled after brief exposure to the isotopes appears in three peaks other than S-RNA and DNA, none of which is exactly coincident with the two peaks of ribosomal RNA (figs. 42 and 44). This distribution does not, however, reflect a separation into RNA's of different nucleotide composition. The relative proportions of label among the three peaks is entirely reproducible provided that the conditions of phenol extraction are such that they preserve the normal proportions of the two sizes of ribosomal RNA. If the RNA is extracted in the presence of 10^{-4} *M* magnesium some of the 23S RNA may be converted to 16S RNA. Such treatment also changes the distribution of the early-labeled RNA among the three peaks. Figure 42 shows such an elution diagram of P^{32} pulse-labeled RNA of *P. vulgaris*. There is still no fractionation into materials of different nucleotide composition (table 23). A similar result is obtained for *B. subtilis* (table 24). Thus, whereas it appears that the three peaks of radioactivity may be aggregates of the pulse-labeled RNA with the 16S and 23S material, or with itself, partition of the D-RNA among them is closely similar.

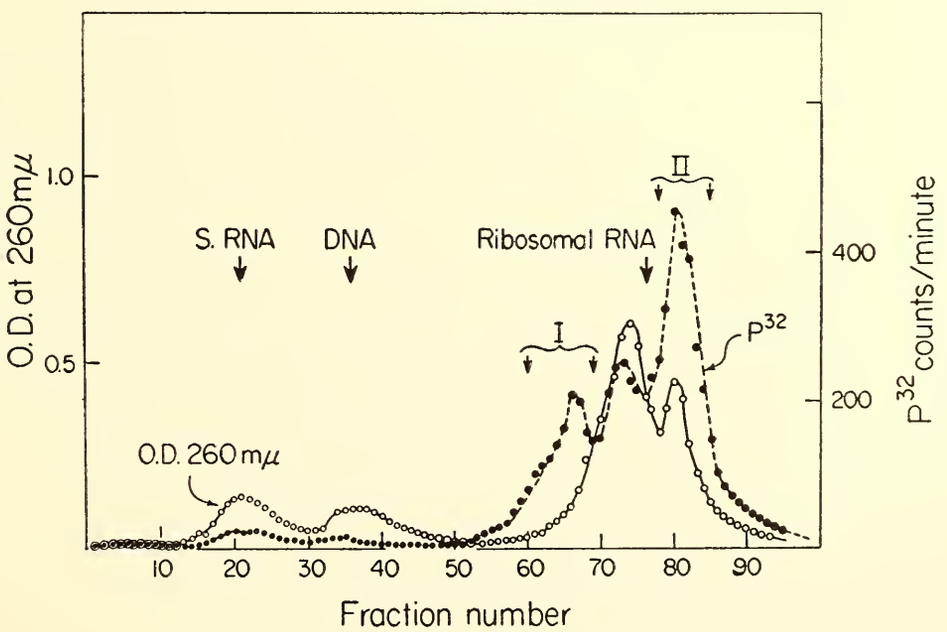

Fig. 42. Elution diagram from a column of methylated beef albumin on kieselguhr of a sample of RNA from *Proteus vulgaris* labeled for 90 seconds with P^{32}. RNA extracted from cells broken in tris-HCl 0.01 *M*, *p*H 7.4, containing 10^{-4} *M* $MgCl_2$. Elution with 300 ml of 0.02 *M* phosphate buffer, *p*H 6.7, containing a linear gradient of sodium chloride from 0.4 to 1.1 *M* NaCl.

TABLE 23. Nucleotide Compositions of Two Fractions of *Proteus vulgaris* 90-Second P^{32} Pulse-Labeled RNA Resolved on a Methylated Beef Albumin Kieselguhr Column

	C	A	G	U(T)
Total P^{32}-labeled RNA	23.5	26.9	26.7	22.9
Peak I	23.5	25.7	27.2	23.6
Peak II	24.7	25.5	27.0	22.8

TABLE 24. Nucleotide Composition of Two Fractions of *Bacillus subtilis* 3-Minute P^{32} Pulse-Labeled RNA Resolved on a Methylated Beef Albumin Kieselguhr Column

	C	A	G	U(T)
Total P^{32}-labeled RNA	23.4	25.4	27.5	23.7
Peak I	23.7	25.0	27.2	24.1
Peak II	23.3	25.5	27.6	23.6

The appearance of essentially all the early-labeled RNA in this region makes it very convenient to compare the kinetics of labeling of this mixture of D-RNA and R-RNA with those of the S-RNA and DNA. Two series of samples of *E. coli* RNA were chromatographed in this way, one prepared from cells that had been pulse-labeled with C^{14}-uracil and the other from P^{32} pulse-labeled cells.

Figure 43 shows two of the eight analyses made of RNA prepared from

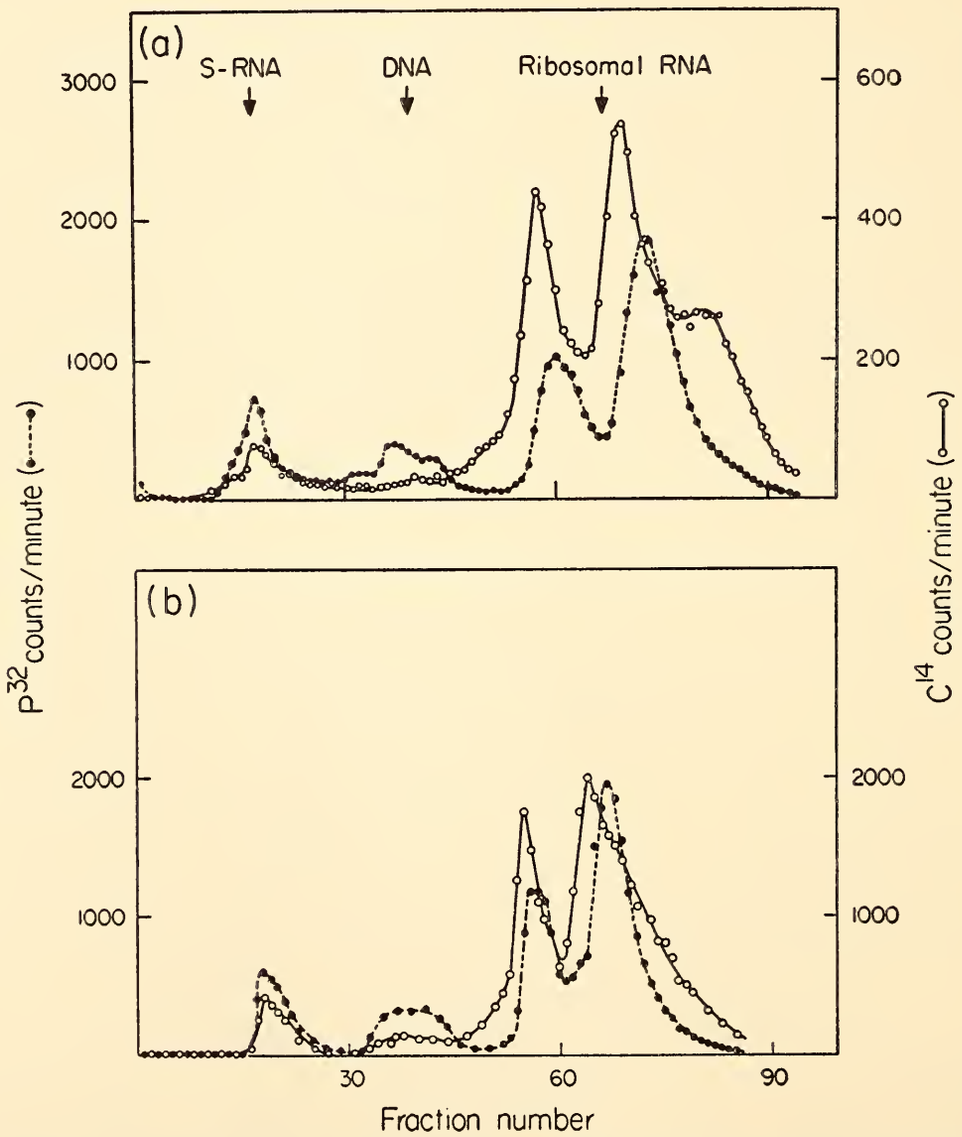

Fig. 43. Elution diagram from columns of methylated beef albumin on kieselguhr of two samples of RNA from *E. coli* grown for three generations in $P^{32}O_4^{\equiv}$ and given (*a*) 1 minute, (*b*) 3¼ minutes. Elution as in figure 42.

cells labeled with P^{32} for three generations, and for periods up to 15 minutes, with C^{14}-uracil. Figure 43(*a*) (1-minute C^{14}-uracil incorporation) shows the characteristic three peaks of pulse-labeled material in the high-molecular-weight region not coincident with the two peaks of P^{32}-labeled RNA. The specific radioactivity, here given by the ratio of C^{14} counts/minute to P^{32} counts/minute, is about three times higher in the high-molecular-weight region, taken as a whole, than in the S-RNA. By a labeling time of 3¼ minutes, the time of the second analysis shown in figure 43(*b*), the difference in specific radioactivity is not as noticeable. At even later times, figure 47(*b*), most of the C^{14} radioactivity appears under the two main peaks of P^{32}-labeled RNA.

Similar elution diagrams were obtained for the other five points after 40 seconds, 2 minutes, 4½ minutes, 7 minutes, and 10 minutes. The specific radioactivities of S-RNA and the mixture of D-RNA and R-RNA were obtained by summation of the counts per minute throughout the whole region. After the appropriate correction had been made the results were plotted in figure 44. The entry of uracil into S-RNA is subject to a delay of just over 1 minute, not shown in the labeling of high-molecular-weight RNA. The

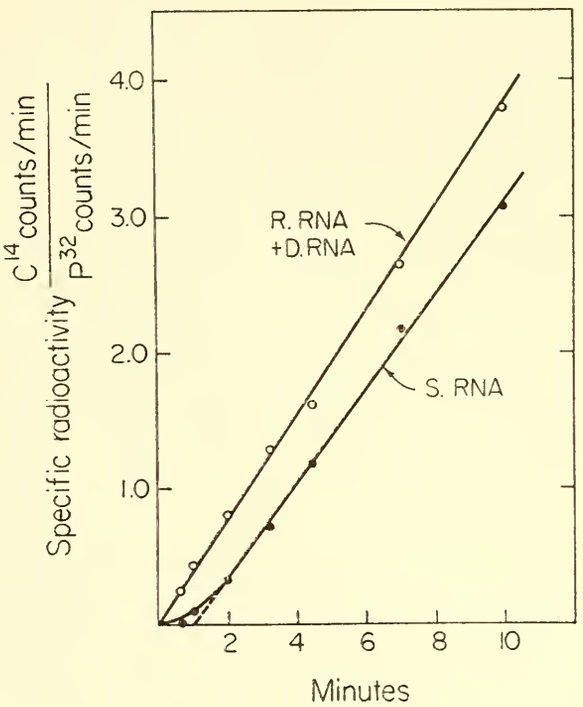

Fig. 44. Plot of the specific radioactivities of S-RNA (solid circles) and the mixture of D-RNA and R-RNA (open circles) as ratios of C^{14} cpm to P^{32} cpm against time. Data from figure 43 and five other analyses.

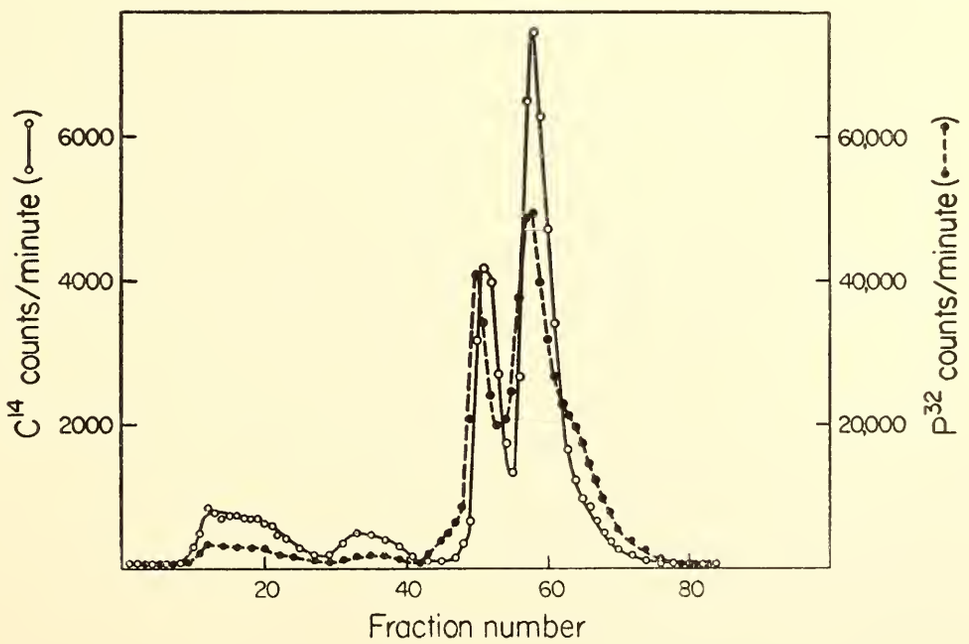

Fig. 45. Elution diagram from a column of methylated beef albumin on kieselguhr of a sample of *E. coli* RNA from cells labeled for three generations with C^{14}-uracil and for 5 minutes with P^{32}.

labeling of DNA is delayed to a similar extent.

The relative delay in S-RNA synthesis is not a special feature of uracil incorporation. When the identical experiment was performed using P^{32} as the pulse label and C^{14}-uracil as the steady label very similar results were obtained. Figure 45 shows an elution diagram of RNA labeled with P^{32} for 5 minutes. The lower specific radioactivity, i.e., ratio of P^{32} cpm to C^{14} cpm, and the separation of P^{32} counts from the steady C^{14} label, are immediately evident. Similar analyses were made after 1, 2, 3, 7, and 10 minutes. The specific radioactivities of S-RNA and the mixture of D-RNA and R-RNA are plotted in figure 46. In spite of the

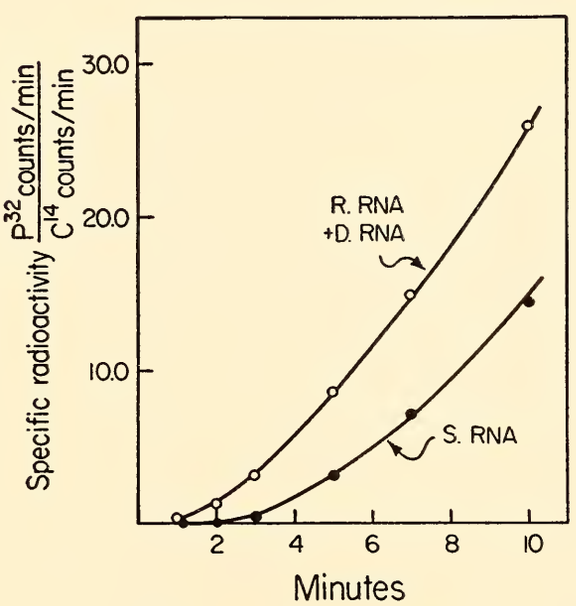

Fig. 46. The specific radioactivities of S-RNA and the mixture of D-RNA and R-RNA as a function of time plotted as ratios of P^{32} cpm to C^{14} cpm. Data from figure 45 and five other analyses.

curvature brought about by the large pool of acid-soluble P^{32} it is possible to see a delay in the entry of P^{32} into S-RNA and DNA relative to that of R-RNA and D-RNA of about 2 minutes.

Thus the labeling of both the pyrimidine bases and the phosphorus atoms of newly synthesized S-RNA and DNA is delayed relative to other RNA by a pool of nucleotide material equivalent to 1 or 2 minutes' worth of RNA. The conclusion will be drawn, after the presentation of further results, that this delay is a consequence of the degradation of a fraction of the high-molecular-weight RNA, identified with D-RNA.

The effect of chloramphenicol on the synthesis of D-RNA. It has been known for a number of years that the overall nucleotide composition of RNA synthesized in the presence of chloramphenicol is similar to that of normal bacterial RNA. Fractionation of *E. coli* chloramphenicol RNA on columns of DEAE-cellulose gives two components, one having the nucleotide composition of S-RNA and the other approximately that of ribosomal RNA. After 10 minutes' exposure to C^{14}-uracil and chloramphenicol, analysis of the extracts on the sucrose gradients shows all the radioactivity in either S-RNA or the 14S precursor peak. It therefore appeared that chloramphenicol brought about an accumulation of the 14S material here under investigation. Further experiments were directed at determining the relative quantities and behavior of the D-RNA and R-RNA moieties.

A detailed analysis was made of the rate of change of the nucleotide composition of the RNA in the presence of chloramphenicol. Chloramphenicol at 200 mg/l was added to exponentially growing cultures of *E. coli* and *P. vulgaris*, and was followed 5 minutes later by P^{32}. Samples were taken for nucleotide analysis as described previously; the results are shown in table 25. They should be compared with those for uninhibited cultures shown in tables 16 and 18.

The compositions at the earliest times compare well with those for control cultures, and they indicate the synthesis of about one-third D-RNA and two-thirds R-RNA. Although the composition changes toward that for total RNA in the early stages as in the control, the nucleotide composition at late times differs

TABLE 25. Composition of Newly Formed RNA during Incubation with (200 mg/ml) Chloramphenicol

Time, minutes	C	A	G	U(T)	$\frac{G+C}{A+U}$
		Escherichia coli			
2	22.5	25.0	29.8	22.7	1.05
4	22.9	25.6	29.4	22.1	1.06
7	22.7	25.3	29.4	22.6	1.09
20	21.7	25.0	31.4	21.9	1.13
40	22.4	25.0	31.2	21.4	1.15
60	22.8	25.3	31.9	21.0	1.16
Total RNA	22.1	25.2	32.5	20.2	1.20
		Proteus vulgaris			
5	22.6	26.0	28.0	23.4	1.02
10	21.5	25.4	31.2	21.9	1.11
20	21.9	25.2	30.7	22.2	1.11
40	21.6	26.0	30.8	21.6	1.10
60	21.4	26.2	30.9	21.5	1.10
Total RNA	22.6	24.6	32.0	20.8	1.20

significantly from that of total RNA. In *E. coli* the difference in G + C/A + U between 1.15 or 1.16 and 1.20 is hardly significant (although it also appears in the data of Horowitz et al.), but in *P. vulgaris* we can be confident of the reality of the difference between 1.10 and 1.20. If these apparent compositions are taken at their face value the RNA formed after 1 hour's exposure to chloramphenicol would consist of some 10–20 per cent D-RNA. Thus these observations suggest that D-RNA and R-RNA are synthesized in the normal proportions in chloramphenicol but that the breakdown of D-RNA is greatly reduced.

Further studies of the nature of RNA synthesis in chloramphenicol were made by means of chromatographic analysis of the RNA. Chloramphenicol at 200 mg/l was added to exponentially growing *E. coli* prelabeled for three generations with P^{32}, and was followed 5 minutes later by C^{14}-uracil. Control cells received no chloramphenicol. Samples taken at 1, 2, 4, and 8 minutes were washed and broken in the usual way, and the purified RNA was analyzed on columns of methylated beef albumin as described above.

The two analyses made after 8-minute exposures are shown in figure 47. By this time RNA from uninhibited cells shows a high degree of coincidence between the C^{14} and P^{32} counts. On the other hand, the RNA from the inhibited culture has a higher proportion of the newly made RNA in the region of 16S RNA. Moreover, the remaining C^{14} radioactivity appears in a peak much broader than that given by the 23S P^{32}-labeled RNA. In fact, the specific radioactivity in this region is lowest at the peak, suggesting the existence of two overlapping components of C^{14} radioactivity.

The analyses of chloramphenicol RNA at the earliest times, 1 and 2 minutes (not shown), are quite similar to those of the control except for the absence of the third peak of C^{14} radioactivity; see figure 43(*a*). At 4 minutes a degree of coincidence of the peaks of radioactivity lower than that in the control is already apparent.

Of greater interest is the plot of the specific radioactivities of S-RNA and D-RNA plus R-RNA shown in figure 48. It appears that the entry of C^{14}-uracil into S-RNA takes place without significant delay. A similar effect was apparent in the labeling of DNA. The data from

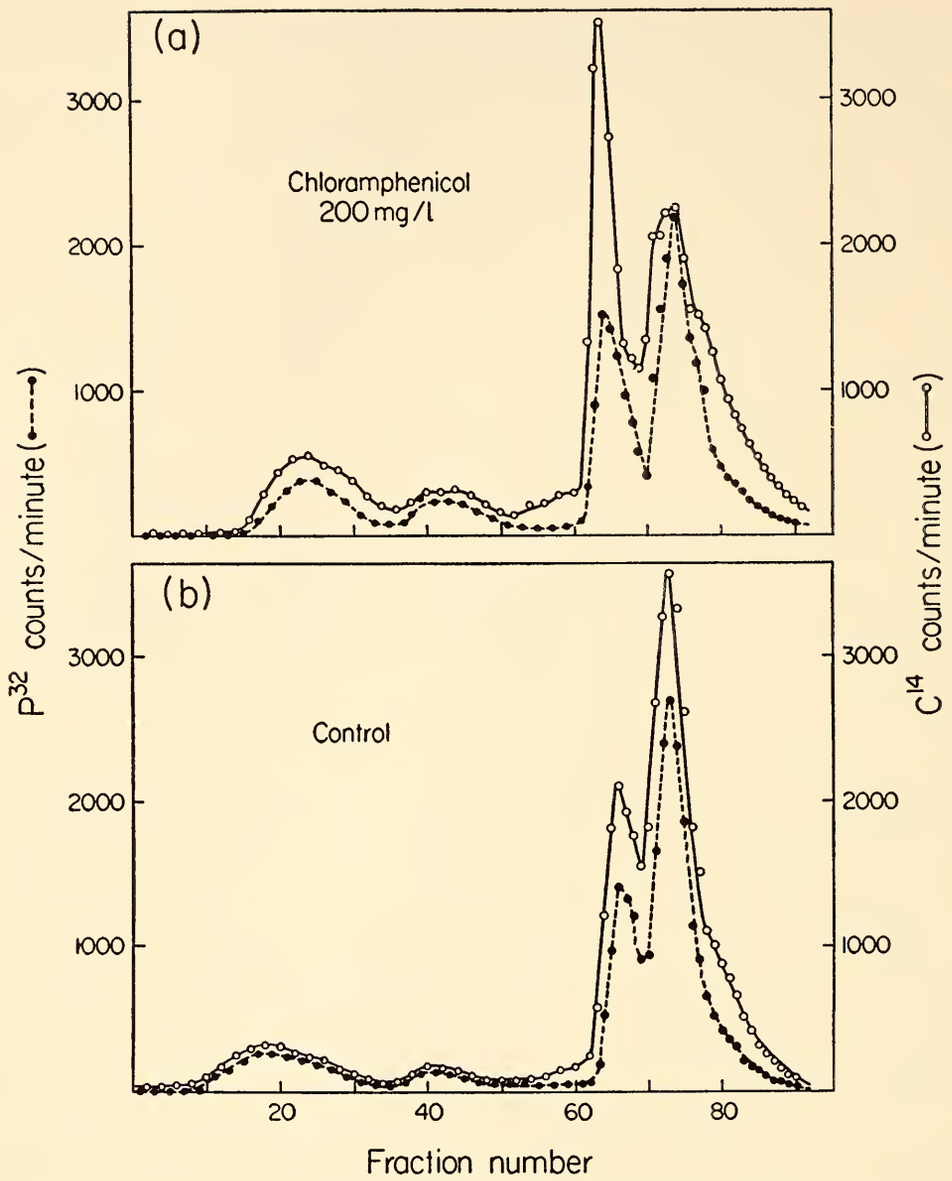

Fig. 47. Elution diagrams from columns of methylated beef albumin on kieselguhr of two samples of RNA from *E. coli* cells labeled for three generations with P^{32} and 8 minutes with C^{14}-uracil: (*a*) in the presence of 200 mg/l of chloramphenicol; (*b*) control.

the control cultures gave the same 1-minute delay as shown in figure 44. As the delay is abolished by chloramphenicol it does not appear to be the result of a special small-molecule precursor pool. On the other hand, the lack of a delay may be correlated with the stability of D-RNA in chloramphenicol-inhibited cultures.

Discussion. A possible consequence of the messenger RNA hypothesis of Jacob and Monod is that these RNA molecules may survive long enough to specify the synthesis of only one protein molecule. Since these molecules would presumably be included in the D-RNA fraction, knowledge of its rate of synthesis is of major importance in considering the mechanism of protein synthesis.

There is, however, no evidence for an extremely rapid rate of turnover of the D-RNA fraction such that the rate of incorporation into these molecules is many times that into the stable RNA. In fact, nucleotide compositions measured at very early times are consistent with a flow of material into D-RNA equal to half that into stable ribosomal RNA. There is, of course, as in all isotope-

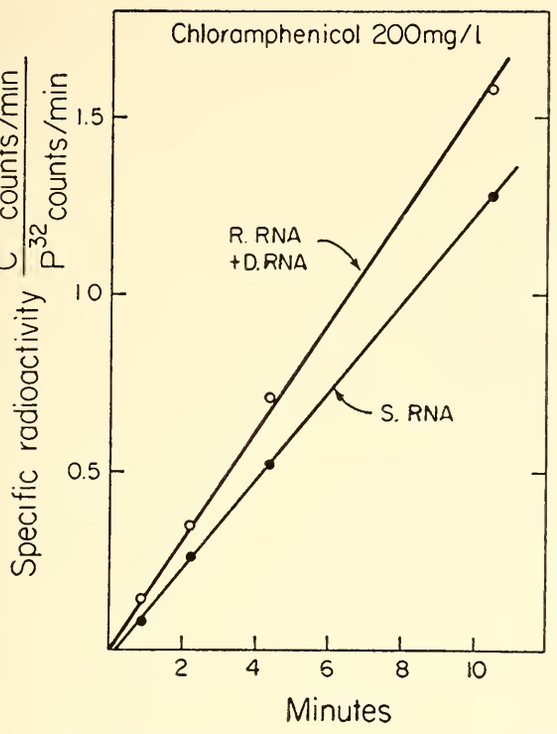

Fig. 48. The specific radioactivities of S-RNA and the mixture of D-RNA and R-RNA as a function of time from chloramphenicol-inhibited cultures plotted as ratios of C^{14} cpm to P^{32} cpm. Data from figure 47(*a*) and three other analyses.

labeling experiments, the possibility that the rate of uptake of label does not measure the true rate of synthesis. Thus, in the present experiment, although it appears that the rate of RNA synthesis is 50 per cent higher than the flow into stable RNA, we may argue that D-RNA is being synthesized and degraded much more rapidly, in equilibrium with a chemically or physically isolated pool. A similar argument must be applied to the incorporation of C^{14}-uracil and other bases. Since this would imply rigid separation between two or more pools of nucleotides and inaccessibility to labeling by either C^{14}-uracil or P^{32} this process has not been considered further in the present discussion.

In all the five organisms studied the composition of the newly synthesized RNA is intermediate between that of D-RNA and R-RNA. Fractionation by water treatment of the 14S component of *Ps. aeruginosa* and of *E. coli* into two RNA fractions provides a strong indication that it consists of a mixture of two types of molecule, one with a base composition like that of the DNA of the cell (D-RNA) and the other pure R-RNA. Although the fractionation was not as successful with RNA's of other bacteria, results are consistent with the existence of two molecules with different compositions.

Detailed analyses of changes in nucleotide composition of the newly formed RNA indicate that the two types of molecule have very similar kinetics of incorporation. The apparent composition of the 14S RNA fraction remains constant, and the changes in overall nucleotide composition appear to result from the degradation of the D-RNA moiety and the conversion of R-RNA to stable ribosomal material. Thus the compositional changes can be correlated with the appearance of radioactivity in stable ribosomal material which dilutes a 14S component of constant composition. In effect, the lifetimes of D-RNA and R-RNA molecules in the 14S fraction are closely similar, one being removed mainly by degradation and the other by the addition of protein and conversion to ribosomes.

The relative amounts of D-RNA and R-RNA synthesized in each of the five organisms studied appear to be the same. This ratio may have stoichiometric significance or it may merely be a function of the conditions of growth, since other authors report considerably higher fractions of D-RNA in pulse-labeled RNA. Bacteria undergoing a downward transition in growth rate and nongrowing yeasts produce higher proportions of D-RNA. The G + C/A + U ratios for the DNA-like materials reported in either of these two conditions are, however, consistent with an equal mixture of D-RNA and R-RNA.

Chromatography of pulse-labeled RNA on methylated beef albumin columns does separate the label from the bulk RNA but does not give any separation between

D-RNA and R-RNA. Each of the three peaks obtained appears to consist of mixtures of the two components. The relative quantity appears to depend on conditions of extraction.

The separation between S-RNA and the other RNA has proved useful, however, for kinetic studies. Studies with C^{14}-uracil and P^{32} showed a delay in incorporation of label into S-RNA relative to the other RNA of about 1 to 2 minutes. Rather than being the result of a precursor pool of nucleotides, this delay seems to be a reflection of the turnover of D-RNA. The apparent difference between the delays experienced in C^{14}-uracil and P^{32} labeling may be attributable to the special features of uracil incorporation. Thus some of the C^{14}-uracil could enter S-RNA directly so that the initial rate of entry is not zero and the delay resulting from the utilization of nucleotides derived from D-RNA breakdown is apparently reduced.

A unified picture of the flow of label into RNA would be the following. The initial flow is accounted for by a one-third entry into D-RNA and the remaining two-thirds into R-RNA. The former is degraded and the nucleotide material used to some extent for S-RNA and DNA synthesis. Of the nucleotides originally entering D-RNA some would serve to make S-RNA (20 per cent of the total RNA) and DNA (about 15 per cent of the cell nucleic acid) and possibly R-RNA. If D-RNA were degraded to nucleoside 5′-diphosphates or monophosphates the conversion of D-RNA to DNA could proceed by the mechanism described by Cohen et al. Thus, rather than being an obligatory precursor of R-RNA the D-RNA is the precursor of S-RNA and DNA and only to a more limited extent the precursor of R-RNA. This picture would fit all the kinetic experiments, and it finds support from the studies of chloramphenicol inhibition.

The presence of chloramphenicol in the growth medium during the incorporation of C^{14}-uracil into RNA removes the kinetic delay of the entry of label into S-RNA. Thus more C^{14} enters S-RNA and DNA directly. It is unlikely that the removal of the delay by chloramphenicol can be adequately explained by changes in a small-molecule precursor pool feeding S-RNA. This effect has been shown to be associated with an accumulation of D-RNA. Although the rate of synthesis of D-RNA remains the same, its degradation is markedly reduced. The conversion of "chloramphenicol RNA" to soluble material when the antibiotic is removed may reflect the renewal of the degradation process, although it is not clear whether both the D-RNA and R-RNA fractions are lost.

Purification of D-RNA

When the report year was drawing to a close it was found that single-stranded DNA could be immobilized in agar, and complementary RNA could be hybridized with this DNA. In applying this principle, which was discovered through a lead provided by Bautz and Hall of the University of Illinois, the D-RNA of several phages and bacteria has been purified and chemically characterized. Kinetic studies of the synthesis of D-RNA were carried out with *Proteus vulgaris*. It was found that D-RNA has a half-life of about 2 minutes and comprises 1 per cent of the total cellular RNA. Assuming D-RNA to be template RNA, it would therefore function catalytically to take part in the synthesis of perhaps fifty polypeptide strands.

Kinetics of Labeling of Turnip Yellow Mosaic Virus

Turnip yellow mosaic virus (TYMV) is a spherical plant virus containing 36 per cent RNA and 64 per cent protein. Preparations of the cell sap of Chinese cabbage plants infected with this virus contain small amounts of noninfectious viruslike particles. The protein complement of these particles is serologically indistinguishable from that of the infec-

tious unit, but the RNA complements are smaller. The RNA content appears to be "quantitized," and a series of particles containing about 24, 12, and 6 per cent RNA can be separated. In addition, another particle is found that contains no RNA. The existence of such a series of particles in the cell sap of infected plants implies that the particles may be functionally related, perhaps serving as sequential stages along a pathway of biosynthesis leading to the infectious unit itself. This hypothesis was tested by Dr. R. E. F. Matthews, who discovered the RNA-containing viruslike particles at the Plant Diseases Division of the Department of Scientific and Industrial Research, Auckland, New Zealand. Such a hypothesis was in fact consistent with his results. Since the experiments did not demonstrate precursor-product relationships, an attempt was made in Dr. Matthews' laboratory to improve upon the design of the tracer experiments in the hope that they would yield a definitive answer to the problem of TYMV assembly.

Initially, "chase" experiments of the type used in our studies on bacterial nucleoprotein synthesis were tried, by which means it was hoped to follow the flow of radioactive precursor through the synthetic sequence. After a number of unsuccessful trials with P^{32} labeling of the virus it was found that chase experiments were impractical because the pool of low-molecular-weight compounds in Chinese cabbage was very large and practically unalterable in size, either as a result of extreme phosphorus starvation or by forcing phosphate into the host plant. Therefore, the incorporation of radioactivity was followed over a long enough period of virus development to permit comparisons of the early rates of incorporation with those occurring later.

In a typical experiment twelve young Chinese cabbage plants that had been inoculated with TYMV 12 days earlier were trimmed to two well expanded systemically infected leaves. The roots were washed and placed in 1 ml of P^{32}-labeled orthophosphate. In about an hour, when the fluid was completely absorbed, a large volume of water was added and the plants were maintained in the greenhouse. At intervals after administration of the tracer, disks were taken from each half-leaf with a cork borer. Then with a smaller cork borer a sample was obtained from the center of the pile of disks previously taken. The smaller disks were extracted with hot ethanol, and the extract was analyzed for phosphorus and radioactivity. The larger disks were ground to a pulp, the pulp was clarified in the centrifuge, and the resulting cell sap was treated with an antiserum specific for viral protein and nucleoprotein. The specific precipitates were digested in alkali; the resulting viral nucleotides were separated chromatographically, and their specific radioactivities were determined.

The partition of phosphorus and the incorporation of radioactivity in the infected plant are shown in figure 49. The phosphorus content expressed in milligrams per leaf appeared to remain about constant in the ethanol extract, decreased to half in the nonviral alcohol-insoluble fraction, and rose linearly fourfold in the virus. The specific radioactivities in these fractions rose rapidly at first, then appeared to level off. In this particular experiment the specific activity of the alcohol-soluble fraction reached the maximum value at 5 hours. In other experiments, however, labeling of this fraction reached saturation in an hour or so.

The fixation of radioactivity in the alcohol in soluble macromolecular components of the infected plant is a slower process, and the specific radioactivity does not reach that of the alcohol-soluble low-molecular-weight fraction during the course of the experiment. After 40–60 hours there is little, if any, further increase in specific activity of the viral or host nucleic acid. Nevertheless, the virus continues to be synthesized at the same rate as before. This might be taken to mean that nucleic acid was turning over,

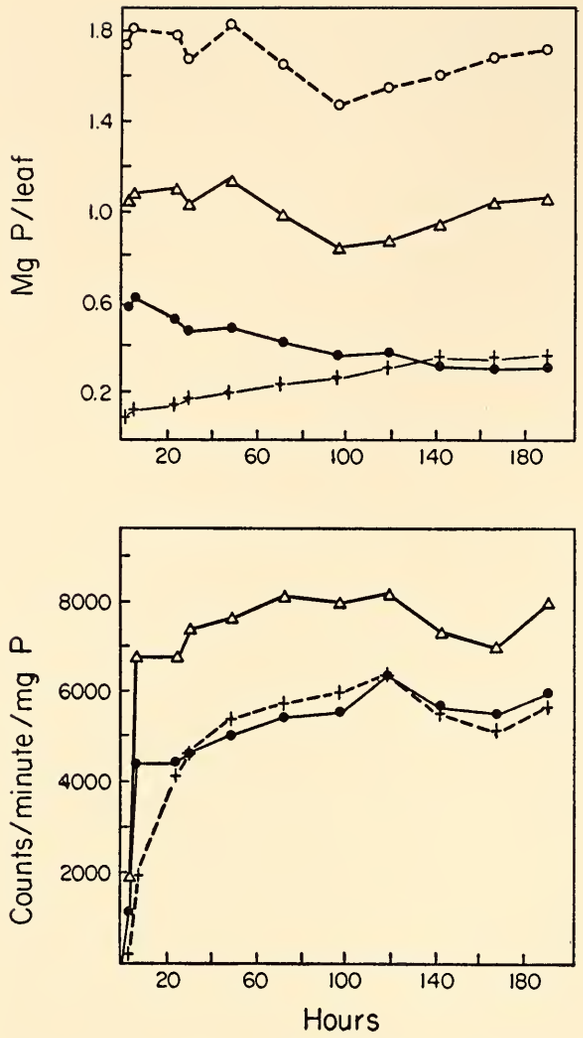

Fig. 49. TYMV 1. Phosphorus content and specific radioactivities in TYMV-infected Chinese cabbage leaves. *Upper*: open circles, total; triangles, alcohol soluble; solid circles, nonvirus alcohol insoluble; crosses, virus. *Lower*: triangles, alcohol soluble; solid circles, nonvirus alcohol insoluble; crosses, virus.

but the result can equally well be explained by assuming that there is a small pool of phosphorus precursor which "bypasses" a large pool. In this way phosphorus of relatively high specific radioactivity could enter the virus at early times whereas later phosphorus of low specific activity would be derived from the large pool, thus causing a leveling-off in the specific-activity curve.

S^{35}-labeled sulfate was used to follow the synthesis of virus protein. In an experiment, 24 previously infected plants were arranged in sets of 3 and provided with S^{35} through their roots. At intervals after the administration of the tracer a set of plants was harvested and the leaf laminae were ground to a pulp. The viral protein was pelleted in the ultracentrifuge. The pellet was then fractionated in a cesium chloride density gradient in the swinging-bucket ultracentrifuge. The top component (T) is the empty protein shell. Next in order are the minor components (B_{00}, B_0), the infectious virus (B_1), and a noninfectious particle (B_2) that is chemically indistinguishable from the virus. The specific-activity data for the empty protein shell and the virus are shown in figure 50. Another experiment

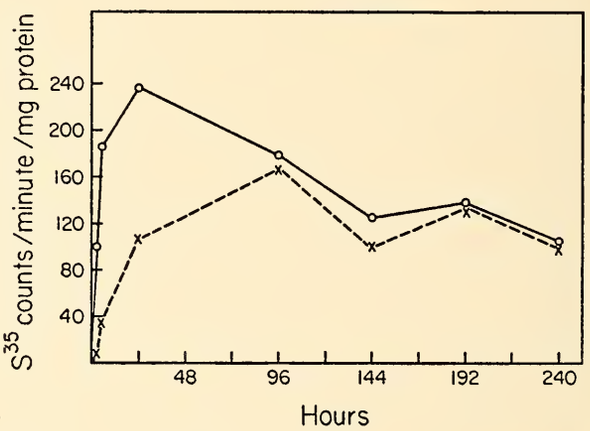

Fig. 50. TYMV 2. S^{35} labeling of the empty protein shell T, and of TYMV (B_1).

was performed similarly except that nonradioactive plants in the same stage of growth and infection as the radioactive ones were added to the radioactive plants during preparation of the virus to provide enough of the minor components for analysis. The results are shown in figure 51.

The shapes of the curves for S^{35} incorporation resemble those for P^{32} incorporation in the nucleoproteins B_{00}, B_0, B_1, B_2, but the S^{35} curve for the empty protein shell is markedly different. The specific radioactivity of this shell rises very rapidly to more than twice that of the nucleoproteins, then decreases slowly. The amino acid compositions of T and B_1 are known to be very similar. Never-

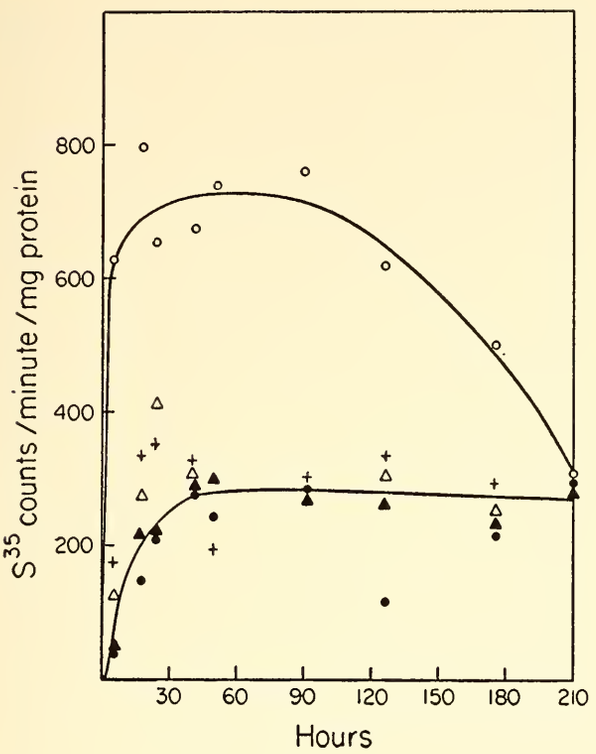

Fig. 51. TYMV 3. S^{35} labeling of the empty protein shell T, the minor nucleoproteins B_{00}, B_0, B_2, and TYMV (B_1).

theless the S^{35} of T is in the amino acids cysteine and methionine, and the radioactivity cannot be released from the shell by treatments with mercaptoethanol, urea, or sodium sulfate. The high specific radioactivity could be explained if T is turning over rapidly and draws on sulfur-containing compounds having a high specific radioactivity at early times. T is continually synthesized during the experiment and is one-fifth to one-fourth of B_1 in amount. Hence, the decrease in its specific activity is strange. It could be explained if the empty protein shell were breaking down at the same time that new shells were being made from sulfur compounds whose specific activity was now low compared with that at early times, perhaps as a result of a bypass mechanism as suggested for phosphorus incorporation. This suggestion would also account for the shape of the nucleoprotein curves. From the data of figures 50 and 51 it appears highly unlikely that T is a precursor of B_1. In addition, the minor components, which are 1 to 3 per cent of the nucleoprotein, could not be precursors between T and B_1 because their specific activities are too low at early times.

The hope of definitely establishing the mechanism of how TYMV is assembled has not been realized. Nevertheless, these experiments make clear the fact that a much deeper knowledge of plant physiology—especially knowledge of the kinds, amounts, and kinetic behavior of low-molecular-weight precursors—is required before any satisfying model of TYMV synthesis can be proposed. It is also clear that "chase" experiments, which have proved a powerful tool for biosynthesis studies in microorganisms, are practically impossible in higher plants.

Control Mechanisms

In the bacterial cell, protein synthesis and RNA synthesis are closely interlocked. DNA synthesis, in contrast, is relatively independent. Thus, in the 15 T-A-U- mutant the lack of thymine (T) prevents DNA synthesis without any immediate effect on protein or RNA synthesis. Conversely, the lack of arginine (A) and uracil (U) causes no immediate change in the rate of DNA synthesis. The lack of either U or A brings both protein and RNA synthesis to a halt. The requirement of RNA synthesis for the continued formation of protein is a natural consequence of the short lifetime of protein-forming templates. However, there is no obvious reason why amino acids are needed for RNA synthesis.

Protein synthesis is not essential for RNA synthesis, as RNA continues to be synthesized in the presence of concentrations of chloramphenicol that block the incorporation of amino acids into protein. Such results have been interpreted as showing a "catalytic role" of amino acids in RNA synthesis or a "derepression" of RNA synthesis either by amino acids or by their activated forms.

These interpretations do not seem entirely satisfying. The action of chlor-

amphenicol is extremely rapid and much faster than would be expected on this basis. Also, chloramphenicol can remove the inhibition of RNA synthesis caused by an amino acid analog, 5-methyltryptophan. Puromycin allows continued RNA synthesis in the absence of protein synthesis, but it is not as effective as chloramphenicol in eliminating the need for amino acids. Such investigations are being continued in the hope of gaining a better understanding of cellular control mechanisms.

Effects of virus infection. Last year it was shown that a study of the time course of enzyme synthesis after the addition or removal of an inducer provided valuable clues to the induction mechanism and to regulatory processes in the synthesis of protein. The addition of inducer immediately accelerated the synthesis of β-galactosidase, and after 2.5 to 3.0 minutes a steady rate many times greater than that of the uninduced cells was observed. It was concluded that the brief period of accelerating synthesis was the time required to produce the new enzyme-forming units (EFU) necessary for the induced rate of synthesis of enzyme. Similarly, the removal of inducer caused an immediate deceleration in the rate of synthesis, and within 3 minutes the system returned to the rate observed for uninduced cells. Such a result would be expected if the induced enzyme-forming units were unstable and decayed with a time constant of 2.5 to 3 minutes.

This year other means of markedly altering the rate of protein synthesis were investigated. In collaboration with Dr. Maury Miranda at the Instituto de Biofísica, Universidade do Brazil, Rio de Janeiro, Brazil, studies were carried out to determine the capacity of *E. coli* cells to synthesize host protein after phage infection.

Wild-type T4 bacteriophage rapidly lyses K12λ *E. coli*, but rII mutants of it are unable to cause lysis of this organism even though infection occurs. Furthermore, Benzer has shown that the rII region of the T4 DNA can be divided into the A and B cistrons, the two subunits having different mutational and functional characteristics. Two rII mutant phages were selected for our studies: 164, a mutant of the A region; and 196, a mutation of the B cistron. K12λ mixedly infected with both these rII mutants causes efficient lysis of this host and the rapid production of mature phage. Single infection with either the A or B rII mutants does not result in lysis, although the bacteria are no longer capable of cellular replication. Phage development within the host cell could not be demonstrated, and shaking infected cells with chloroform liberated no infective particles.

Infection with rII A phage followed at a later time by a second infection with the complementary rII B phage rendered the K12λ cells capable of subsequent lysis and phage production. After an elapsed time of several hours between infections phage production still resulted. Evidently the initial infection provides the host cells with certain latent characteristics that can be stored and maintained. This system seemed an ideal one for the investigation of the effect of phage infection upon the capacity of the cell to synthesize host and viral proteins.

The data in figure 52 illustrate the effect of rII phage upon the induced synthesis of β-galactosidase. In this

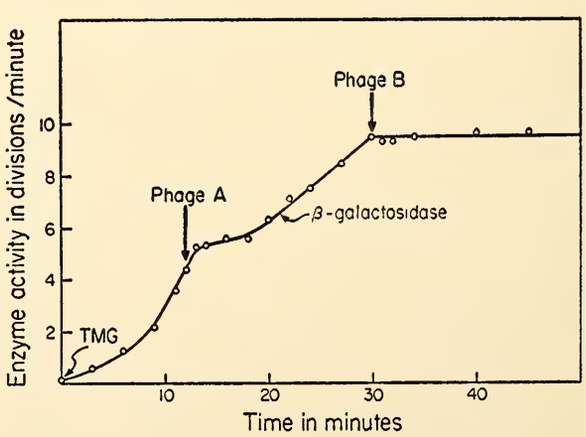

Fig. 52. Time course of induction of β-galactosidase in K12λ *E. coli* after infection with rII mutants of T4 phage. (A = mutant 164; B = mutant 196.)

experiment an exponentially growing culture of K12λ cells was induced for β-galactosidase synthesis with 3.5×10^{-4} *M* methyl-thio-β-D-galactoside (TMG). Nine minutes later rII A phage particles (4 phages per cell) were added to the culture. Figure 52 indicates that the β-galactosidase synthesis continues for several minutes after phage infection. Enzyme production then slows down or stops and later resumes. Plate count analysis revealed that 99 per cent of the cells were infected with the rII A phage. Growth of the culture after infection could not be detected.

In figure 53 it is shown that these enzyme kinetics are quite similar to those

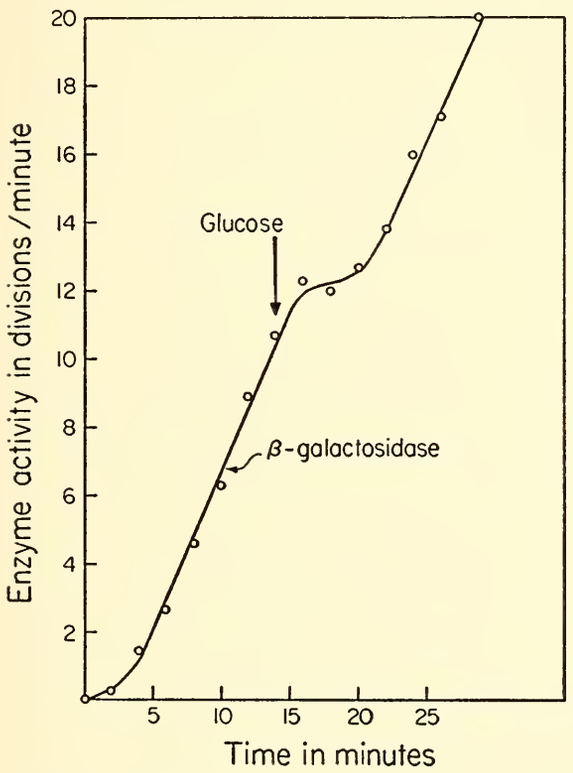

Fig. 53. Effect of glucose on β-galactosidase induction.

observed when glucose (10^{-2} *M*) was added to K12 cells after the induced synthesis of enzyme had been initiated. The infection with rII A phage (or the addition of glucose) appears to stop the synthesis of active enzyme-forming units. Those already present at the time of infection continue to synthesize enzyme until they decay or become inactive. It is interesting that each of these very different treatments of the culture permits the resumption of the induced synthesis of enzyme. Identical results are obtained if the culture is infected with rII B mutants instead of the rII A phage.

The temporary inhibition of the synthesis is not caused by low-molecular-weight contaminant material contained in the added phage lysate. Dialysis of the phage suspension does not reduce the effectiveness of the infection. More significantly, nondialyzed preparations added to the culture in which no tryptophan was present caused no effect. Tryptophan is required for the adsorption of these phages to the host.

The synthesis of β-galactosidase can be abruptly stopped after the initial infection with rII A phage if the complementary rII B mutants are added to the culture later. These kinetics are shown in figure 54. Preinduced K12λ cells were

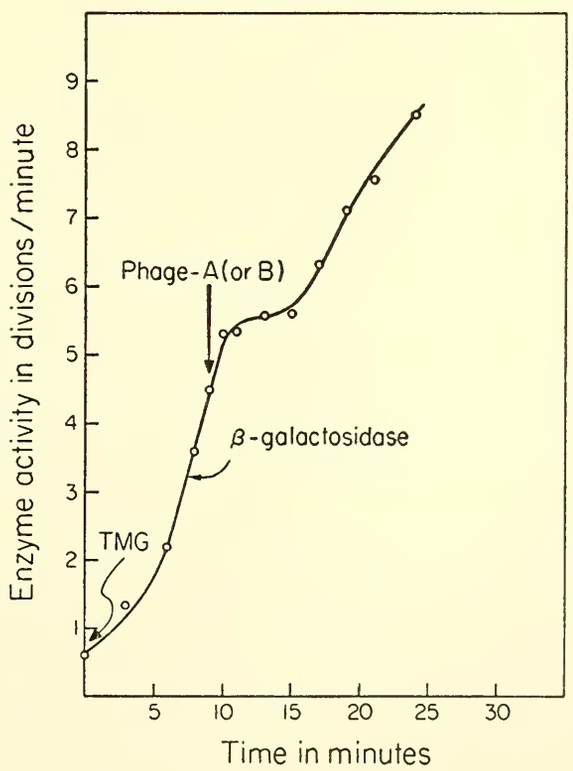

Fig. 54. Kinetics of β-galactosidase synthesis after an initial infection with an rII mutant, A (164 phage), and subsequent infection (36 minutes later) with rII mutant B (196 bacteriophage).

first infected with rII A phage and 18 minutes later with rII B phage. The immediate cessation of synthesis of β-galactosidase is observed as normal phage development begins. Presumably, not only are no new enzyme-forming units synthesized but, more significantly, those previously existing and functioning after the initial infection are inactivated within seconds.

The rapidity with which phage can stop the synthesis of β-galactosidase can be seen from the data of figure 55. Wild-type

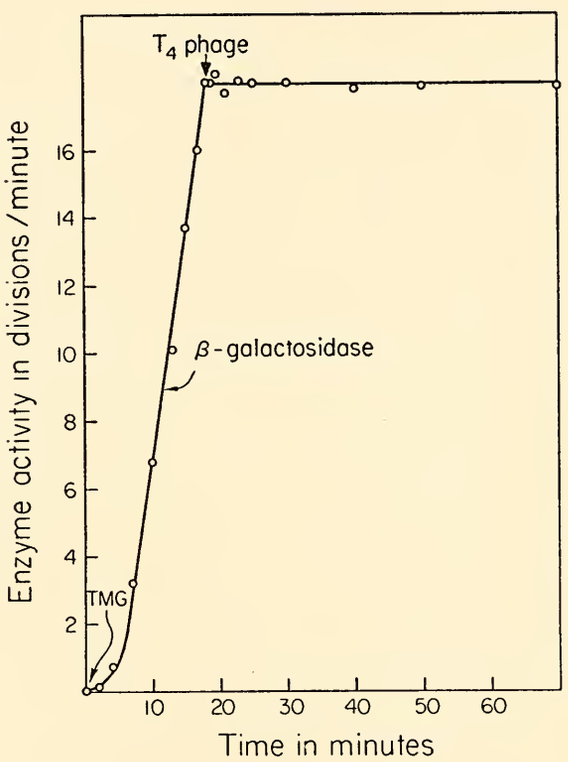

Fig. 55. The kinetics of β-galactosidase induction in K12λ *E. coli* after infection with wild-type T4 bacteriophage.

T4 phage were added to a preinduced culture of K12λ. Within seconds all synthesis of β-galactosidase ceased.

It is extremely puzzling that the injection of viral DNA into the host cell should cause instantaneous alterations in the capacity of the cell to synthesize host protein. Ribosomes are presumably the sites for protein synthesis, and it is extremely difficult to imagine a *direct* action of injected DNA on all β-galactosidase-forming units. It is also difficult to imagine why the *complete* phage genome is required for permanent suppression of host protein synthesis.

The information in the injected DNA of one of the rII mutants may remain dormant for long periods but can be expressed at the moment of entry of the second rII mutant DNA. Our current research is directed toward determining how and where such information is maintained and the mechanisms by which it is finally expressed.

Cell-Free Synthesis

In spite of the successful use of cell-free systems by other laboratories, this technique has had little application here. One particularly important use of such systems is to provide an assay for templates capable of synthesizing a biologically active protein. In *Year Book 60* two apparently successful experiments of this type were reported. Broken cells, incapable of synthesizing β-galactosidase, synthesized a small quantity of the enzyme when incubated with purified RNA extracted from induced cells. Numerous attempts were made to repeat and extend these results, but no RNA preparation other than the initial one gave positive results.

Doublet Code

In 1954 Gamow formulated as a coding problem the role of nucleic acid in specifying the order of amino acids in protein. A year ago an experimental attack on the coding problem became possible when Nirenberg found that synthetic polynucleotides could serve as templates for protein synthesis. This year the "code letters" for 19 of the 20 amino acids were experimentally determined. Almost invariably the experimental findings have been interpreted in terms of a "three-letter" or triplet code.

As the experiments progressed and the code letters for the amino acids were recognized, a serious difficulty developed

TABLE 26. Calculation of Hypothetical Template

	Proportions in *E. coli* Proteins*	Code	Common U	Proportion of Bases Expected in Template			
				U	G	A	C
Ala	14.7	UCG	14.7		14.7		14.7
Arg	7.0	UCG	7.0		7.0		7.0
Asp	14.4	UAG	14.4		14.4	14.4	
Cys	0.9	UUG	0.9	0.9	0.9		
Glu	15.6	UAG	15.6		15.6	15.6	
Gly	12.0	UGG	12.0		12.0		
His	2.9	UAC	2.9			2.9	2.9
Ileu	7.5	UUA	7.5	7.5		7.5	
Leu	12.4	UUC	12.4	12.4			12.4
Lys	8.5	UAA	8.5			8.5	
Met	4.3	UAG	4.3		4.3	4.3	
Phe	4.8	UUU	4.8	4.8			
Pro	5.8	UCC	5.8				5.8
Ser	6.5	UUC	6.5	6.5			6.5
Thr	7.8	UAC	7.8			7.8	7.8
Try	----	UGG	----				
Tyr	3.9	UUA	3.9	3.9		3.9	
Val	10.0	UUG	10.0	10.0	10.0		
			139.0	50.8	90.9	73.4	62.9
Composition of template (including common U)				45.1	21.8	17.6	15.1
Composition of template (excluding common U)				18.3	32.7	26.4	22.6
Composition of *E. coli* 50S ribosomal RNA†				19.6	33.5	25.4	21.5

* Data of Sueoka.
† Data of Midgley.

in the triplet interpretation. If the composition of the product protein is known, it is simple to calculate the nucleotide composition of the template according to an assumed code. Table 26 shows such a calculation of the templates needed to direct the incorporation of amino acids into the proteins of *E. coli*. When the uridylic acid (U) common to all the triplet code words is included, the templates would include 45 per cent U. No RNA of this nature is found in *E. coli*. In fact, the template material of *E. coli* may be DNA-like or ribosome-like in composition, but clearly not of high U content. The same difficulty holds with other RNA's that can act as templates. The RNA of tobacco mosaic virus, which acts as a template in cell-free systems, does not have the high U content required by the present triplet code.

This failing has been rationalized on the theory that the code is highly degenerate and contains many other still undiscovered code words lacking U.

There is the further difficulty that a UC polymer containing 60 per cent nonsense words (according to the triplet interpretation) is very effective as a template. It is increasingly difficult to believe that enough code words low in U have remained undiscovered to compensate for the high U content of the words readily discovered.

If, however, the common U of the code words is discarded and the code is considered to be a doublet, these major failings disappear. The predicted templates for a number of bacteria (table 27) are strikingly close in their composition to the ribosomal RNA.

The same calculation applied to the protein of six plant viruses gives predicted templates somewhat like the RNA of the corresponding virus (table 28). Exact agreement would not be expected, because

TABLE 27. Comparison of RNA's

Organism	GC Content of DNA, %	Type of RNA	Mole per cent			
			U	G	A	C
B. subtilis	42	Template*	18.5	32.1	27.5	21.8
		50S†	19.3	32.0	26.5	22.5
		Newly synthesized†	23.7	27.3	25.5	23.5
E. coli	50	Template	18.3	32.7	26.4	22.6
		50S	19.6	33.5	25.4	21.5
		Newly synthesized	22.6	29.5	25.0	22.9
A. aerogenes	57	Template	18.1	33.4	25.2	23.2
		50S	21.2	31.2	25.6	22.0
		Newly synthesized	21.5	30.3	24.8	23.4
Ps. aeruginosa	65	Template	17.4	33.9	24.5	24.1
		50S	21.3	31.2	26.3	21.2
		Newly synthesized	20.5	31.9	21.4	26.2

* Hypothetical template calculated according to doublet code from amino acid analyses of Sueoka.
† Observed RNA compositions, Midgley.

TABLE 28. RNA of Viruses Compared with Template Calculated by Doublet Code

	Measured RNA Composition					
	TBSV	PV	CV	TYMV	SBMV	TMV
U	25	25	30	22	25	27
G	28	24	26	17	26	26
A	28	29	26	23	26	29
C	21	22	19	38	23	19
	Calculated Template					
	TBSV	PV	CV	TYMV	SBMV	TMV
U	21.8	20.4	27.0	20.8	21.2	22.8
G	30.2	27.5	25.2	21.4	28.2	28.4
A	21.4	25.3	19.0	25.8	23.4	21.4
C	26.6	26.8	28.9	31.9	27.3	27.4

TBSV, tomato bushy stunt virus.
PV, polio virus.
CV, cucumber virus.
TYMV, turnip yellow mosaic virus.
SBMV, southern bean mosaic virus.
TMV, tobacco mosaic virus.

only a part of the product of the template appears in the virus protein.

The doublet code obtained by discarding the common U of the triplet code is shown in table 29. The observed degeneracies were omitted. They can be attributed to errors in distinguishing leucine from isoleucine and valine, since these three amino acids are well known to be confused by the entry mechanisms in living cells. The order was chosen to fit amino acid replacement data.

It is striking that all the possible spaces are filled and that all the ambiguities lie on the purine pairs.

In addition to correcting the failings of

TABLE 29. Doublet Code

	G	A	C	U
G	gly try	glu gluN	ala	val
A	asp aspN	lys met	thr	ileu
C	arg	his	pro	leu
U	cys	tyr	ser	phe

the triplet code, this code has several other features. It is equivalent to the triplet code in predicting the incorporation of any amino acid relative to phenylalanine, since the ratio XUU/UUU = XU/UU. It is nearly equivalent again in correlating amino acid replacements, because the amino acid data assign U to a common position in the triplet code. The doublet code eliminates the unnecessary U and with it a large set of possible mutations that have not been observed.

The doublet code is not usually considered seriously because of the difficulty in imagining how 4 letters could provide more than 16 different words. Perhaps this view is too abstract, and the proper question is how many of the possible configurations of any doublet are sufficiently stable. The difficulty can still be avoided by a mixed code in which a purine pair indicates the beginning of a 3-letter word. Alternatively, the purine pairs could carry additional information.

At present no certain assignment of the coding ratio can be made. The pure doublet requires a mechanism for transmitting and preserving some as yet unrecognized property of purine bases; the triplet disagrees with well known data. Further experiments should eliminate one or the other or both in the near future.

Cooperation

The Biophysics Section has benefited greatly from collaboration both by visits of our staff to other laboratories and by visitors who carried out experiments here. Bolton spent six months working with Dr. R. E. F. Matthews in New Zealand and visiting numerous laboratories in Australia and Japan. Cowie spent three months working with Dr. Miranda at the Biophysics Institute, Rio de Janeiro, after visiting several leading South American laboratories.

We have continued our close relationship with Drs. L. B. and J. B. Flexner and follow with the greatest interest their experiments on protein synthesis in the brain. Visitors who carried out experimental work in our laboratory include Dr. McQuillen, University of Cambridge, England; Dr. Hotta and Dr. Van Holde, University of Illinois; Dr. Sager, Columbia University; Dr. Brown, Department of Embryology; and Dr. Hendler, National Institutes of Health. In addition, we have carried out collaborative experiments with Dr. Nirenberg of the National Institutes of Health.

Conclusions

Figure 56 summarizes our present conception of the flow of material in nucleic acid synthesis. To arrive at a diagram of such complexity it has been necessary to combine evidence from a variety of experimental approaches. The boxes enclosed in dashed lines show how the problem has been separated into analyzable parts, and the titles indicate the classes of evidence used to establish the existence of various elements shown. Low-molecular-weight (acid-soluble) compounds are shown by clear boxes, and macromolecular (acid-precipitable) by crosshatched boxes. The areas of the elements shown on the diagram correspond to the relative quantity of each present in a steadily growing cell.

There are four major regions on the diagram. Region 1 concerns the pool of low-molecular-weight RNA precursors. Although, of course, many chemical steps and a large number of pool compounds are involved, one dominant feature becomes clear from an examination of the incorporation of labeled nucleic acid bases into the pool and the total RNA. A major fraction of the compounds entering the cell are rapidly incorporated into RNA, bypassing the relatively large nucleoside-phosphate pools. Labeled compounds entering these pools do, however, ultimately become incorporated into RNA.

Region 2 concerns the steps in ribosome synthesis, or assembly, carrying the process only up to the 30S and 50S

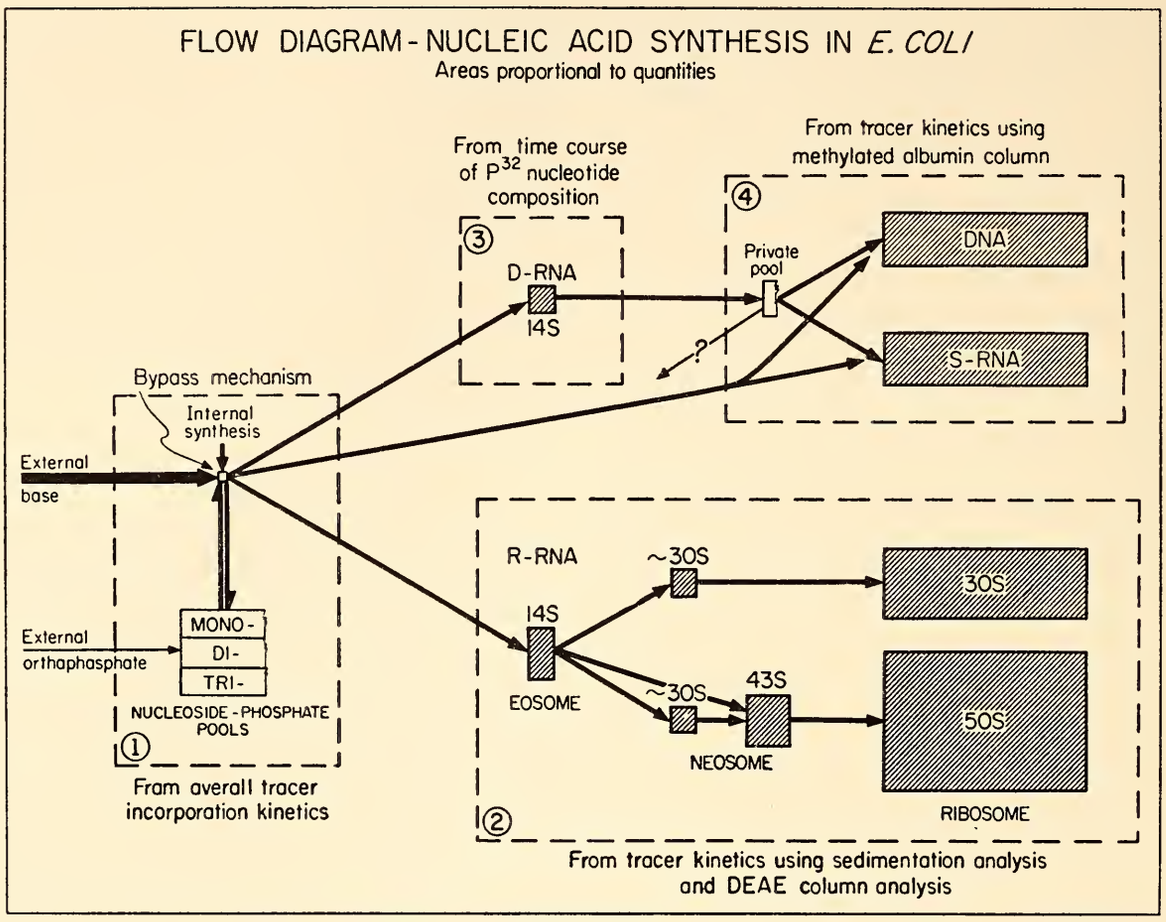

Fig. 56. Flow diagram for nucleic acid synthesis in *E. coli*. The areas of the boxes represent the relative quantities of the different elements present in exponentially growing cells. Open boxes represent low-molecular-weight (acid-soluble) compounds, and the crosshatched boxes represent macromolecular fractions. The dashed lines show how the pattern has been separated into analyzable parts, and the titles indicate the classes of evidence used.

subunits of the larger (70S, 85S, and 100S) ribosomes. Studies of the kinetics of labeling of fractions of RNA resolved by sedimentation analysis and DEAE column chromatography show that there is a set of sequential steps starting with the relatively small (14S) eosome and progressing to the completed ribosome.

It appears, however, that there are two distinct classes of RNA of about 14S, which are the first to be labeled and are not resolvable from each other by sedimentation analysis or column chromatography. This is shown by the fact that the apparent nucleotide composition of this fraction (pulse P^{32} labeling) does not correspond to that of ribosomal RNA. Further special procedures permit its partial resolution into two subfractions having, respectively, a composition similar to that of DNA (substituting uracil for thymine) and a composition similar to that of ribosomal RNA. These subfractions have been termed D-RNA and R-RNA, and the nucleotide composition of the 14S fraction in a number of bacteria indicates that their relative quantities are in the ratio of 1 to 2. The D-RNA has been placed on the diagram in box 3.

Since the nucleotide composition of the D-RNA differs from that of any major RNA fraction, and it is rapidly labeled, it must at least in part be degraded to low-molecular-weight fragments. The fate of these degradation products is somewhat uncertain. However, the entry of labeled compounds into the DNA and

S-RNA is delayed, and very likely these nucleic acids are in part synthesized from the degradation products. This evidence combined with various other arguments has led to the pattern of flows shown in box 4.

From the studies summarized in this diagram the quantity of newly synthesized RNA and its distribution between the D-RNA and the R-RNA components can be estimated. Two per cent of the total RNA is in the newly formed R-RNA, and 1 per cent is D-RNA. Both components are labeled with the same time constant of 2½ minutes.

A number of lines of evidence indicate that this newly formed RNA fraction carries the template for protein synthesis. Its lifetime is the same as that of the enzyme-forming unit for β-galactosidase, and Nirenberg has found that this fraction is the most active in stimulating protein synthesis in his cell-free system. There is no clear evidence, however, whether it is the D-RNA fraction (as was postulated by Jacob and Monod) or the R-RNA fraction or both that act as templates.

In table 30 we have calculated the rate of synthesis per template. Since it is not certain which fraction provides the template or whether the coding ratio is 2 or 3, the calculation has been carried out for two cases. For purposes of calculation the template has been assumed to have a length of 600 nucleotides. According to either model the rate of amino acid incorporation is rapid, 2–10 seconds to complete a peptide strand, or 10–30 milliseconds per amino acid if they are added sequentially.

The rate of RNA synthesis can also be related to the sites for its synthesis. If these are assumed to be the cells' DNA there are 15,000 sites of 600 nucleotide lengths per nucleus. The rate of D-RNA synthesis corresponds to the production of 2 copies of the entire complement of DNA during each generation. Thus the rate per site need only be 1 copy per 30 minutes.

A much faster synthesis of templates must occur during the induction of an enzyme. An increase in the rate of β-galactosidase synthesis can be observed within 30 seconds after the inducer is added. As an induced enzyme may account for 2 to 5 per cent of the cells' protein when fully induced we may assume that 2 per cent of the templates are required for that particular enzyme. If all these templates are made at one of the 15,000 sites the rate per site must be 600 copies per generation or 1 copy per 6 seconds. The factor of 300 from the minimum rate to the maximum corresponds to the ratio from the uninduced to the induced level of enzyme.

It seems reasonable that the templates for many of the cells' active enzymes

TABLE 30. Calculated Rate of Synthesis per Template

	Template Material			
	$E_D + E_R$		E_D	
Coding ratio	2	3	2	3
Templates per cell	5000	5000	1666	1666
Length of peptide	300	200	300	200
Time for peptide synthesis, seconds	10	7	3	2
Milliseconds per peptide bond	33	33	10	10
Average peptides per template	15	22	45	67

* Average template is assumed to contain 600 nucleotides.

would be synthesized at an intermediate rate between these extremes. If 1000 sites (1/15 of the total) were active and produced an average of 60 copies per generation this rate would be sufficient to account for the production of the R-RNA.

The questions as to which fraction of the RNA serves as template and how the activity as a template is terminated still require experimental answers. At present we can only speculate and try to find the most reasonable hypothesis consistent with the known facts.

One other fact is very pertinent in these speculations. The proteins of a series of bacteria having widely different DNA's and correspondingly different D-RNA's have very nearly the same amino acid contents. Thus if the D-RNA acts as template there must be a highly degenerate code to allow quite different templates to produce very similar products. The code as now worked out in cell-free systems has not shown the needed multiplicity of symbols.

The R-RNA fraction, on the other hand, is constant in composition, so that a single nondegenerate code would suffice if the R-RNA were the template for the greater part of the cells' protein. The doublet code does provide a correlation between the amino acid composition of the protein and the nucleotide composition of the R-RNA.

If it turns out that the R-RNA fraction can act as template, the termination of template activity could be ascribed to the covering of the template during the course of ribosome synthesis. More definite answers, based on experiments instead of speculation, should be available in the near future.

IMAGE TUBES FOR LARGE TELESCOPES

The activities of our Department include a vigorous participation in the development of photoelectric image tubes to extend the sensitivity and range of medium-sized and large telescopes, based on the fact that modern photoelectric surfaces are more than a hundred times more sensitive than the best photographic emulsions used in astronomy. The complete record of these activities, including the full-time work of Dr. W. K. Ford, Jr., of the DTM staff, and others, is found in the report of the Committee on Image Tubes for Telescopes, which follows.

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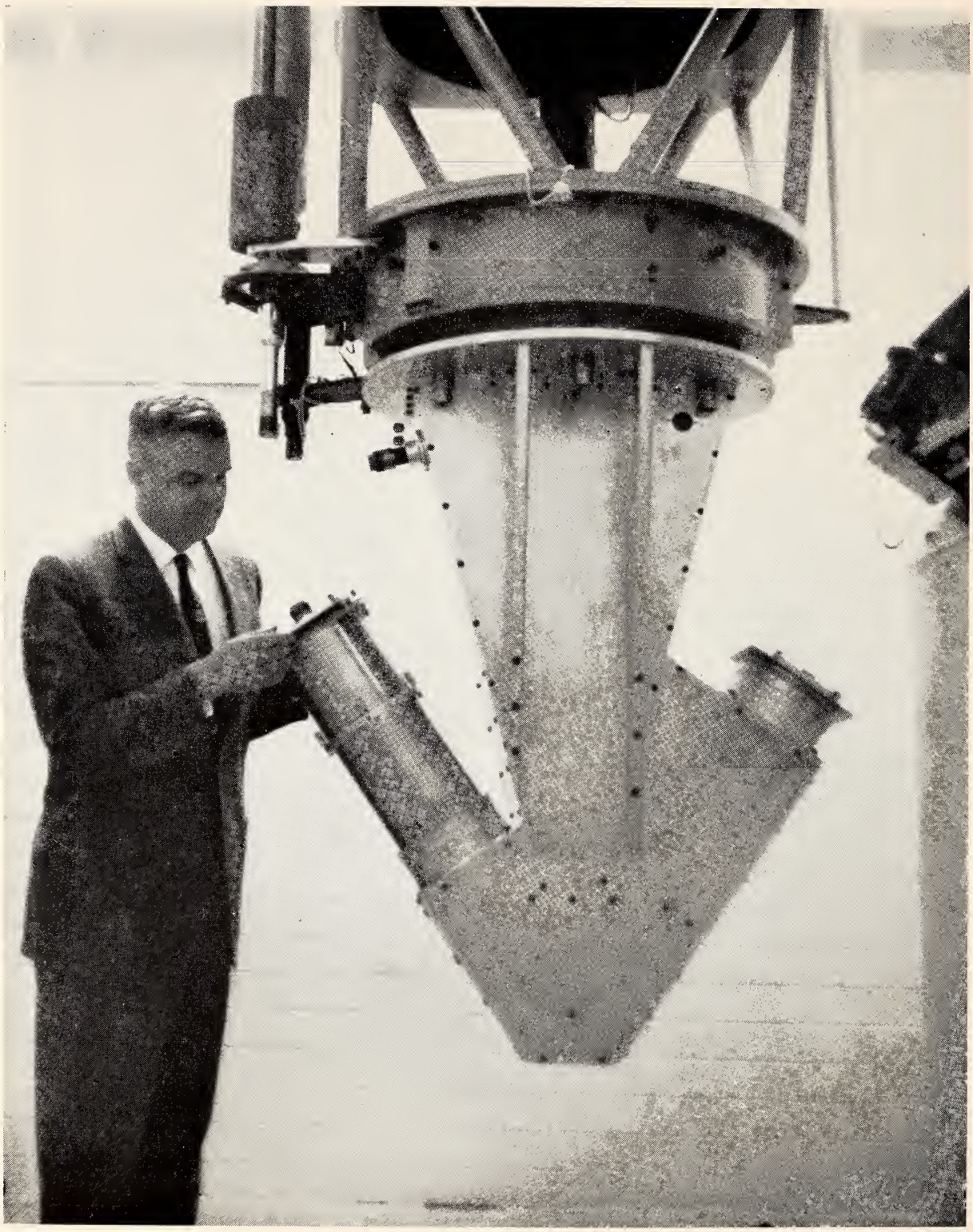

The DTM spectrograph on the Morgan 24-inch reflector, Lowell Observatory, Flagstaff, Arizona. A cascaded image intensifier system is mounted on the left side of the spectrograph. This unit consists of the spectrograph camera lens (just below the mounting flange), a cascade tube and focusing magnet (just above the flange), the relay lens system and plateholder (shown here with an eyepiece for viewing the phosphor screen).

INTRODUCTION

Improved samples of magnetically focused image tubes were tested and evaluated by the Carnegie Image Tube Committee during the report year. We have found that these tubes tend to have better operating characteristics than we had hoped for three years ago.

Our tests of these tubes have been made primarily with the DTM spectrograph on the Morgan 24-inch reflector at Lowell Observatory. In October 1961 and April 1962 a blue-sensitive cascaded tube made by ITT Laboratories was used to obtain good-quality spectra, but exposures were limited to 20 minutes by spurious emission. In March 1962 a mica-window tube made by RCA proved to be highly successful. It gave very good definition over 35 mm of spectrum. In April and early May an RCA cascaded tube with unusually high sensitivity was tested and compared with the tubes previously tested. Each of these tubes provided a gain of roughly 5 over direct, unaided plates.

While observations at the telescope demonstrated the reliability and effectiveness of these devices, laboratory tests were helpful in distinguishing the relative merits of the various tubes. In particular, resolution and screen quality were evaluated with an excellent test target designed by Dr. Baum.

On the basis of these spectrographic and laboratory tests, the Committee believes that both the mica-window and the cascaded tubes will have wide application in astronomy because of their advantages over conventional photography. To help meet the high cost of completing the development of these tubes the Committee applied for and has been awarded a new grant from the National Science Foundation.

APPLICATION OF IMAGE TUBES

Image intensifiers are of importance because of the exceedingly low light levels with which astronomers must work. Image intensifiers make use of the high quantum efficiency of the photoelectric process to allow information to be recorded more rapidly on conventional photographic emulsions or, in other applications, to allow a better measurement of light intensity to be made.

The advantages of the photoelectric type of image converter have been demonstrated primarily by Professor Lallemand and his colleagues. In his device, however, the operation of the tube occupies an undue amount of the astronomer's time. This Committee indeed was organized to explore possible ways of providing a simple and reliable alternative to the Lallemand tube.

The image tubes with which we are now concerned are completely sealed off and are reliable in operation under actual observing conditions as well as in the laboratory. Good photocathodes, once they are made, are well protected and have an indefinitely long life. Many of the electrostatic tubes evaluated by this Committee three or four years ago are still quite sensitive and are still being used on some problems such as double star photography, where the smallness of the field in good focus is not a serious handicap.

The first application that comes to mind, perhaps, for an image intensifier is the photographing of fields of faint stars. Here the brightness of the night sky limits the exposure that can be made with a fast optical system. To see fainter stars a longer-focal-length telescope is required, but with present instruments this involves going to focal ratios too large for the rather low quantum efficiency of photographic plates.

With image intensifiers, such as the

cascaded tubes made by RCA and ITT, long-focal-length telescopes too slow for photography can be used to record faint images against the background of the night sky. The long-focus instruments provide greater magnification, and the total number of photons from 1 square minute of the night sky are spread over a larger area. When exposures are made to a given optical density of the night-sky background with the aid of an image intensifier, a longer time is therefore permitted, there are more photo events in the still concentrated stellar image, and the signal-to-noise ratio is improved. Consequently, fainter stars can be detected.

Perhaps the most important application of image intensifiers in astronomy will be in obtaining stellar spectra. These devices, when perfected, should make it possible to obtain with telescopes of moderate aperture spectra that previously have been difficult to obtain with even the largest instruments. Moreover, image tubes are efficient in the infrared region of the spectrum, where emulsions are relatively insensitive.

TESTS OF SAMPLE TUBES

The image intensifiers tested were experimental tubes which are still being improved. Some of the defects in these first samples have been corrected in more recent tubes. The remaining shortcomings can, we believe, be eliminated with continued effort.

Infrared converter tubes. The infrared converter has an S1 photocathode with useful sensitivity from 0.7 to 1.4 microns. One such tube is the FW132 image converter made by ITT Laboratories (a picture of this tube appeared in last year's report). This compact tube is characterized by a high-quality S1 photosurface, a series of accelerating electrodes, and, finally, a phosphor screen capable of high resolution. Under optimum conditions, 50 line pairs per millimeter can be resolved with these tubes. A conventional camera with a fast relay lens system is used for photographing the phosphor screen. These tubes provide advantages over infrared photographic plates because of their improved resolution, their simplicity in operation compared with hypersensitization processes, and their efficiency compared with the infrared photographic sensitivity. Because conventional optics are used for photographing the phosphor screen, and since there is no internal multiplication of electrons, the quantum efficiency of the photocathode is not fully utilized in these tubes. Nevertheless, they are of value for photographing infrared fields (provided that moderately fast F ratios are available), for high-resolution spectroscopy where bright sources are involved, and finally for specialized work such as photographing the infrared coronal lines of the sun.

An infrared converter, FW132-1221, made by ITT Laboratories, was tested on the Morgan telescope in April and more extensively in October 1961. The tube had a good photocathode with a sensitivity of 4.5 μA/lumen to 2870°K light with a no. 2540 filter. The photocathode was cooled with dry ice to reduce thermionic emission, and, at 15 kv, 20-minute exposures on IIa-0 plates showed no spurious background. The relay lens was a pair of $f/1.5$ Zeiss Biotars mounted front-to-front.

With a 12-inch camera lens on the DTM spectrograph (2-inch collimator), spectra at a dispersion of 45 angstroms/mm at 1.0 micron were obtained at 15 kv. Typical exposure times at 1 micron on IIa-0 plates were 20 minutes for Jupiter and 20 minutes for β Andromedae (2.4 mag_v, MO III). The resolution of the system was better than 40 line pairs per millimeter.

The gain of this system relative to

infrared photographic emulsions is difficult to judge, owing to variations in sensitivity of the emulsions with hypersensitization. We estimate that roughly a gain of 30 in exposure time is achieved over typical I-Z hypersensitized emulsions, accompanied by a slight improvement in resolution and granularity.

Slightly greater gains could be achieved if the tube could be operated at higher voltages. ITT Laboratories has now developed an improved version of this tube, known as the FW167, which is twice as long as the FW132. This tube has not yet been tested.

Cascaded image tubes. In a cascaded image tube, photoelectrons from the first cathode are multiplied by a phosphor-photocathode sandwich; the "secondary" photoelectrons thus produced are imaged on a phosphor screen. The screen is then photographed with a relay lens system. The multiplication process is an efficient one, and resolution is limited principally by the granularity of the phosphor.

Several cascaded tubes made by ITT Laboratories have been tested at DTM, and the best of them, FW152-37, was used with the spectrograph on the Morgan telescope in October 1961 and April 1962. The resolution of this tube was close to 25 line pairs per millimeter. The cathode was 40 mm in diameter, but the Zeiss Biotar relay system was effective in imaging only a 25-mm field. The tube was focused with a permanent-magnet system and gave magnification close to unity.

At an operating voltage of 18 kv, exposures of 20 minutes could be made without the background due to spurious emission becoming excessive. There was no artificial cooling in these tests. The S11 photocathode had a sensitivity of about 40 μA/lumen, and the multiplication across the sandwich was approximately 12. Baked IIa-0 plates were used to photograph the phosphor screen.

Spectra were taken with a 12-inch camera lens which gave a dispersion of 22 A/mm at the photocathode. These spectra were compared with direct spectra taken with a 7-inch lens and were judged to be similar in quality. The speed gain of the image tube system in this comparison ranged from 3 to 5, depending on the quality of the seeing.

Work in progress at ITT Laboratories is directed now toward improving the background in this tube type to permit operation at higher voltages. Improvements have been made in the control of phosphor deposition, and more recent tubes have somewhat better resolution. Additional tubes are scheduled for early delivery.

RCA has also developed for us a two-stage cascade intensifier, the C70 056. This tube is similar in size to the ITT FW152 but has the voltage per stage divided between a series of four accelerating electrodes. This results generally in less spurious background. The cathode is S20 (multialkali) and is 40 mm in diameter. The tubes we have received have had P20 screens.

The RCA tube we have tested (C70 056-1) had 140 μA/lumen photosensitivity and a large electron gain across the sandwich. It was operated in a permanent-magnet system at an overall voltage of 16.5 kv. At this voltage the background due to ion scintillations limited exposures to 2 hours. On axis visual resolution of the system in these tests was slightly better than 20 line pairs per millimeter.

Test exposures were made with this tube with the 12-inch camera on the DTM spectrograph. The speed gain over direct spectra obtained with the 7-inch lens was about 5. The actual information gain was probably somewhat less, owing to the limited resolution of the intensifier system. Improved phosphors and less background due to ion scintillations (which will permit operation at higher voltages) will increase considerably the gain that can be obtained. Delivery of several more RCA cascaded tubes of slightly different types is in progress.

Mica-window tubes. A single-stage image converter with a phosphor screen

deposited on a thin end window provides an efficient method of image intensification. By pressing a photographic emulsion into contact with the window, good definition can be maintained, and most of the light produced in the screen by impinging electrons reaches the emulsion.

A magnetically focused mica-window tube (C70 026-6) made for the Committee by RCA, Lancaster, was tested by Ford and Fredrick with the DTM spectrograph on the Morgan telescope. This tube was similar in size to the ITT FW117 pictured in the Annual Report of the Committee on Image Tubes in *Year Book 60*. The mica window was 40 mm long by 2 mm wide and approximately 8 microns thick. The tube had an S20 photocathode (76 μA/lumen) and a P11 phosphor screen.

Preliminary tests had previously been made with ITT magnetic tubes with infrared photocathodes. Unfortunately the preliminary tubes were characterized by excessive field emission, optical distortion, and slumping photocathodes. The tube used in these RCA tests was the first one fabricated for the Committee that had acceptably low background and a reasonable sensitivity. Under optimum conditions, 45 or 50 line pairs per millimeter could be resolved visually on the phosphor screen. With Plus-X film, 25 line pairs could be resolved in contact exposure.

Exposures were made with a film transport mechanism built in the DTM shop. With this mechanism, pieces of 35-mm film, approximately 35 mm long, are loaded onto a cylindrical mandrel, and the mandrel is pressed into mechanical contact with the thin window. With the apparatus in its present form, six mandrels are available for preloading, and a single exposure is made on a piece of film. A box with six partitions and individual dark slides is available for storing the mandrels with film. Less than 1 minute is required to change mandrels between exposures. In the tests at Flagstaff, all exposures were made on Plus-X film developed in D76 for 5 minutes at 68°F.

Test exposures were made at 10, 12½, 14½, and 17 kv. At the higher voltages, scintillations were visible when the phosphor screen was viewed with a microscope. They are believed to be due to residual gas atoms becoming positively ionized, bombarding the photocathode, and releasing bunches of electrons. The scintillations due to these bunches of electrons could be recorded at 14½ kv on the Plus-X emulsion, and this limited the duration of the exposures. It was determined that 12½ kv approached an optimum operating voltage from the point of view of gain and background. At this voltage 1-hour exposures could be made. The magnetic field required to focus the tubes was provided by an array of cylindrical rod magnets, and the magnetic field was varied in strength by adding or removing rods. No attempt was made to minimize the "S" distortion of the image, and the magnification of the system was slightly greater than unity.

The first exposures were made with the 7-inch spectrograph camera, and were compared with direct plates made with the same camera. It is estimated that for wavelengths in the range 4000–5500 A the gain in exposure time for equal densities over 103a-G plates was 20 to 25 times, accompanied, of course, by a loss in resolution. This gain in exposure time for equal density is estimated for similar slit widths, widening, and seeing conditions.

A second series of exposures were made with a 12-inch spectrograph camera lens, giving a dispersion of 22 A/mm at the photocathode. These exposures were compared directly with direct photographs at 39 A/mm made with the 7-inch spectrograph camera. It was found that, with the mica-window tube and the Plus-X film, the spectra obtained with the 12-inch camera were comparable in quality to those obtained directly on 103a-G plates with the 7-inch lens.

The information gain in this system is estimated to approach 5 at 12½ kv. The

tube used had a 75-μA/lumen photocathode, which is somewhat less than average for the S20 photosurface.

The Carnegie Committee has two more tubes of this type on order from RCA. The prospects look good that they will be useful for astronomical observations. In addition, four tubes of similar type having infrared photocathodes are on order at ITT. One sample tube having a very good cathode has been received and will be tested soon.

ACKNOWLEDGMENTS

Many of the tests described in this report were conducted by Dr. Ford of the Department of Terrestrial Magnetism. The development of special tubes in industrial laboratories, under the direction of the Committee, is supported by a generous grant from the National Science Foundation.

Department of Plant Biology

Stanford, California

C. Stacy French
Director

Contents

INTRODUCTION

For the last few years there has been increasing interest in the functional nature of the two-pigment system now considered to be of basic importance in the process of photosynthesis. This year the subject has become the focal point of investigations in several laboratories. At the Department of Plant Biology a particularly striking illustration of a multipigment system was studied in isolated leaf chloroplasts. Dr. David C. Fork found that chlorophyll *b* was more effective than chlorophyll *a* for the evolution of oxygen by isolated spinach chloroplasts suspended in a medium free of added oxidants. This endogenous production of traces of oxygen by illuminated chloroplasts has been known for about eighty years but only recently was discovered to be due more to the action of chlorophyll *b* than to that of chlorophyll *a*.

In 1937 Hill found that oxidants like ferricyanide added to chloroplast suspensions would tremendously increase their capacity to evolve oxygen. This process, in contrast to the endogenous reaction, is driven by chlorophyll *a* and chlorophyll *b* about equally. Since Hill's discovery very little attention has been given to the slower and short-lasting endogenous process.

When chloroplasts without added reagents are first illuminated the rate of oxygen evolution is reasonably high, but it drops in a few seconds to a very low value. Presumably some material in the chloroplast structure is used up by the light reaction. The ability to give off oxygen is regenerated by storage in the dark for some minutes. Dr. Fork found furthermore that the recovery process is greatly hastened by far-red light. The participation of two pigments in the endogenous evolution of oxygen by chloroplasts is therefore particularly clear. Chlorophyll *b* drives off oxygen from some intermediate which is regenerated by a pigment with a longer-wavelength absorption band. This intermediate reaction, which may turn out to involve plastoquinone, or perhaps the copper protein plastocyanin, appears to be driven to the reduced state by chlorophyll *b* and to the oxidized state by the far-red pigment. The effectiveness of different wavelengths in driving the reaction in one direction or the other is complicated, therefore, by the overlapping of the absorption bands of the two pigments which have opposite effects.

Up to the present time the identification of a specific pigment as responsible for a measurable photochemical event connected with the photosynthesis process has depended mainly on matching the wavelengths of peaks in the action spectrum to the wavelengths of absorption maxima of known pigments. However, the action spectrum for a reaction being driven in opposite directions by two pigments may be so complex as to make the identification of the functional pigments through their characteristic absorption spectra impossible.

It is becoming evident that the diverse photochemical functions of different pigments as well as the shapes of their absorption curves must be better understood in order to account for the various action spectra measured for processes related to photosynthesis. Advances in the study of action spectra require further information about both the light-absorbing and the photochemical properties of the various functional plant pigments.

The lack of sufficient information about the absorption spectra of the different pigments and the specific reactions driven by them has become emphasized through the attempt to find a satisfactory explanation for two additional action spectra determined by Dr. Fork for other light effects in leaf chloroplasts. The four effects giving very different action spectra

with the following characteristic peaks in the red region were: the evolution of oxygen (650 mμ, 680 mμ shoulder); the regeneration of the ability to do so (730 mμ); the uptake of oxygen, apparent when the oxygen-evolving system is poisoned (690 mμ); and the evolution of oxygen from chloroplasts with added ferricyanide (678 mμ, 650 mμ shoulder).

Of these peaks only two can be identified with a reasonable degree of certainty. The 650-mμ action peak is attributed to chlorophyll *b*, and that at 678 mμ to chlorophyll *a*. However, it is not yet certain whether both the 670-mμ and the 683-mμ absorbing forms of chlorophyll *a* contribute to the 678-mμ action maximum for the evolution of oxygen when ferricyanide is added. Furthermore, this action spectrum, for this reaction, which is also typical of green plant photosynthesis, shows structure characteristic of two pigments. The maximum is at 678 mμ, but a shoulder at 650 mμ shows that chlorophyll *b* as well as chlorophyll *a* is involved. Since two chlorophylls, *a* and *b*, are believed to drive separate steps of the overall reaction, the relative heights of the maxima in action spectra should be capable of experimental variation. This could in principle be accomplished by varying the amounts of the intermediate substances linking the two photochemical reactions or by the partial inhibition of the reaction rates of certain nonphotochemical steps in the process.

A further hope for the future is that it may be possible to add certain intermediates to disintegrated chloroplasts in such a way that the action of the separate photochemical steps may be clearly distinguished. So far the attempts to do this in other laboratories have resulted in rather complex action spectra indicating that the separation of the effects of the individual pigments has not been complete.

The importance of the complete separation of one effect from another lies not only in elucidating the chemical nature of the individual steps but also in using the action spectra to identify the fractional absorption of each pigment at various wavelengths. By simple absorption spectroscopy, and even with derivative spectroscopic measurements, it has not been possible to determine the shape of the complete absorption curves of the individual pigments. Perhaps in the future more precise measurements and deeper understanding of action spectra for the various photochemical effects of different pigments may be used to extend the now very incomplete knowledge of the absorption spectra of the functional plant pigments in their natural state.

The need for more specific information about the shapes of the complete absorption spectra of the individual pigments is illustrated by the action spectrum for oxygen uptake by poisoned chloroplasts. Is the 690-mμ peak to be attributed to a particularly active form of chlorophyll *a* with its maximum at that wavelength? Or is the position of this maximum determined by the wavelength of minimum overlap between two other forms of chlorophyll having opposing functions? Furthermore, what is the absorbing entity giving the maximum recovery of the oxygen-evolving capacity of chloroplasts at 730 mμ? Does this wavelength correspond to a pigment like phytochrome, or could 730 mμ perhaps be the wavelength at which the ratio of the absorption by two pigments most strongly favors one of them? Such questions are not easy to answer with certainty, yet they are of the greatest significance in understanding the functional relations of the pigments responsible for photosynthesis.

To study further the relation between the two light reactions in the red alga *Porphyridium*, which was reported on last year, measurements were made with two light beams of different colors separated in time. Red light absorbed by chlorophyll *a* and green light absorbed by phycoerythrin were given in flashes of a few seconds' duration. A green flash was found more effective in oxygen produc-

tion if it was immediately preceded by a red flash. The material produced by red light which enhances the succeeding green flash had a half-life of about 18 seconds. Within the time intervals studied, however, the yield of oxygen from a red flash was slightly decreased rather than enhanced by a previous exposure to green light. Such studies can certainly be used to evaluate the lifetimes of intermediate products disappearing in side reactions and may give information on relative quantities of the several intermediates.

Flash experiments may have only a limited value in telling which photochemical reaction comes before another. A cyclic process has no "first step" from the point of view of the permanent machinery. The concept of a first step is reasonable with respect to material flowing through the system, such as a labeled compound. For the cycling part of the system, however, the idea of a first step has significance only in deciding which intermediate piles up in a ready state during a dark period.

Last year we used a particular model scheme to compute hypothetical time-course curves for the relative rates of oxygen exchange of illuminated photosynthetic systems. This scheme gave reasonably realistic results. The model differs from others, currently popular, in predicting that the time-course curve for gas exchange immediately following a light exposure should have a second-order shape which should vary with the color of the light used, whereas the other models predict a first-order decay curve whose shape should be independent of the light color.

The difficulty in settling this question experimentally lies in the fact that concomitant with oxygen evolution there is also the production of variable amounts of easily oxidizable material. The increased rate of oxygen uptake by this material after a light period complicates the study of the shape of the decay curve for the lingering oxygen evolution. Some experiments are in progress in the hope of finding a way around this difficulty.

Dr. J. S. Brown has studied how greatly the conditions of growth and measurement influence enhancement and photostimulated oxygen uptake in the red alga *Porphyridium*. The initial values in a series of measurements are strongly affected by the light used for culturing the algae. During the first 5 hours photosynthesis and enhancement may vary inversely; the photostimulation of oxygen uptake declines. After 5 hours all three processes reach constant values.

Dr. Brown also found that the 710-mμ absorbing compound of aged *Euglena* cultures leaks out of the chloroplasts into the cytoplasm. The 685-mμ absorbing form of chlorophyll, however, remains in the chloroplasts. The 670-mμ form of chlorophyll *a* accompanies the 710-mμ pigment. In collaboration with Dr. J. H. C. Smith she found the 710-mμ pigment to be pheophorbide *a* or a closely related compound. It arises from the 695-mμ form of chlorophyll *a*.

An investigation of the production of free electrons induced by light in the green alga *Chlamydomonas* started in Zurich by Drs. Harry and Ellen Weaver was continued at the Department in collaboration with Varian Associates. The measurements were made by the electron paramagnetic resonance method. Exposure to light produced a broad slowly disappearing signal and an intense narrow rapidly disappearing signal. The action spectrum for the narrow signal approximated the absorption spectrum of chlorophyll *a*. These observations agree with recent work in other laboratories. Earlier results on action spectra had been distorted by using preparations with too great absorption.

For the narrow rapidly decaying signal the *g* value was 2.0025, which is close to that for a pure conduction electron. The half-width was 8.3 gauss. For the slowly decaying signal the *g* value was 2.0046 and the half-width was 20 gauss. This signal appears to be due to plastoquinone.

The Experimental Taxonomy group is attaining increased experimental precision in different aspects of the study of plant relationships. Several races and species of *Mimulus* are being used for comparative growth studies at the altitudinal field stations, for cytogenetic analysis, for controlled growth chamber experiments, and for quantitative comparisons of photosynthetic rates under varied conditions. All these studies lead to an improved understanding of evolutionary mechanisms and of natural selection in different environments.

One of the most striking observations in the transplant work is the enhanced vigor in first-generation hybrids between climatic races of the same species. The vigor shown by such a hybrid depends on the environment in which it is grown and on the relation of that environment to the native climates of its parents. The approximate degree of genetic difference needed between two climatic races to yield first-generation progeny capable of surviving over a wider range of climates than either parent is now being systematically studied in *Mimulus*. This basic question has not been adequately examined experimentally before. Reciprocal crosses have been made between each pair of eight key races native to eight different altitudes. The hybrids and parental races are being established for observation this year as cloned transplants at the three altitudinal transplant stations.

Milner and Hiesey have completed an examination of the photosynthetic response of six races of *Mimulus cardinalis* originating from different climates. The measurements covered a temperature range from 0°C to the high temperature at which apparent photosynthesis ceases, about 50°C.

The light intensity required to saturate photosynthesis varies from 3700 to 5900 footcandles for the different races at 40°C, and from 300 to 600 footcandles at 0°C.

All six races showed their maximum photosynthetic rate at 30°, and differences between races were small from 20° to 40°. Racial differences became apparent at the low and high ends of the temperature range. At 0° the photosynthetic rates of the six races vary from 11 to 20 per cent of the maximum rates. At high temperature, apparent photosynthesis becomes zero at 46° for one race, 47° for three races, and 49° for the other two.

The races also differ in their ability to maintain a high rate of photosynthesis for a long time. Measurements of continuous photosynthesis for 12 hours under optimum light intensity and temperature were made. At the end of this time the rates for the different races were from 92.5 to 74 per cent of the maximum rates.

Much greater variability was found for the rates of carbon dioxide production in the dark than for rates of its photosynthetic uptake in the light. All the photosynthetic rates referred to above are rates of apparent photosynthesis, uncorrected for the rate of carbon dioxide evolution in the dark. Because of the intrinsic interest in the latter value, however, it was measured many times during the course of the work.

A single measurement of the rate of dark carbon dioxide evolution is found to be of doubtful value, and making enough measurements to provide a trustworthy mean is very time consuming. For these reasons, and also because the rate of dark carbon dioxide evolution reflects the operation of other processes besides respiration, we question the validity of "correcting" photosynthetic rates of *Mimulus* leaves for it.

Studies on the growth and development of *Mimulus* in controlled growth chambers by Hiesey and Milner are being coordinated with the quantitative measurements of their photosynthetic rates. At present the first objective—developing an effective working laboratory for this type of comparison—has been achieved. The marked effects of the interaction of temperature, light intensity, and carbon dioxide concentration on the

growth and development of *Mimulus* clones are being studied. Clones of distinct climatic races show differential patterns of response that undoubtedly are linked with differences in internal physiology.

From the light-saturation and temperature curves derived from short-term quantitative measurements, predictions can be made about expectations from growth cabinet experiments lasting several weeks to months. Some such predictions have been supported by experimental results, but others have been reversed, indicating the influence of other factors that remain to be examined. Enhancement of growth has been observed by enriching the carbon dioxide in the atmosphere, the degree of enhancement differing with races.

An important step was made this year by Mrs. Ruth Elliott in establishing tissue cultures from *Mimulus* clones that are also being used for related studies. The significance of the work with cultures of parts of plants is that quantitative growth and photosynthesis measurements can be made of tissue from races with contrasting environmental requirements. It should thus be possible to examine the physiological requirements of tissue from the various organs of plants as well as for the entire plant to localize the site of the physiological differences that determine survival or extinction of the races.

We are collaborating with Dr. Axel Nygren, Dr. Olle Björkman, and Dr. Paul Holmgren of the Institute of Plant Systematics and Genetics at Uppsala, with whom Dr. Nobs has been working during most of the year. The Swedish group works mostly on races and species of *Solidago*, a goldenrod genus common to North America and to Europe. Although *Mimulus* and *Solidago* belong to different plant families, both are well suited to a wide variety of parallel experimental studies and are of particular value for cross comparison in two distinct and complementary climatic regions of the world.

Findings of considerable interest have been made by Drs. Björkman, Holmgren, and Nobs on two races of *Solidago virgaurea*, one a shade race from Sweden at 56°N and the other an alpine race from Norway at 69°N. The two races have very different photosynthetic responses to different light intensities. The northern alpine race has a much higher requirement for light saturation than the southern, and its chloroplasts remain normal when exposed to a light intensity of approximately 3500 footcandles in contrast to the southern race whose chloroplasts disintegrate at this moderately high light intensity. The leaves of the southern shade race become twice as large when grown in light of 700 footcandles as when grown in light of 3500 footcandles. The direction of the corresponding leaf modifications in the northern race is just reversed. The rates of photosynthesis measured under controlled conditions on the modified leaves of both races are also changed, but the significant racial differences remain.

Dr. Clausen has been reviewing some of the earlier data on *Poa*, completing records on the fertilities and performance of interspecific hybrids. The older data are being supplemented by some new observations from current field plantings made at Stanford. Some of the tentative conclusions about the fertilities and characteristics of hybrid progenies, arrived at during earlier years on the basis of less complete information, are being reexamined. The unpredictable outcome of interspecific crossings between species and races of the highly polyploid and predominantly apomictic species of *Poa* becomes increasingly evident.

PERSONNEL

Biochemical Investigations

Staff: C. Stacy French, *Director*, Jeanette S. Brown, David C. Fork, James H. C. Smith, *Emeritus*
Visiting Investigators: Paul Latimer, Ellen C. Weaver
Technical Assistants: Robert A. Clair, Harriet M. Fulk

Experimental Taxonomy

Staff: Jens C. Clausen, *Emeritus*, William M. Hiesey, Harold W. Milner, Malcolm A. Nobs
Visiting Investigators: Thomas R. Pray, Henry J. Thompson
Summer Research Assistants: Steven N. Gilborn, Andrew N. Lenz
Technical Assistants: Ruth F. Elliott, Frank Nicholson
Clerical Assistant: Marylee H. Eldredge
Gardeners: Joseph S. Chang, Emmett R. Clagg, Wesley B. Justice

Department Secretary

Wilbur A. Pestell

Administrative Assistant

Wiley Knight, Jr.

Mechanic

Richard W. Hart

Custodian

Jan Kowalik

Wilbur A. Pestell retired on June 30, 1962, after 42 years with the Institution. During this period he was in the Division of Publications in Washington; secretary in the Desert Laboratory at Tucson, Arizona, and at the Coastal Laboratory at Carmel, California; and since 1929 secretary of the Department of Plant Biology at Stanford, California.

Dr. Jens C. Clausen was made a Knight of the Order of Dannebrog by the King of Denmark in October 1961. In July–August 1961 Dr. Clausen presented a series of five lectures on evolution at the Summer Institute for College Teachers of Botany, Washington State University, Pullman, Washington.

Drs. James H. C. Smith and C. S. French spent the summer of 1961 visiting European laboratories of plant physiology and biochemistry that are concerned with the study of photosynthesis, and attended international congresses on the subjects of biochemistry, biophysics, and plant physiology.

Dr. David C. Fork spent the month of March in the Photosynthesis Laboratory of Professor A. Moyse at Gif-sur-Yvette near Paris. There he collaborated with Mr. Y. de Kouchkovsky, who had also been working on the endogenous evolution of oxygen from isolated chloroplasts. Since then Dr. Fork has been at the Department of Physical Chemistry of the Philips University in Marburg to work with Dr. H. T. Witt. He has been applying Dr. Witt's methods for measuring the changes in the absorption spectra of cellular components, induced by light, to algae and chloroplasts, whose time course of oxygen evolution he has previously analyzed. This work is expected to clarify further the relations between the pigment systems and several of the intermediate compounds involved in the process of oxygen evolution.

Dr. Malcolm A. Nobs worked at the Institute of Plant Systematics and Genetics at the Royal Agricultural College of Sweden from October 1961 to June 1962 in collaboration with Dr. Axel Nygren, Dr. Olle E. Björkman, and Dr. K. Paul Holmgren. The program of our Experimental Taxonomy group on the comparative physiology of climatic races and that of the Uppsala group are closely related. Dr. Nobs' time in Sweden was devoted to comparative studies of physiological characteristics of latitudinal species and races of *Solidago*, native both to North America and to Europe. The

newly constructed phytotron at Uppsala is being used for this purpose. The findings of the Uppsala group in *Solidago* and those of our group in California on altitudinal and latitudinal races of *Mimulus* are of much mutual interest because the two cover distinct but complementary regions of the world.

EXPERIMENTAL TAXONOMY INVESTIGATIONS

New Vistas in Experimental Taxonomy

William M. Hiesey, Harold W. Milner, and Malcolm A. Nobs

Current developments in techniques, and new information from long-term experiments begun years ago, extend the horizon of experimental taxonomy so markedly that a reevaluation of what this field now encompasses appears to be timely. The basic objectives formulated during earlier years remain the same; but the leverage afforded by more precise means of investigation opens exciting fresh avenues for the experimental study of plant relationships.

The entire spectrum of expression of higher plant species, ranging from the multitudinous aspects they present to the observer in the wild to the detailed analysis of the functioning of their many component parts down to the cellular level, is now within reach of systematic experimental study. In prospect is a truly integrated plant science whereby contributions from the various specialized fields, including taxonomy, ecology, cytology, genetics, physiology, developmental morphology, and biochemistry, can be incorporated in a panoramic view of plant relationships and evolution not only scientifically satisfying but also aesthetically inspiring.

A restatement of objectives. The enlarged perspectives in experimental taxonomy call for a revised statement of its objectives. The idealized goal toward which we are working is a fully integrated understanding of the chain of mechanisms that underlie the end products of plant evolution in terms ranging from those used in purely descriptive classification to those of genetics, developmental morphology, physiology, and biochemistry. The mechanisms are studied in successive steps, starting with populations of plants in their native habitats and proceeding to more sharply focused stages down to the cellular level.

The experimental steps. The importance of studies on the growth and development responses of plants from diverse natural habitats when grown at the altitudinal field stations is well established from earlier publications, and such studies may be considered to be the first step. The necessity for cytological and genetic investigations has likewise been demonstrated through numerous examples; they are the second essential step. The inclusion of experiments in controlled environments and of quantitative physiological measurements, as outlined in *Year Book 58*, pages 344–346, constitutes a third vital step. The fourth step—study of detached tissues of higher plants grown under aseptic conditions—has been started during the current year.

All four steps are directly linked through a common basic unit of study, the cloned individual, which assures genetic identity. Clones from the virtually unlimited diversity of climatic races of the same or of different species that occur in the wild, or of controlled hybrid combinations between them, are the experimental materials required for supplying the information needed to piece together the overall picture.

Methods and materials. Each of the experimental steps needs to be examined in considerable detail to bring to light the innumerable bits of information essential for assembling the comprehensive picture of relationships and evolu-

tionary mechanisms that we seek. Data need to be reexamined from many points of view before their place and significance in this picture can be evaluated. The importance of selecting materials fulfilling the exacting requirements for such studies has been emphasized in previous year books (*Year Books 46*, pp. 103–104; *53* 157–158), together with reasons why our current work is centered principally on latitudinal races of the *Mimulus cardinalis-lewisii* complex.

The possibilities of utilizing other plant groups are also being kept in mind. Especially promising from the point of view of comparing the physiological characteristics of ecological races from contrasting latitudes are forms of the goldenrod, especially the *Solidago multiradiata-virgaurea* complex of North America and Europe. Dr. Nobs has spent the greater part of the current year working on this group of plants at the Institute of Plant Systematics and Genetics at Uppsala, Sweden, where, in cooperation with Dr. Axel Nygren, Dr. Olle E. Björkman, and Dr. K. Paul Holmgren, he has been studying rates of photosynthesis of races of *Solidago* from Scandinavia from 56° to 69°N latitude, and from California at 38° to 39°N.

Transplant Station Activities

Malcolm A. Nobs, Jens Clausen, William M. Hiesey, and Frank Nicholson

The systematic testing of various combinations of F_1 hybrids between different altitudinal and latitudinal races of the *Mimulus cardinalis-lewisii* complex mentioned in last year's report (*Year Book 60*, pp. 381–382) has been carried to the garden planting stage this year. Cloned individuals both of parental and of hybrid plants have been established at Stanford, Mather, and Timberline. In most combinations, reciprocal F_1 hybrids are included in the tests as well as progeny resulting from selfing the parents.

The preliminary evidence indicates that F_1 hybrids derived from crosses between the same race of *M. lewisii* and different races of *M. cardinalis* from low and middle altitudes have markedly different capacities to survive at the three altitudinal stations. From the new plantings it should be possible to establish approximately the degree of genetic differentiation needed in parental forms to confer a given amount of tolerance to progeny for survival in contrasting climates.

Cloned propagules of the same parental and hybrid plants used in these altitudinal tests have been sent to Dr. Robert K. Vickery, Department of Botany, University of Utah, Salt Lake City. He and his associates are studying the flower pigments by paper chromatography in an effort to determine the mode of inheritance of the various chemical constituents that govern flower color variations in this group of plants. Flower color, as described in *Year Book 57*, pages 270–271, is one of the morphological characters that appear to be linked with capacity for survival at the transplant stations in segregating F_2 progeny resulting from crosses between coastal *M. cardinalis* and alpine *M. lewisii*.

Seeds and living plants of a race of *Solidago multiradiata* from Umiat, Alaska, were supplied to us by Dr. John Koranda of the Alaska Agricultural Experiment Station at Palmer, Alaska. This race will be studied as transplants at our altitudinal stations. Plants of California forms of *Solidago* have, in turn, been sent to Dr. Koranda for study in Alaska. Information about the responses of latitudinal ecotypes transplanted between California and Alaska is almost wholly lacking. These exploratory plantings may serve to chart the way for more comprehensive efforts later.

Some new plantings of parental and hybrid derivatives of *Poa* have been made at Stanford to clarify questions about the relative fertilities and degrees of apomixis in interspecific combinations made in earlier years. At the altitudinal stations some observations are being

continued on cloned transplants of key parental and apomictic hybrid strains established as early as 1946. The new data supplement the now extensive information obtained from the *Poa* studies in previous years.

During the latter part of the summer of 1961 Dr. Clausen carried on observational vegetational studies in the Harvey Monroe Hall Natural Area. They were aimed primarily at determining vegetational patterns associated with differences in microclimates, soils, and terrain in this typically high Sierran region. Floral components both of circumboreal and of more southern origin meet in this area. Observational studies of this kind help to orient experimental work not only with alpine but also with lowland plants.

Visitors at the transplant stations during the current year included Dr. John Koranda of the Alaska Agricultural Experiment Station at Palmer and staff members and graduate students from the University of California at Davis headed by Dr. Charles M. Rick. A class of graduate and undergraduate students in botany from Stanford under the guidance of Dr. John Thomas and Mrs. Roxana S. Ferris of the Department of Systematic Biology conducted field work at Mather during the spring of 1962. Dr. Alexander Sokoloff of the Department of Genetics of the University of California, Berkeley, collected *Drosophila* material at Mather for Dr. Th. Dobzhansky, who is continuing his studies with species and races from along the Sierran Transect at the Rockefeller Foundation.

Physiology of Climatic Races

Harold W. Milner, William M. Hiesey, and Malcolm A. Nobs

Different climatic races of the *Mimulus cardinalis-lewisii* complex show different responses in their rates of photosynthesis to changes in light intensity, temperature, and duration of continuous photosynthesis. Following the lines described in earlier year books, we have now completed the examination of six races of *M. cardinalis* over a temperature range of 0° to 50°. The results are being prepared for publication and are summarized here.

Light intensity. The photosynthetic rate of each plant was determined at high light intensity and then at a series of intensity levels each 80 per cent of the preceding intensity. The true light saturation level lies between the last measured intensity at which the maximum photosynthetic rate was maintained and the next lower intensity where a decrease in rate was found. This procedure was followed at each 5-degree temperature interval from 0° to 40°. At each temperature the light intensity levels between which saturation occurs were plotted. A smooth curve was then drawn to fall within the appropriate light levels at each temperature. Above 40° the photosynthetic rate is not stable over the length of time required to make satisfactory light saturation measurements.

Light saturation curves were determined for three clones each of the Los Trancos, San Antonio Peak, Jacksonville, Priest's Grade, and Yosemite races and for five clones of the Baja California race. Within each race the clones showed a very similar response of light saturation to temperature.

Mean values for the clones of each race are shown in figure 1 to represent the light saturation requirement of the races. The Los Trancos and Baja California races have the lowest light saturation, from 500 footcandles at 0° to 3700 footcandles at 40°. At these temperatures the range in footcandles for the other races is: San Antonio Peak, 600 to 4300; Jacksonville, 400 to 4800; Priest's Grade, 300 to 5700; and Yosemite, the highest, 600 to 5900. The saturating light intensity at 0° relative to that at 40° ranges from 5 per cent for the Priest's Grade to 14 per cent for the San Antonio Peak race.

The effect of high and low light intensity at different temperatures over a period of weeks was tested on plants in

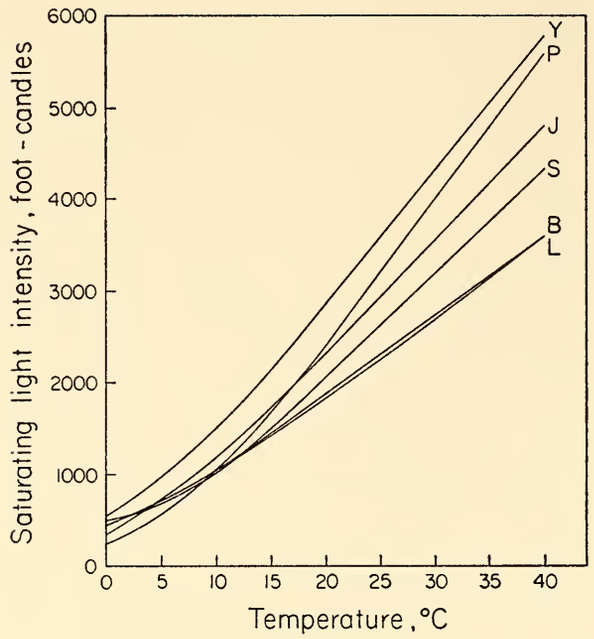

Fig. 1. Saturating light intensity versus temperature. The curves are identified by the initial letters of the race names: Yosemite, Priest's Grade, Jacksonville, San Antonio Peak, Baja California, and Los Trancos. Reading down at 0° the curves are *Y*, *S*, *B* and *L* (equal), *J*, and *P*.

controlled growth cabinets. The results with clones of the Los Trancos and Priest's Grade races are described in the following section of this report.

Temperature. The rate of light saturated photosynthesis was measured at 5-degree intervals from 0° to 40°, then at 1-degree intervals from 40° to the temperature at which apparent photosynthesis became zero, the temperature compensation point. These measurements were made on the same plants that were used for the light saturation measurements described above. The six races have their maximum rate of photosynthesis at 30°. This is true for 19 of the 20 clones. The exception is a Los Trancos clone that has a slightly higher rate at 35° than at 30°.

Between 20° and 40°, where photosynthesis is about 75 per cent of its maximum rate, there are small differences between the curves of the six races. At the low and high ends of the temperature range the races show considerable differences in their ability to photosynthesize effectively. Figure 2 shows these differ-

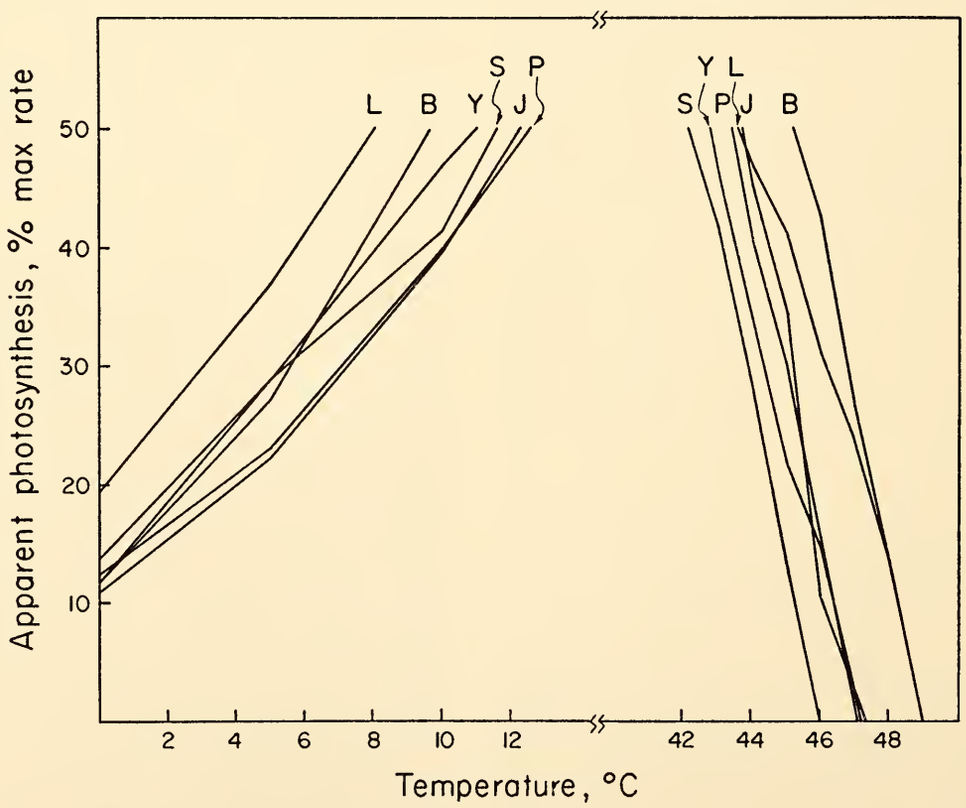

Fig. 2. Rate of light-saturated photosynthesis versus temperature. Identification of the curves same as in figure 1.

ences on an expanded scale by omitting the top half of the rate curves and the rates between 12° and 42°. The photosynthetic rate at 0° varied from 7 to 27 per cent of the maximum rate among the 20 clones. The mean values for the clones of each race were about 20 per cent for the Los Trancos race and between 11 and 14 per cent for the other five races. At low temperatures the Los Trancos race has the highest photosynthetic rates, Priest's Grade and Jacksonville the lowest. Above 40° the Baja California race has the highest rates, and San Antonio Peak the lowest. The temperature compensation point (mean of the clones) is 46° for the San Antonio Peak race; 47° for Priest's Grade, Jacksonville, and Yosemite; 49° for Los Trancos and Baja California.

Continuous photosynthesis. Each plant was allowed to photosynthesize continuously for 12 hours under saturating light intensity and at constant temperature. The photosynthetic rate was measured every 5 minutes. All the plots of rate against time in these experiments have the same general shape. The rate rises to its maximum, then decreases during the remainder of the 12 hours. The curves differ in the time taken for the rate to reach its maximum and in the extent of the subsequent decrease in rate. Each plot shows some random fluctuations of several per cent in rate during the time after the maximum is passed. There is wide variation in the amount of such fuzziness observed with different clones. This effect tends to conceal the true trend of the curves.

Comparison and evaluation of the curves were facilitated by applying a smoothing procedure to them. At each 5-minute point on the time scale we plotted the mean of the five rates determined in the interval from 10 minutes before to 10 minutes after the designated point. This largely eliminated the sawtooth appearance of the original curve and made the trend readily visible. Comparison of different curves was further improved by plotting each of the above mean rates as percentages of the maximum rate.

Continuous light saturated photosynthesis was measured for 12 hours at several temperatures on the three Los Trancos and three Yosemite clones. Within the probable reproducibility of the curves, no difference in shape was observed at 20°, 25°, 30°, and 35°. Therefore, the clones of the other races were all run at 30°, the temperature giving the maximum photosynthetic rate.

The body of data now available indicates that the rate versus time curve during 12 hours' continuous photosynthesis is not a critical measurement for detecting small differences between the performance of various *Mimulus* clones. Duplicate curves for the same clone may differ as much as those made on different clones of the same race.

Mean curves were calculated for the clones within each race. These curves, illustrating the performance of the six races, are shown in figure 3. To make the small differences between the curves visible, only the top third of the vertical scale is shown in the figure. There is a marked difference, 0.5 to 3.5 hours, in the time taken by different races to reach their maximum photosynthetic rate. The least time is taken by the Priest's Grade race, followed by the San Antonio Peak and Jacksonville races in the first hour. The Yosemite race reaches its maximum rate in about 1.5 hours. The Baja California and Los Trancos races require 3.0 and 3.5 hours, respectively, to reach their maxima.

After reaching its maximum, the photosynthetic rate of each race declines in an irregular fashion during the rest of the 12 hours. The least loss in rate is shown by the Los Trancos race, which at the end of 12 hours photosynthesizes at 92.5 per cent of the maximum rate. There is probably no real difference between the curves for the Priest's Grade and the Jacksonville races. They follow nearly the same course during the 12 hours, ending at 87 and 85 per cent of the maximum

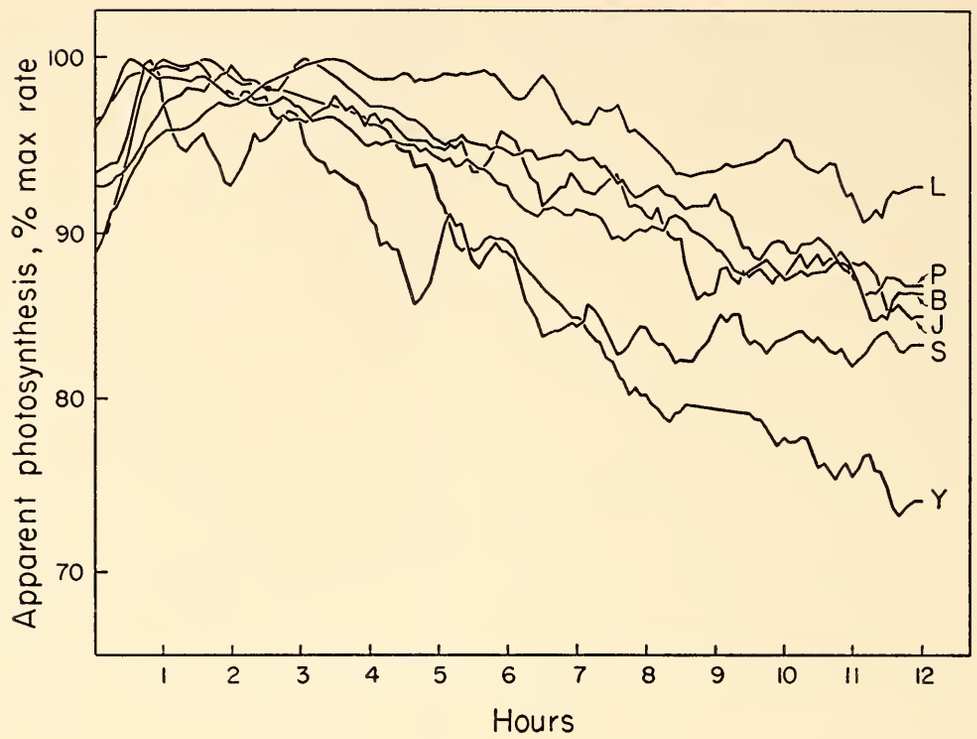

Fig. 3. Course of photosynthetic rate with time. Identification of the curves same as in two preceding figures. Reading down at zero time the curves are *Y*, *P*, *S*, *B*, *J*, and *L*.

rate. Except for taking longer to reach its maximum, the curve for the Baja California race is hardly distinguishable from the Priest's Grade and Jacksonville curves. Although it ends at 82 per cent of maximum rate, the curve for the San Antonio Peak race is decidedly below the curves of the races just mentioned during most of the 12 hours. The Yosemite race shows the sharpest decline in rate, its curve ending at 74 per cent of the maximum rate.

Dark carbon dioxide evolution. At the start of our work we used the classical approach, alternating measurements of photosynthetic rate with measurements of dark CO_2 evolution and correcting the apparent rate of photosynthesis for the dark CO_2 evolution rate. We soon abandoned this procedure, for three reasons. First, it is the net rate of CO_2 uptake that is of greatest interest to us. Second, after a dark period it takes too long to reestablish a steady photosynthetic rate, particularly at low temperature or low light intensity. Third, the reproducibility of measurements of dark CO_2 evolution is unsatisfactory.

Because of the intrinsic interest in dark CO_2 evolution, however, we measured it as time permitted on different plants under different conditions. Enough data have now been accumulated to permit a critical evaluation of the dark CO_2 evolution by *Mimulus* plants. The general unreliability of these measurements is illustrated by two examples.

Using 12 members of one clone of the Yosemite race, 113 rates of dark CO_2 evolution were determined at different times and temperatures. Each measurement was made immediately after a period of light saturated photosynthesis. The rates of dark CO_2 evolution were plotted against the immediately preceding photosynthetic rates. Two plots were made, one in which both rates were expressed as milligrams of CO_2 per square decimeter per hour and the other in which milligrams of CO_2 liberated in the dark was expressed as percentage of milligrams of CO_2 absorbed in the light.

The distribution of the points in both plots showed no correlation between the rate of dark CO_2 evolution and the immediately preceding photosynthetic rate.

The 113 measurements were at 5-degree steps from 0° to 40°. The rates at each temperature were averaged, and a line was drawn through the means. The rate of dark CO_2 evolution followed an irregularly rising course from 0° to 40°. There was a wide scatter in the values at each temperature. The 35 values at 20° showed a sevenfold variation from the lowest to the highest. By elimination of the 5 highest values, the scatter of the remaining 30 was reduced to 2.2-fold. The wide scatter at other temperatures was also caused by a few values much higher than the range of the majority. The cause of occasional very high values of dark CO_2 evolution is unknown.

An equally confused picture was obtained by applying the same treatment of data to 41 measurements of dark CO_2 evolution made on one clone of the Los Trancos race.

These apparent irregularities in the rate of dark CO_2 evolution were confirmed by a few experiments designed to test the reliability of such measurements. The generally accepted trends of dark CO_2 evolution were confirmed qualitatively. It is greatest just after a period of vigorous photosynthesis. It decreases as the plant remains dark, and reaches a roughly uniform level overnight. The rate is not measurably affected by CO_2 concentrations between 75 and 500 ppm in the air surrounding the plant. But, even with a plant that had been dark overnight, duplicate measurements of dark CO_2 evolution differed by as much as 25 per cent. When the plant had not been in the dark for some time, duplicate rate measurements showed up to twofold differences.

A single measurement of the rate of dark CO_2 evolution is of doubtful value, and making enough measurements to provide a trustworthy mean is very time consuming. For these reasons, and also because the rate of dark CO_2 evolution reflects the operation of other processes besides respiration, we question the validity of "correcting" photosynthetic rates of *Mimulus* leaves for the rate of dark CO_2 evolution.

Growth Studies in Controlled Environments

William M. Hiesey, Harold W. Milner, and Malcolm A. Nobs

The facilities for growing plants under controlled conditions have been improved during the year. Four small cabinets as described in earlier reports (*Year Books 54*, p. 350; *59*, p. 319) have been completed. Recent modifications in the cabinets provide for different light intensities and also for alternating day and night temperatures. The power supply was increased to permit full-time operation of four units utilizing artificial light and an additional two units utilizing natural daylight in the greenhouse. Dr. French and Mr. Clair have improved the performance of the CO_2 controller mentioned in *Year Book 58*, p. 352. Our operational facilities for the study of cloned plant materials in controlled environments are beginning to approach an optimum level for the present size of our staff. Studies utilizing these facilities are being closely coordinated with quantitative measurements on rates of apparent photosynthesis and dark CO_2 evolution.

Some interaction effects between light intensity, temperature, and carbon dioxide concentration on the growth of Mimulus races. Among the questions that arise immediately in the comparative study of ecological races are those basic to formulating effective experiments designed to reveal the characteristics of the climatic races. An example of some of the interacting effects of three external variables—temperature, light intensity, and CO_2 concentration—on two clones of *Mimulus cardinalis*, one from Los Trancos along the cool coastal area of central California,

the other from Priest's Grade at 800 feet elevation in the warm interior foothill region of the Sierra Nevada, will serve to illustrate the method.

The marked differences in light saturation values of the two races as a function of temperature are shown by curves *L* and *P* of figure 1. The curves of figure 1 are based on quantitative measurements made in a short time. In experiments in cabinets in which both light intensity and temperature were held at different values the growth in the clones was compared over a 35-day period, starting with small rooted cuttings and ending with plants in the flowering or near-flowering stages. Some of the results of such an experiment are illustrated in figure 4, plate 1.

At the low light intensity of approximately 2300 footcandles (fig. 4, lower row) the clone from Los Trancos, 6546-5, is light-saturated both at 20° and at 30° C, but the clone from Priest's Grade, 7210-1, is barely light-saturated at 20°C and not nearly saturated at 30°C. At the high light intensity of 4200 footcandles (fig. 4, upper row) both clones are fully light-saturated at both temperatures. The differential growth responses of the two clones are evident in the figure, especially at 30°C, where under low light intensity the total growth of the Los Trancos clone is much less than that of the clone from Priest's Grade. Increasing the light intensity to 4300 footcandles at 30°C increases the growth of both clones expressed in total dry weight, but the amount of increase is relatively much greater on the Los Trancos clone, as is shown in table 1.

The cooler temperature of 20°C markedly increases growth of both races as compared with 30°C, both at low and at high light intensities, but the enhancement is greater on the coastal Los Trancos clone than on the inland Priest's Grade clone. An unpredicted result is an actual reduction in dry weight at the high light intensity on the clone from Priest's Grade when grown at 20°C, as shown in table 1. In contrast, the dry-weight yield on the Los Trancos clone was the same at both light intensities at this temperature, verifying a prediction based on the fact that under both intensities this clone was light-saturated.

When the same two clones are subjected to high day temperature (30°C) and cool nights (15°C) at both low and high light intensities, a marked increase in total growth per unit time results in both clones, the increase being the great-

TABLE 1. Differential Effects of Light Intensity and Temperature on Growth of Two *Mimulus* Clones

See text and figure 4.

Clone	20°			30°		
	Low Light Intensity (2300 fc)	High Light Intensity (4200 fc)	% Difference at High Intensity	Low Light Intensity (2300 fc)	High Light Intensity (4200 fc)	% Difference at High Intensity
6546-5, Los Trancos (coastal race)	1.26 ± 0.07*	1.33 ± 0.11	0.0	0.37 ± 0.08	1.07 ± 0.22	+66.3
7210-1, Priest's Grade (interior race)	1.67 ± 0.10	1.26 ± 0.09	−27.1	1.10 ± 0.14	1.73 ± 0.21	+36.4

* Mean dry-weight increase in grams per propagule over a 35-day period; 10 propagules of each clone used in each experiment.

When the same two clones are subjected to high day temperature (30°C) and cool nights (15°C) at both low and high light intensities, a marked increase in total growth per unit time results in both clones, the increase being the great-

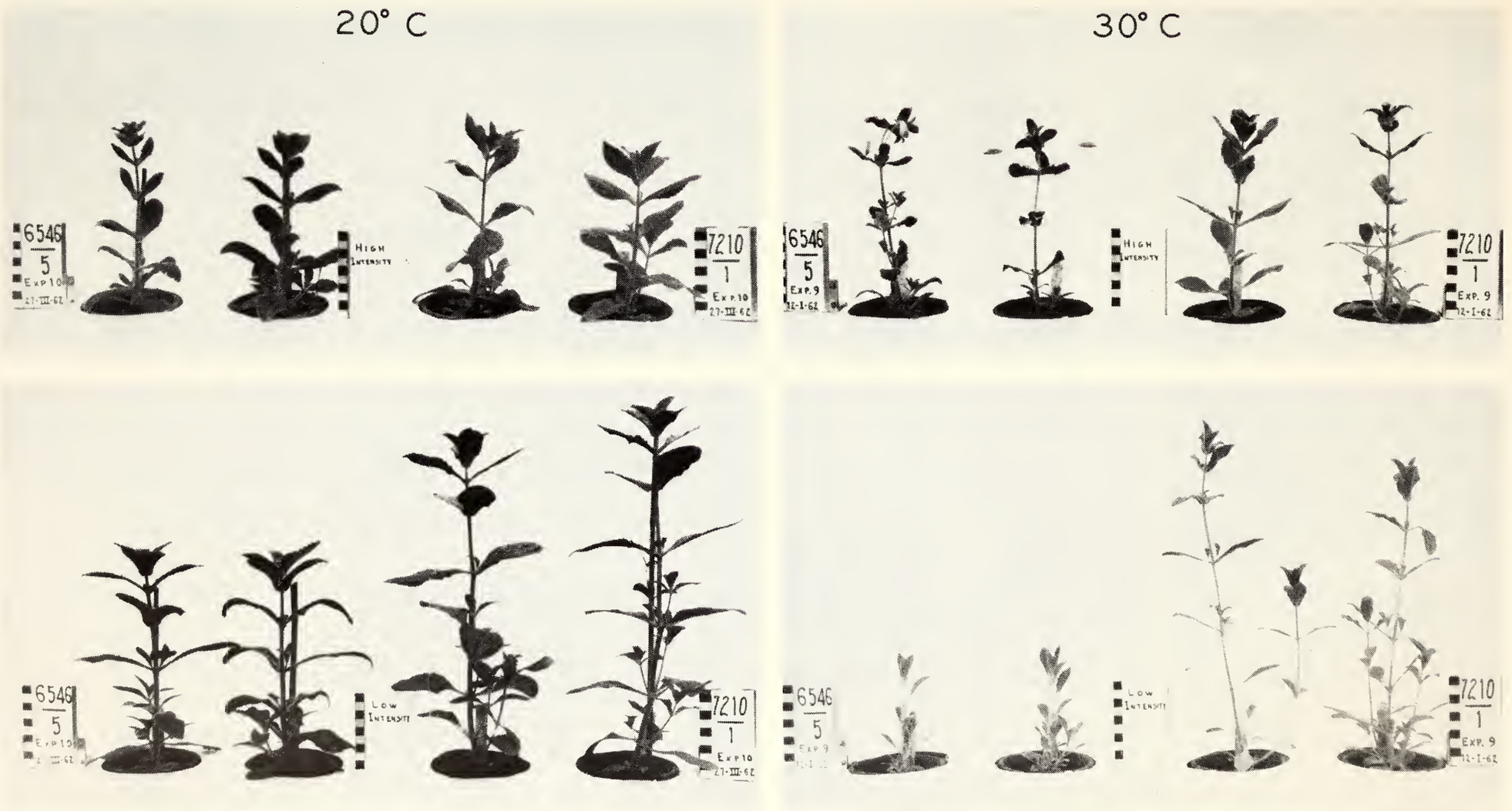

Fig. 4. Responses of two *Mimulus* clones to two temperatures and two light intensities. Propagules on the left grown at 20°C, on the right at 30°C; those on the upper row received 4300 fc light intensity, those on the lower row 2300 fc. Illumination period 12 hours daily. Markers are 10 cm high. See text and table 1.

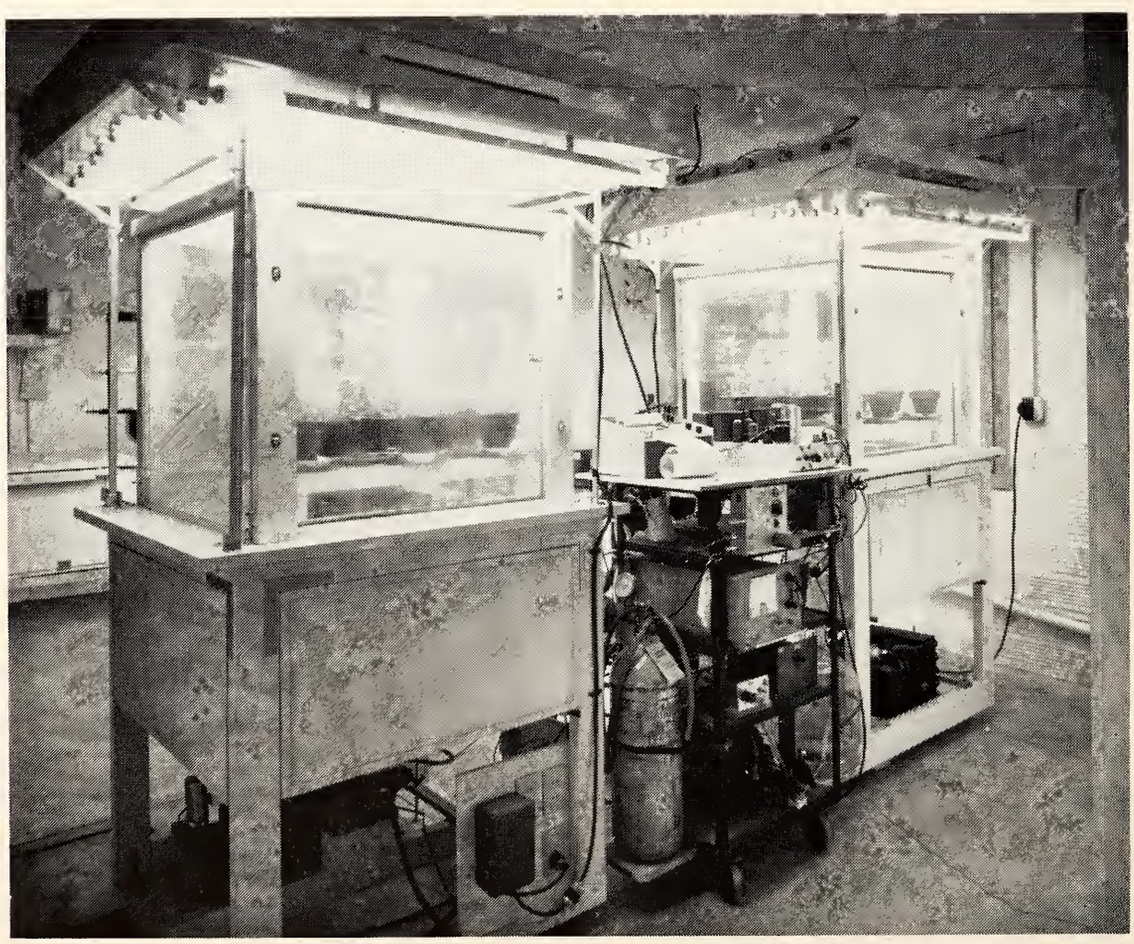

Fig. 5. *Upper:* Clones of *Mimulus cardinalis* grown in an atmosphere containing a high (1250 ppm) and a normal (300 ppm) CO_2 concentration. Temperature held constant at 25°C and the light intensity at 2000 fc during 12-hour days. The scales are 10 cm high. *Lower:* The plant cabinets and the CO_2 controller.

est in the Los Trancos clone at the low light intensity. These spectacular increases are thought of as being associated with a reduced rate of respiration during the colder night period that conserves stored food reserves for increased growth. The increase in both clones was much less marked at the high light intensity, an observation that challenges further study.

The importance of CO_2 concentration as a variable in influencing the rate of photosynthesis of *Mimulus* was mentioned in *Year Book 59*, pages 316–317. Some effects on relative growth are illustrated in figure 5, discussed in the section below. It is still too early to formulate theoretical interpretations about internal mechanisms or the ecological significance of these observations, but as further information accumulates about these and the other races of *Mimulus* and their hybrid derivatives a sound basis for doing so may be expected.

A Control system for Carbon Dioxide Concentration in Plant Growth Chambers

C. S. French, R. W. Clair, and W. M. Hiesey

For several years we have experimented with various types of controllers for maintaining desired concentration levels of CO_2 in the atmosphere of plant growth chambers.

The device now in use is shown schematically in figure 6. It appears to work reasonably well, and two successful runs of several weeks' duration have been completed with different climatic races of *Mimulus*. A description of the model now operating and a discussion of the models previously tested are being prepared for publication.

The responses of two races of *Mimulus* to high and normal CO_2 concentration in a 15-day experiment are illustrated in

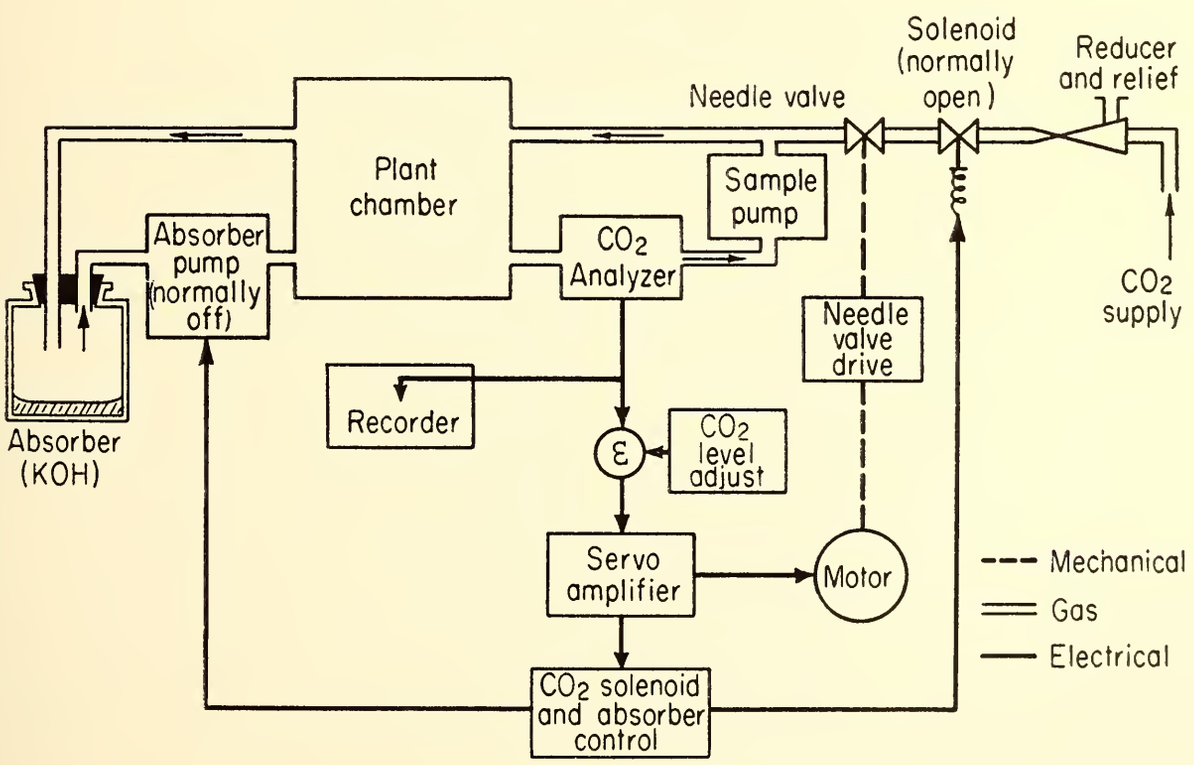

Fig. 6. Diagram to show the operating principles of the CO_2 controller. The infrared analyzer continuously monitors the CO_2 concentration in the plant chamber. When the concentration deviates from the desired level a motor adjusts the rate of CO_2 input by a needle valve. When the apparatus is operating below the normal atmospheric level some of the gas is pumped through a CO_2 absorber whenever the concentration is too high; when it is set above the atmospheric level the concentration is reduced by taking in outside air.

TABLE 2. Dry-Weight Increase of *Mimulus* Clones of Two CO_2 Concentrations
Temperature, 25°C; light intensity, 2000 footcandles; initial mean dry weights, 0.14 and 0.17 gram for clones 6546-1 and 7210-1, respectively. Mean increases in grams per propagule during 15 days.

Clone	CO_2 Concentration, ppm		Increase at 1250 ppm, %
	1250	300	
6546-5, Los Trancos (coastal)	1.24 ± 0.05	0.65 ± 0.04	90.7
7210-1 Priest's Grade (foothill)	1.45 ± 0.08	0.94 ± 0.07	54.3

figure 5. In 10 replicated clones CO_2 enrichment resulted in an average dry-weight increase of 90.7 per cent in a coastal race (clone 6546-5) and of 54.3 per cent in a Sierran foothill race (clone 7210-1), as shown in table 2. Whether the difference between the races is of ecological significance requires further study. What is immediately apparent is that CO_2 concentration is a variable that needs to be carefully controlled in growth experiments.

Ecotypic Differences in Response to Light Intensity in *Solidago virgaurea*

Olle E. Björkman, K. Paul Holmgren, and Malcolm A. Nobs

The light intensity, varying widely between different habitats, is often a highly important factor in determining the local distribution of different races and species of plants. At the Institute of Plant Systematics and Genetics at Uppsala, Sweden, a study is under way on the effect that different light intensities during development have upon the photosynthetic process. This program was designed to supply basic information in a cooperative study of the physiology and genetics of ecotype differentiation which is being conducted by the Institute of Plant Systematics and Genetics and the Department of Plant Biology of Carnegie Institution.

The genus *Solidago* was selected for these Swedish investigations. In the northern half of the eastern hemisphere this genus is primarily represented by one species, *Solidago virgaurea* L., which is found nearly continuously from North Africa to the Arctic Ocean and from the Atlantic coast to the Pacific. The species is particularly well suited for these studies, for it contains a great number of distinct ecological races within its wide distribution, occupying habitats ranging from dense forests to open heaths and meadows.

The studies were focused on two contrasting races that differ widely in the intensity of the light received. The Hallands Väderö race grows in the subdued light of the oak forests on the island of Hallands Väderö, just off the coast of southern Sweden. The Beskades race grows in a habitat with high light intensity, an alpine heath at 600 meters elevation in Finnmark in the Beskades region of northern Norway.

Two identical series of each race were vegetatively propagated. In the Institute's phytotron one series was cultivated under a low light intensity of 3×10^4 erg cm^{-2} sec^{-1} (400–700 mμ), about 700 footcandles; the other was grown under higher light intensity, 15×10^4 erg cm^{-2} sec^{-1} (400–700 mμ), about 3500 footcandles. Other conditions were identical: a photoperiod of 16 hours, a temperature regime with a 20°C day and a 10°C night, and constant air humidity at 70 per cent.

From 4 to 8 weeks was allowed for the establishment and development of the plants. After this period the leaf morphology and anatomy, the amount of chloro-

plast pigments in the leaves, the light dependence of photosynthesis, and the temperature dependence of photosynthesis were compared in the plants from the two treatments.

The mean values for the structural modifications in three clones of each race are summarized in table 3. The anatomical modifications were studied in thin sections of living leaves. The gross leaf shape, proportions, and venation pattern are unaltered for each clone in either treatment. The effect of the different light intensities on leaf area, however, is strikingly different between the two races. In Hallands Väderö the leaf area produced by the weak light plants is nearly twice that produced by the same plants in the strong light treatment. In Beskades, on the other hand, the leaf area in the weak light plants is only about half that of those grown under the higher light intensity. No differences were found in the proportions and size of the epidermal cells either between the races or between the treatments. A parallel modification is found in the leaf thickness, the depth of the spongy parenchyma, and the number of cell layers in the palisade parenchyma. In both races the greater leaf thickness in the strong light plants is the result of an increase in the number of palisade layers coupled with an increase in the thickness of the spongy parenchyma.

A striking difference between the two races is evident in the nature of the chloroplasts in the upper layers of the palisade parenchyma in the plants grown under high light intensity. In the Hallands Väderö race grown under high light conditions, the chloroplasts in all plants were irregular, fragmented, and often disintegrated in the two upper layers of the palisade parenchyma. These abnormalities in the chloroplasts are even present in the first layer of palisade cells in young leaves that have not fully expanded. This suggests that in this race the chloroplasts are photolabile. All plants from the Beskades race had normal chloroplasts in the mesophyll tissues. An analysis of the plastid pigments from the Hallands Väderö race shows that the plants grown in the higher light intensity have about half as much chlorophyll on a fresh-weight basis as the corresponding plants grown in low light intensity. In the Beskades race, plants from both strong and weak light treatments have about equal amounts of chlorophyll, which also indicates that in this race the chloroplasts are photostable under high light intensities.

These differences between the races are connected with differences in the light and temperature dependence of photosynthesis. Figure 7 illustrates typical light saturation responses of these two races grown under different light intensities. The solid lines indicate plants grown under low, and the broken lines those

TABLE 3. Comparison of Structural Modifications Induced in Leaves of Two Races of *Solidago virgaurea* Cultivated under Two Light Intensities

Light 10^4 erg cm^{-2} sec^{-1} (400 – 700 mμ)	Hallands Väderö				Beskades			
	Leaf Area, cm^2	Leaf Thickness, μ	No. Palisade Layers	Depth of Spongy Parenchyma, μ	Leaf Area, cm^2	Leaf Thickness, μ	No. Palisade Layers	Depth of Spongy Parenchyma, μ
3 weak	39.0	210	2	80	3.0	190	2	80
15 strong	20.5	290	4	120	6.5	260	4	110

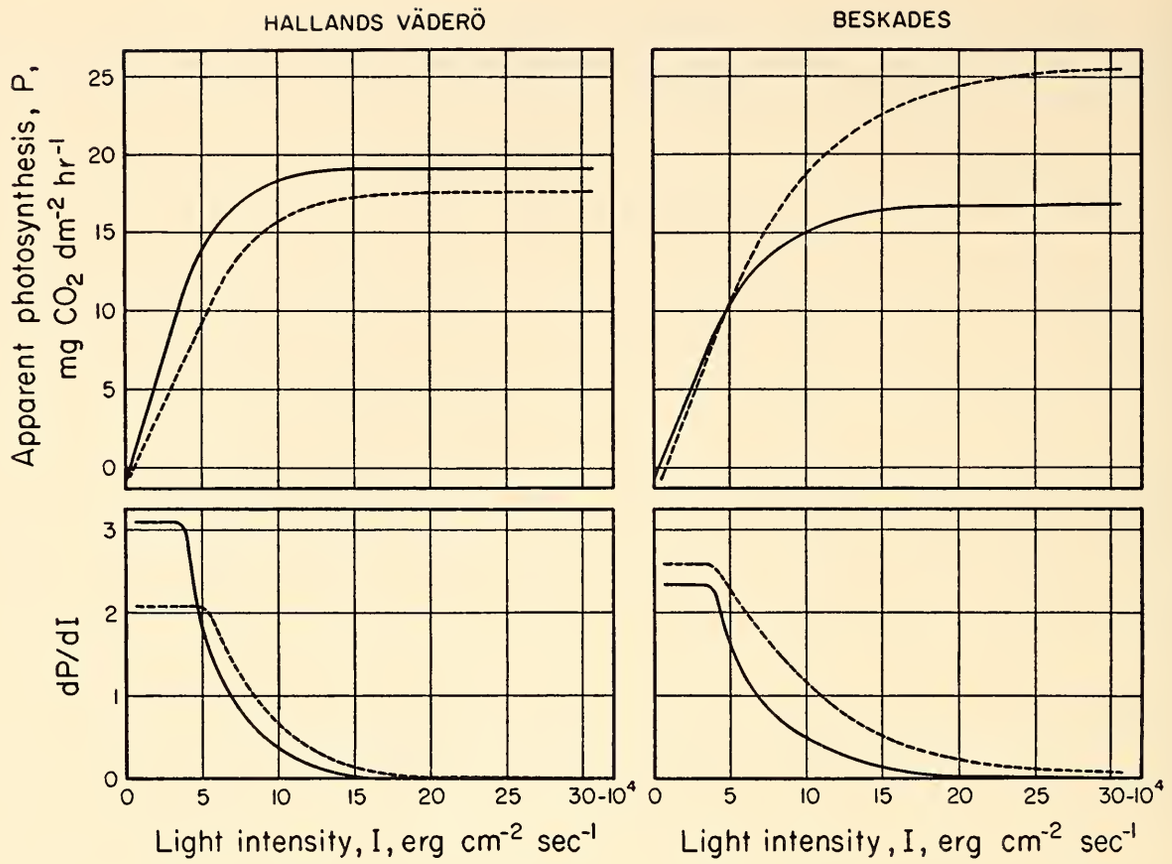

Fig. 7. Light curves of photosynthesis in clone plants of the two ecotypes, cultivated at one low light intensity, 3×10^4 erg cm^{-2} sec^{-1} (solid lines), and one high light intensity, 15×10^4 erg cm^{-2} sec^{-1} (broken lines).

under high, intensity. The leaf temperature was held constant at 22°C. In the Hallands Väderö race the plants grown under low light intensity consistently had a higher photosynthetic rate than those grown in the higher light, which was particularly evident at low light intensities. In the Beskades race the plants cultivated under the high light have the higher photosynthetic rate. In both treatments light saturation for photosynthesis occurs at a higher light intensity in the Beskades race than in the Hallands Väderö race, and in both races light saturation occurs at higher light intensities in the plants grown at high light conditions.

The temperature dependence of photosynthesis in these two races, shown in figure 8, was determined at a constant light intensity of 15×10^4 erg cm^{-2} sec^{-1} (400–700 mμ), about 3500 footcandles. In both races the plants cultivated under weak light have the higher temperature optimum.

The racial differences between these two ecotypes in their response to the light conditions during growth are consistent and reflect the ecological characteristics of the natural environments from which they were obtained. The photosynthesis of the Hallands Väderö race from the shade of the oak forests is highly efficient under low light intensities, while the photolability of the chloroplasts in the leaf tissues places it at a disadvantage under high light conditions. The Beskades race from the exposed alpine heath, on the other hand, has photostable chloroplasts and requires high light intensities for maximum photosynthesis.

Studies on another Scandinavian population from an exposed alpine habitat at Tarfala, Sweden, show a pattern essenti-

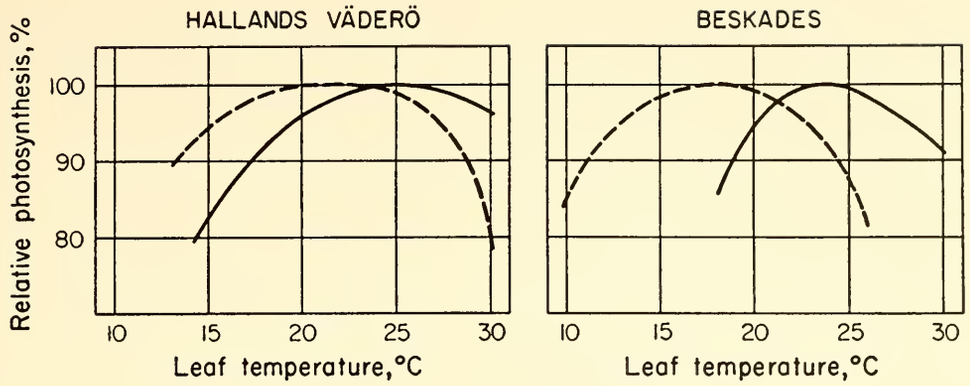

Fig. 8. Temperature curves of photosynthesis measured at a light intensity of 15×10^4 erg cm^{-2} sec^{-1} in clone plants of the two ecotypes, cultivated at one low light intensity, 3×10^4 erg cm^{-2} sec^{-1} (solid lines), and one high light intensity, 15×10^4 erg cm^{-2} sec^{-1} (broken lines).

ally identical to that found in Beskades. Additional studies, still incomplete, on other races of *Solidago virgaurea* and closely related species from California indicate that the adaptations found in response to different light intensities are generally valid.

The Aseptic Culture of Excised Tissues of *Mimulus*

Ruth F. Elliott,[1] *Frank Nicholson, and William M. Hiesey*

Recent advances in the culture of plant tissues under aseptic conditions prompted us to explore the application of these techniques to clones of *Mimulus*. The encouraging results to date indicate the possibility of making studies at the cellular level on the same clones of higher plants that are being used in transplant, cytogenetic, and physiological investigations.

The work of the current year has been devoted mainly to developing methods for isolating and growing callus tissues from the cambium of stem internodes. The practicality of growth cultures on agar slants and in liquid media has been demonstrated.

Culture media. The culture media for these experiments are based on the mineral solution in which Dr. W. M. Laetsch and Dr. Winslow R. Briggs of Stanford grow sporelings of *Marsilea*. Their basic solution was chosen because it is essentially the same one used in other tissue culture work, and with appropriate additives it was known to support growth of a green callus tissue isolated by Dr. Laetsch from *Euonymus*.

Laetsch's solution was based on Knop's solution and Berthelot's trace element solution as modified by Gautheret. Molybdenum was added according to Ball and Street. Calcium and magnesium were added in half the amount specified for Knop's solution, and NaFeEDTA instead of ferric sulfate was the iron source. The last two modifications, suggested by Sheat and co-workers for White's medium, eliminated the precipitate produced in Gautheret's mineral solution on autoclaving and reduced the drop in *p*H that normally occurs during autoclaving. The *p*H was initially adjusted to 6.0 by the addition of 0.1 *N* NaOH.

To the mineral solution was added 3 per cent sucrose and 10 or 15 per cent coconut milk, a fluid that may reactivate quiescent cells and cause their renewed growth.

Supplements added to this basal medium were of three types: (1) amino acids or casein hydrolysate or peptone; (2) yeast extract; and (3) growth regulators

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of the auxin type, including indoleacetic acid or naphthalene acetic acid, kinetin, and 2,4-D.

Methods for preparing the explants. Internode material prepared by a modification of the method described by Blakely and Steward[2] was successfully established. Internodes from actively growing plants which were fully firm were cut from the stem with a razor and dropped in 95 per cent ethyl alcohol for a few seconds to wet the surface. They were then placed in 1 per cent hypochlorite for 10 minutes and washed twice in sterile distilled water. After this washing, the "epidermis" was stripped aseptically, and the explant was placed in the culture medium. In the internodes used, secondary growth of the stem had started, but the vascular tissue still formed only a narrow zone. In preparing the explants, a longitudinal incision, as far in as the woody layer, was made in the stem, and the outer layer was peeled off. This removes all the phloem and cortex, as the tissue splits at the cambium. Thus the explants consisted of a core of pith surrounded by a narrow zone of xylem and traces of cambial tissue.

Apical tissues also were established from actively growing tips having approximately two short undeveloped internodes. The attached leaves were removed before the apices were sterilized. Attempts to culture rhizome tissues from plants grown in soil were unsuccessful because of difficulties in sterilization. Root tissues were later successfully established, however, from subcultures by transfer from established internode tissues that had developed roots.

Conditions for growth. In most tissue culture work reported in the literature, light intensities of about 100 footcandles were used, and this procedure was followed in the present preliminary studies. The temperature was maintained between 20° and 25°C. Agar slants were placed in small cabinets in which the relative humidity was maintained at approximately 70 per cent to prevent excessive drying. The best results were obtained when inocula were taken from young, vigorous plants started from cuttings and grown in one of the controlled cabinets. Explants from greenhouse-grown plants were also successful when taken from actively growing young stems.

Early results. The first attempt at establishing stem tissue cultures of *Mimulus cardinalis* and *M. lewisii* from greenhouse plants was unsuccessful. In the second trial explants were taken from young *M. cardinalis* plants grown in a controlled cabinet at 20°C under a light intensity of approximately 2000 footcandles. The tissues were placed in twenty-one modifications of the basic medium using the supplements mentioned above, and successful growth was attained on six of the modifications. The most rapid growth was made on a medium containing 10^{-7} μ per liter indoleacetic acid as a supplement, and this medium was used for subsequent work. Successful cultures were later established for eight races of *M. cardinalis*, five of *M. lewisii*, and one of *M. verbenaceus*. Of the three species, *M. lewisii* was the most difficult to establish. Apical tissues of *M. lewisii* were found to grow successfully, first developing green shoots, then roots.

Origin and structure of isolated tissues. Some preliminary transverse sections and smears of explants suitable for microscopical examination were made. One of the cultures was callus tissue that had grown from the cambium of stem internodes. The callus tissue develops either on the surface or at one end (the morphological base) of the explant, but sometimes there is a proliferation of pith cells.

Externally the new tissue appears as small groups of cells which eventually form ball-like outgrowths covering the whole surface of the explant. Microscopically the callus is at first more or less serially organized, like young xylem or phloem tissue, but with increased activity

[2] *American Journal of Botany, 48,* 351–357, 1961.

the cells become more irregularly organized. The larger "balls" of tissue consist of large, loosely organized parenchyma-like cells, interspersed with some recognizable phloem-type cells, and a central core of thickened, scalariform tracheids.

Current studies. The present work is directed toward obtaining cultures of discrete tissues of selected clones of contrasting altitudinal and latitudinal races of *Mimulus*. Work with these cultures will be coordinated with the transplant and physiological studies. One of the first considerations is to explore the rate of growth in liquid as compared with solid media as a means of speeding up studies on relative growth rates for comparative experimental purposes.

Mimulus roots in rotated liquid cultures were found to grow much faster than similar explants of the same clones established on agar. Both the liquid and solid media are useful, the liquid for comparative growth studies and the solid for maintaining stock cultures.

The necessity of adding indoleacetic acid to the medium for growing root tissues of *M. cardinalis* has been demonstrated in tests using four clones. Studies on the effects of controlled external environmental variables on the relative growth of tissues of selected clones are planned.

Studies in *Poa* Hybridization

Jens Clausen, William M. Hiesey, and Malcolm A. Nobs

The *Poa* program was initiated in 1943 as a study of a genus that has developed mechanisms for asexual (apomictic) production of seed in many of its species. The approximately 600 species of this large genus contribute prominently to the plant cover of the earth from the warm temperate to the arctic zones of both the northern and southern hemispheres. Many of these bluegrass species are of economic interest to man.

When our *Poa* program was started the reproductive mechanisms of apomixis in general were little known, having been studied primarily in a descriptive way. The biological relationships between the species of the *Poa* genus were largely unknown. The Department of Plant Biology was in a strategic position to conduct an investigation on the biological relationships of a genus of this kind because of its background experience in other groups of plants and its unique transplant facilities.

The bluegrasses constitute one of the biologically most complex genera of higher plants. In the course of the investigations it was found that the apomictic processes are in an intricate balance with the still existing, although suppressed, sexual processes. Crossings between apomictic species often restore sexuality, resulting in a phase of partial recombination between parental characters. Through selection the apomictic processes can be restored in later generations, and in such progenies there are many different expressions of balance between apomixis and sexuality. Wild forms tend to stabilize at either a high degree of sexual or a high degree of asexual reproduction. Apomictic plants store a great deal of potential variability, but spontaneous crossings occasionally occur that release and recombine this variability.

In the bluegrasses apomixis combined with high polyploidy makes it possible to add the heredities of highly distinct wild species, even though the chromosome numbers and the morphological characters may differ widely in the parents. In sexual species with fewer sets of chromosomes this is usually not possible. It was found that, through selection in later generations of apomicts crossed with apomicts, the hybrid derivatives can become as vigorous and as apomictic as wild species of *Poa*, and such derivatives can reproduce themselves as seed clones that can readily be tested in diverse climates. The convenient handling of seed apomicts made it possible to conduct transplant experiments with *Poa* on a worldwide scale.

The success of the *Poa* crossings is probably related to the fact that species from remote and contrasting habitats were crossed, producing hybrids that combined heredities that complemented each other and that had not previously been subjected to hybridization followed by natural selection. These experiments demonstrated that it is sometimes possible to develop new hybrid apomicts able to compete with natural apomicts in their native habitats even though the natives have presumably been subjected to crossing and natural selection through geologic ages. In addition, a few of the new hybrids were found to have a wide range of tolerance to contrasting climates, so that they also could compete with locally established biotypes in other parts of the world.

In apomictic high-polyploid plants the inheritance is governed not so much by individual genes as by the component sets or partial sets of chromosomes, the genomes. It is now clear that in crossing apomictic plants fertilization does not occur at random. Strong selection appears to occur among the masses of pollen deposited on a single stigma and among the ovules to be fertilized, which may be either sexual or asexual, reduced or unreduced with respect to chromosome number. There appears to be something beyond a mere chance interplay between the particular genetic-physiologic make-up of the two parents that determines the kind of fertilization that will take place.

In apomictic plants the heredities are genic as in other kinds of plants, but the hybrids do not segregate according to Mendelian ratios. The fertilization process depends upon what can properly be added to the already complex heredity of the maternal parent. Study of the *Poa* genus therefore suggests new concepts about evolutionary processes in plant groups that combine polyploidy with apomixis in establishing what may be regarded as superspecies.

Evaluation of progenies of the 1951 crossings. In working on the monograph reporting the *Poa* experiments, a major part of the year was spent by Clausen in evaluating and completing the accumulated records of a series of crossing experiments that were performed in 1951 (*Year Book 51*, pp. 111–117). The purpose of the 1951 hybridization was to include species belonging to other sections of the genus than those used in the 1943–1946 experiments and to broaden the basis for the conclusions.

In the earlier series of crossing experiments 52 hybrid combinations were attempted, 37 of which actually produced hybrids. In the 1951 crossings, 27 different hybridizations were attempted and 16 new combinations were obtained. In some of these crossings hybrids were obtained in high frequencies; in others, none at all. Our combined crossing data from the *Poas* included representative races from only 15 species, or 2.5 per cent of the approximately 600 species within the genus. Fortunately, however, the species studied represent about one-third of the sections of the genus.

It is now possible to present a clearer summary of the second series of hybridizations than appeared in the preliminary account in *Year Book 52*, table 2, page 113. The present tables 4 and 5 record the revised data and list the fertilities and later-generation progenies. Fifty-five of the second- and third-generation progenies were planted at Stanford during the spring of 1962.

It is obvious from the data that not all the species of *Poa* can be intercrossed and equally clear that it is impossible to predict which will be the successful interbreeders. Whether or not two species will combine depends on how their genomes fit together genetically and physiologically.

The tables indicate widely varying degrees of success in combining the heredities of distinct species of *Poa*. From the 11 unsuccessful hybridizations in table 5 we progress to the least successful hybrids in table 4, such as the rare, barely viable hybrid between *P. scabrella*, Lucerne, and *P. arachnifera*, and to others that are viable but completely sterile,

TABLE 4. Results of Interspecific Crossings in *Poa*

Combination	No. Seedlings	No. F_1's	Per Cent F_1's	F_1 Fertility Range, %	No. F_2 Progenies	Remarks
Poa caespitosa hybrids						
pratensis × *caespitosa*	120	2	1.7	0	2	300 F_1 seeds; 1 F_2 plant
compressa × *caespitosa*	330	5	1.5	10–40	1	96% of F_2 die
caespitosa × *arachnifera*	111	6	5.4	15–40	4	21 F_3 populations, both parents sexual
Poa arachnifera hybrids						
scabrella ($2n = 94$) × *arachnifera*	210	52	29.4	0.2–9	9	Initial sterility high
Previous *scabrella-arachnifera* crossings						
scabrella, Los Posas ($2n = 84$), × *arachnifera*	967	0	0			
scabrella, Watsonville ($2n = 82$), × *arachnifera*	1060	0	0			
scabrella, Lucerne ($2n = 63$), × *arachnifera*	1145	1	0.09	0	0	F_1 exceedingly weak
nervosa ♀ ($2n = 81$) × *arachnifera* ♂	57	55	96.5	0	0	43,000 F_1 "seeds" sown; no germination
Poa scabrella, Paso Robles, $2n = 84$, hybrids						
scabrella × *pratensis*, Groveland ($2n = 56$)	1050	6	0.6	0	0	F_1 weak, sterile
scabrella × *pratensis*, Mono Lake ($2n = 50$)	810	52	6.4	0–2	4	F_1 vigorous, high sterility; $2n = 93, 93$
scabrella × *pratensis*, Las Vegas, N. Mex. ($2n = 65$–74)	630	30	4.8	6–14	8	F_1 vigorous; $2n = 89, 95, 115, 116$
Poa ampla, Albion ($2n = 63$) × *P. compressa*, Crescent Mills ($2n = 42$)						
ampla × *compressa*	503	1	0.2	20	1	Variable F_2
Poa arida × *ampla*						
arida ($2n = 63$) × *ampla*, Albion ($2n = 56$)	630	85	53	10–50	78	F_1 vigorous; few apomicts in F_2; $2n = 90$ to 95
Reciprocal	510	0	0			
arida × *ampla*, Wenatchee	324	33	52	3–15	0	F_1 fairly sterile ($2n = 90$ to 95)
Reciprocal	210	0	0			
Quadruple hybrids						
arida-ampla × *ampla-alpigena*	510	22	4.3	7–15	2	F_1 vigorous, fertile; F_2 germination good but 76% died first year
Reciprocal, line 4683-1	870	5	0.6	64	1	5 aberrants from the line
arida-ampla × *ampla-pratensis*	890	32	3.6	4–20	5	Reduced fertility in F_1's
Reciprocal	126	9	7	1–25	0	
scabrella-pratensis × *ampla-alpigena*	450	88	48	1–90	6	Presence of *ampla-alpigena* pollen increases apparent sexuality
Reciprocal, line 4683-1	540	0	0			
ampla-alpigena (line 4273-9) × *ampla-pratensis*, Heise-Newport	480	9	1.9	1–60	0	
Reciprocal	79	0	0			

TABLE 5. Unsuccessful Hybridizations of Interspecific Crossings in *Poa*

Combination	No. Seedlings	No. F_1's Seen	Per Cent Fertility
ampla, Spokane, × *arachnifera*	600	0	0
Reciprocal	24	0	0
ampla, Albion, × *arachnifera*	840	0	0
Reciprocal	30	0	0
ampla × *caespitosa*	621	0	0
Reciprocal	621	0	0
caespitosa × *scabrella* ($2n = 94$)	1128	0	0
Reciprocal	43	0	0
arachnifera × *pratensis*	30	0	0
arachnifera × *ampla-pratensis*	30	0	0
Reciprocal	30	0	0
howellii × *scabrella*	958	0	0
Reciprocal	90	0	0
howellii × *pratensis*	1771	0	0
howellii × *douglasii*	660	0	0
Reciprocal	150	0	0
douglasii × *arachnifera*	30	0	0
kelloggii × *ampla*, Albion	150	0	0

such as *P. pratensis* × *P. caespitosa*. In contrast with these are the easily produced, highly vigorous F_1 hybrids that are completely sterile, as, for example, *P. nervosa* × *P. arachnifera* in table 4.

Further degrees of increasing fertility of F_1 hybrids are represented by *P. scabrella*, Paso Robles, × *P. pratensis*, Groveland, in which the F_1 was weak and nearly sterile; by *Poa compressa* × *P. caespitosa*, in which an F_1 of normal vigor was moderately fertile but almost all F_2 plants were weak and died; and by *P. scabrella*, Paso Robles, × *P. pratensis*, Mono Lake, that resulted in a fairly high percentage of F_1's which exhibited hybrid vigor and tolerance but nevertheless had a low degree of fertility, an unusual situation in *Poa scabrella-pratensis* hybrids.

Among the unexpectedly successful hybrids were *Poa caespitosa* × *P. arachnifera*, an Australian tussockgrass pollinated by the dioecious Texas bluegrass. Both species are sexual and yielded vigorous and moderately fertile F_1 hybrids from which 23 F_3 progenies were planted in the Stanford garden during the spring of 1962.

We have previously found that the members of the western North American section of bunchgrass poas, such as *Poa ampla*, *P. scabrella*, *P. canbyi*, and *P. gracillima*, combine easily with many races of *Poa pratensis* of the circumboreal section of Pratenses (Stolonosae). The Swedish investigators Dr. Arne Müntzing and Dr. Erik Åkerberg have found that *Poa alpina* of the Alpinae (Subbulbosae) section likewise combines with various forms of *Poa pratensis*, and that natural hybrids occur. These hybridizations have in later generations resulted in successful apomictic hybrid strains that morphologically can be classified as forms of *Poa pratensis*.

Genetic relationship and taxonomic sections. Poa arachnifera from the southern North American Great Plains probably belongs to the predominantly South American section Dioicopoa. In North American floras this species has generally been considered to be a member of the section Pratenses, but it is not easily crossed with *P. pratensis* and also cannot be crossed with either *Poa ampla* or *P. scabrella* or the western bunch poas (table 5).

A dioecious, sexual, and high-chromosome form of *Poa nervosa* from southeastern Washington was easily crossed

with *P. arachnifera,* however, and the hybrids were vigorous although completely sterile (table 4), suggesting some affinity between the two species. *P. nervosa* also has commonly been classified with the Pratenses, but it probably belongs to the section Dioicopoa. High-altitude forms of *P. nervosa* occur in the Sierra Nevada, but they are exclusively female. In previous experiments four female *P. nervosa* plants from near our Timberline station were pollinated by two forms of *Poa canbyi* and by three plants of *Poa scabrella,* Las Posas. The progenies consisted of 2429 plants, all maternal *Poa nervosa,* indicating that the high Sierran form is apomictic and lacks affinity to the species that cross with *Poa pratensis.*

Poa caespitosa of an Australian–New Zealand group of species also does not cross with *P. ampla* or *P. scabrella* (table 5) but crosses readily with *P. arachnifera* and is partly interfertile with it.

From the crossing evidence it therefore appears that genetically the species tested arrange themselves in two major complexes: the *Poa pratensis-alpina-ampla-scabrella* complex and another consisting of *P. arachnifera, P. nervosa,* and *P. caespitosa.*

Although in our hybridization experiments *P. arachnifera* × *P. pratensis,* Mather, did not succeed, there is a connection between these two groups of *Poa.* Dr. Marion E. Brown of the University of Missouri, Columbia, was able from the cross of *Poa arachnifera* ♀ × *P. pratensis,* Troy (Turkey), to develop a vigorous apomictic hybrid line that superficially resembles a large form of *P. pratensis,* suggesting that *Poa arachnifera* may also have entered into the parentage of *Poa pratensis.*

The present and previous evidence from *Poa compressa* of the section Tichopoa suggests that that species is not closely allied to any of the two groups above. Neither is *Poa howellii,* an annual species of the Ochlopoa section, closely related to any of the previous groups. Hybrids of *P. howellii* were attempted with *P. scabrella* of the bunchgrass section, *P. pratensis* of the Pratenses, and *P. douglasii,* possibly of the Dioicopoa section, but none of them succeeded (table 5).

Fertilities in hybrid progenies. Table 4 also lists the range of seed fertilities among the F_1 plants of these *Poa* crossings. The fertilities vary greatly from plant to plant, for example, between 10 and 50 per cent among 78 F_1 plants of *Poa arida* × *P. ampla* of which F_2 progenies have been grown.

In the hybrid *Poa scabrella-pratensis* × *P. ampla-alpigena,* however, the fertilities of the quadruple F_1 plants varied between 1 and 90 per cent, and table 6 lists the frequencies of fertilities among 44 of these F_1 plants. It is immediately evident that approximately 43 per cent of the plants had low fertilities, of less than 10 per cent, but also that 2 F_1

TABLE 6. Seed Fertilities of F_1 Plants of Hybrid *Poa scabrella-pratensis* × *ampla-alpigena*

Seed Fertility, %	No. F_1 Plants
0 – 10	19
10 – 20	5
20 – 30	6
30 – 40	2
40 – 50	3
50 – 60	3
60 – 70	2
70 – 80	2
80 – 90	2
	44

plants had a high fertility, of approximately 90 per cent, the same as in *P. pratensis*, and exceeding the fertilities of the *P. ampla* and *P. scabrella* parents.

Some of the F_1 plants of this quadruple cross were sexual, and others were already apomictic in various degrees. High seed fertility and the ability to reproduce apomictically was recombined with low fertility and sexual reproduction. Two quadruple F_1's, for example, 6310-10 and 6310-313, both had high fertilities ranging between 65 and 75 per cent and a high percentage of germination, but their F_2's were highly variable and weak, as was also the progeny of the equally sexual 6310-8, a plant having a low fertility ranging between 15 and 20 per cent. One moderately apomictic F_1 plant, 6310-1, had a low seed fertility, of about 20 per cent, and another highly apomictic plant, 6310-2, had the highest fertility, approximately 90 per cent. It visibly combines the hereditary characteristics of the four parental species native to highly diverse climates ranging between 34° and 68°N latitude.

Fertilization mechanisms. As indicated by the data of table 4, hybrids in some crossings occur with abnormal frequency and far exceed the frequencies observed in crossings made during the 1943 to 1946 period, in which the frequency of F_1 hybrids ranged between 0.2 and 4.6 per cent of the total progeny. Emasculation was not attempted in any of the experiments, because it does not prevent the development of apomictic embryos.

One of the two sperm cells from the pollen fertilizes the central diploid nucleus of the embryo sac and starts the development of the endosperm that nourishes either apomictic or sexual embryos. The other sperm cell presumably may fertilize a sexual ovule and produce a hybrid, or, as indicated later, may under certain circumstances be able to fertilize the cell which would develop into the apomictic embryo.

The evidence suggesting this type of fertilization is circumstantial and rests upon the abnormal increase in frequencies of F_1 hybrids having high chromosome numbers when *Poa arida* is pollinated by two kinds of *Poa ampla*. For example, *Poa arida*, North Platte, 4262-1 and 4262-11, were open-pollinated at Mather, and the seeds were space-planted at the U. S. Plant Materials Center at Pullman, Washington. Under these circumstances no *ampla* hybrids were obtained; approximately 78 per cent of the progeny were of the apomictic *Poa arida* type, and 22 per cent were weaker aberrants, some of which died early, as shown in table 7. One plant among the progeny from 4262-11, however, was a spontaneous hybrid, *Poa arida* × *P. pratensis*.

When the same two *Poa arida* plants were cage-pollinated at Stanford with a plant of *Poa ampla*, Albion, 5156-23, 45 and 61 per cent of their progeny were hybrids, *Poa arida* × *ampla*, and the percentage of apomictic and aberrant progeny decreased dramatically. A similar result was obtained with *Poa arida*, 4262-13, when cage-pollinated with a morphologically very different plant of *Poa ampla* having 63 chromosomes, from near Wenatchee, Washington. It yielded 52 per cent hybrids, the apomictic *Poa arida* plants being reduced to 42.5 per cent of the progeny.

The chromosome numbers of the hybrids reveal that something different from normal fertilization takes place, as shown in the lower part of table 7. The parents had $2n = 63$ and 56 chromosomes or $2n = 63$ and 63 chromosomes, but 59 F_1 plants had the high chromosome numbers of $2n = 86$ to 96. This suggests that the hybrids arose from unreduced egg cells having 63 chromosomes pollinated by *ampla* sperm cells having a reduced number of chromosomes. Morphologically the hybrids reflected the preponderant influence from *Poa arida*.

Apparently the presence of *Poa ampla* pollen changes the fertilization mechanism of *Poa arida* even when the pollen of *arida* is present in great abundance. When the *Poa arida* plants are exposed to a

TABLE 7. *Poa arida*, North Platte, Progenies from Different Kinds of Pollination

P. arida Parent	*P. ampla* Parent	Per Cent Progeny			Total
		P. arida		F_1 Hybrids	
		Apomicts	Aberrants		
4262-1,	Open-pollinated	77.8	22.2	0	64
$2n = 63$	× *ampla*, Albion, 5156-23, $2n = 56$	43.6	11.0	45.4	64
4262-11,	Open-pollinated	78.1	20.3	1.6	64
$2n = 63$	× *ampla*, Albion, 5156-23, $2n = 56$	29.6	9.3	61.1	54
4262-13	× *ampla*, Wenatchee, 4175-1, $2n = 63$	42.3	5.8	51.9	52

		$2n$ of F_1 Hybrids			Total
		63	77	86 to 96	
4262-1	× *ampla*, Albion, no. plants	----	1	23	24
4262-11	× *ampla*, Albion, no. plants	1	----	19	20
4262-13	× *ampla*, Wenatchee, no. plants	----	----	17	17
	Total no. plants	1	1	59	61

mixture of pollens as in the Mather garden containing a preponderance of different kinds of *Poa pratensis* in the surrounding meadow, the apomictic development proceeds unchanged. The weaker aberrants have *Poa arida* characteristics, presumably resulting from pollination by *arida* pollen. A single spontaneous hybrid *P. arida* × *pratensis* indicates the possibility of pollination by *Poa pratensis*.

It does not seem reasonable that *Poa arida* should produce such high percentages of unreduced egg cells that function only when pollen from *Poa pratensis* is present. Another possibility is that the *Poa ampla* sperm cells can stray away from the normal egg cell and are capable of fertilizing cells that normally would develop into apomictic embryos.

It is also challenging that in the reciprocal cross, *Poa ampla* × *arida*, there were no hybrids among 720 progeny, although when *P. ampla* receives pollen of *Poa pratensis* a fair percentage of hybrids arise. *Poa arida* pollen therefore appears to be ineffective both with sexual and apomictic egg cells of *Poa ampla*, in contrast with the potency of *Poa ampla* pollen when applied to *Poa arida*.

A similar abnormal increase in the percentage of hybrids was observed in the quadruple cross, culture 6310, *Poa scabrella-pratensis*, 4711-3, × *P. ampla-alpigena*, 4683-1. Each of the two parents of this hybrid were recombined F_2 plants of interspecific hybrids that had become apomictic. Morphologically the two parent plants can be classified as forms of *Poa pratensis* having some obvious inheritance from *P. scabrella* and *P. ampla*, respectively. When the hybrid apomictic plant 4711-3 is open-pollinated, approximately 62 per cent apomicts and 38 per cent weaker aberrants develop. When plant 4683-1 was caged with it, however, the seed harvested on 4711-3 produced only about 34 per cent of the apomictic 4711-3 type and nearly 48 per cent hybrids.

This quadruple cross resembles the *Poa arida* × *P. ampla* cross in the abnormally large number of hybrids produced, but the chromosomal situation is very different in the two crosses, as is indicated in the lower part of table 8. The parents of the quadruple cross have higher chromosome numbers, $2n = 68$ and 70, respectively, as compared with $2n = 63$ and 56 in the *Poa arida-ampla* crossings. Considering the irregularities in the distribution of the chromosomes when sex

TABLE 8. Hybrids from a Quadruple Cross
Poa scabrella-pratensis, 4711-1 ($2n = 68$), × *P. ampla-alpigena*, 4683-1 ($2n = 70$)

♀ Parent	Pollinator	Per Cent Progeny			Total
		Line 4711-3		F_1 hybrids	
		Apomicts	Aberrants		
4711-3	Open-pollinated (4 progenies)	61.7	38.3	0	360
4711-3	× 4683-1	33.7	28.5	47.8	92

	$2n$ of F_1 Hybrids of 4711-3 × 4683-1							
	56 to 58	62 to 66	68 to 74	75 to 78	79 to 80	82 to 84	88 to 92	
No. plants	1	6	11	6	1	----	4	29

cells of *Poa* are formed, all 29 F_1 plants of the quadruple cross could have been produced from the union of an unreduced sex cell with another having a reduced number of chromosomes. Most likely, however, the 4 plants having $2n = 88$ to 92 chromosomes were derived from the union of an unreduced sex cell with another having a reduced number of chromosomes.

The high frequency of hybrids in the quadruple cross *Poa scabrella-pratensis* × *P. ampla-alpigena* appears to have a different cause from that in the *Poa arida* × *P. ampla* cross. Fertilization of unreduced embryos obviously did not occur to any great extent in the quadruple cross. Competition in rate of development between fertilized hybrid embryos and apomictic ones would seem to be the factor governing the shift in frequency of hybrids in this case.

The reciprocal cross *Poa ampla-alpigena* × *P. scabrella-pratensis* did not produce a single hybrid among 720 plants, an example that parallels the situation in *Poa ampla* × *P. arida*.

This new evidence on fertilization mechanisms and chromosome numbers in *Poa* brings into focus data reported in *Year Book 49*, page 106, on selections that occur during the pollination of *Poa ampla*. Considerably more information is now available; it is presented in table 9.

When *Poa ampla*, Albion, plant 4183-1, $2n = 64$, is pollinated by *Poa pratensis*, Mather, 4253-4, $2n = 68$, unreduced "ovules" are predominantly being fertilized; but when the same plant is being pollinated by *Poa pratensis-alpigena*, Lapland, $2n = 74$, the hybrids are derived exclusively from ovules having the reduced number of chromosomes. The frequency of hybrids in these two crosses was low, 4.6 and 2.0 per cent, respectively, indicating that the hybrids were derived from unreduced female cells. It is possible, however, that unreduced apomictic cells were also being fertilized.

The data here presented bring into

TABLE 9. Chromosome Numbers in Hybrids of *Poa ampla*, Albion, Plant 4183-1, $2n = 64$

Parents	Number of Plants								Total
$2n$ =	63 to 66	68	70 to 73	77	80 to 84	88 to 100	104	117	
4183-1 × 4253-4, $2n = 68$, *pratensis*, Mather	3	--	2	--	--	14	2	1	22
4183-1 × 4050-1, $2n = 74$, *alpigena*, Lapland	2	1	3	--	--	--	--	--	6

renewed focus the complexities of the intricate balances between the fertilization mechanisms in the genus *Poa*.

The North American Field Pansy, *Viola rafinesquii*

Jens Clausen and R. B. Channell

While Clausen was lecturing at Vanderbilt University in the spring of 1961 (*Year Book 60*, p. 379) an opportunity presented itself to make a field study of the only North American member of the otherwise Old World Melanium section of the violets, which were the subjects of his experiments and a series of papers between 1921 and 1931. Dr. Channell and Dr. Uzi Nur of the Department of Biology of Vanderbilt University, Nashville, Tennessee, have been associated in these studies, which also include the seasonal change in floral morphology and in the mode of fertilization of a species whose geographic isolation from its Old World relatives is puzzling.

In terms of its technical classification the American field pansy has had an unusually confused history: from 1808 to 1958 it was known under various names, such as *Viola tenella rafinesque*, *V. tricolor* L., *V. arvensis* Murr., *V. rafinesquii* Greene, V. *kitaibeliana* R. et S. var. *rafinesquii* (Greene) Fernald, and *V. bicolor* Pursh in Shinners. Alternately, it has also been considered a native North American species or an introduced European weed.

As shown in the research before 1931 most of the European species of the Melanium (pansy) section are genetically interconnected by being able to produce partly fertile hybrids regardless of their chromosome numbers, which vary from species to species between $n = 7, 8, 10, 11, 12, 13, 17, 18, 20, 24$, and 30. The Melanium section of the *Viola* genus is fairly unique in such tolerance to hybridization among nonapomictic species. The single North American species has $n = 17$, determined on plants originally from Kansas (Clausen, 1929, and Gershoy, 1934; from Dr. Ezra Brainerd's stock); from Tennessee (Uzi Nur, 1961); and from Texas (Lloyd Shinners, 1961).

At the Stanford laboratory in 1933 Clausen crossed reciprocally the Kansas stock of the American field pansy with the best "combiners" among the European species, namely, *V. tricolor*, $n = 13$, *V. arvensis*, $n = 17$, and *V. kitaibeliana*, $n = 24$. All seeds resulting from these crossings were empty and did not germinate, and F_1 hybrids were not obtained. In contrast, these three species cross easily and produce highly segregating later generations (Clausen, *Hereditas, 15*, 219–308, 1931). Being an isolated experiment with a negative result, the 1933 crossings were not reported at the time, but the record was clear and was preserved. The negative results in these crossings strongly indicate that the American field pansy is genetically well separated from its morphologically closest European relatives.

The North American species is unique within the Melanium section because it has two kinds of flowers: from March to early April the spring flowers are showy, bluish white to blue, and not unlike the flowers of the European *V. tricolor*, except that in the American species the orifice of the stigma has no lip to protect it against self-pollination. Later in the season the flowers of the North American pansy become inconspicuous and so different that the plant appears like a wholly different species: the late spring flowers never open; their petals are rudimentary and hidden by the sepals; in successively later flowers the four anthers become rudimentary or their pollen sacs abort, but the anther between the two upper petals remains well developed; in the changed flowers the pistil makes a complex twist and places its stigma just below the fertile anther that opens directly into the orifice. The developmental changes within the flower are gradual as the season progresses, although the end result is rather drastic.

This late stage is known as a cleistogamic one, and in this species it has generally been overlooked by botanists.

None of the Old World species of the Melanium section of *Viola* have cleistogamic flowers, but such flowers are known from species belonging to the Nomimium section, such as *Viola mirabilis* L., so named on account of its remarkable shift in seasonal development. In *V. mirabilis*, however, it is the two lower anthers that persist, and the pistil bends forward instead of twisting backward as in *Viola rafinesquii*.

These remarkable seasonal adjustments from open to self-pollination involve systemic and coordinated changes in the growth mechanism of the flower. It is significant to notice that species of distinct sections follow different paths in achieving the same end result, namely, regular alternation between open and forced self-pollination, and extreme economy in pollen production in the cleistogamic stage.

On the basis of both genetical and morphological facts it is now well substantiated that the North American field pansy is a native species and that it is evolutionally distinct from its Old World relatives. A search through botanical literature has established that its name according to international botanical rules is *Viola rafinesquii* Greene. This single American species covers a territory similar in size to that occupied on the other side of the Atlantic Ocean by approximately 50 species. The American species extends through more than ten latitudes from the Atlantic east coast to Colorado and occupies habitats edaphically as distinct as sand, limestone, and ruderal sites.

The *Viola rafinesquii* situation provides some food for thought on the evolution of the pansies. Did the North American pansy cross the Atlantic Ocean in an early geologic age, or did the Melanium section originate in North America with a couple of pioneers making the trip in the opposite direction, or did the continents simply float apart? The posing of such a question is probably more significant than a final answer. Even more intriguing is the hereditary growth mechanism that must regulate the seasonal change from open cross-pollinated to closed self-pollinated flowering. The data will be presented in a joint paper with Dr. R. B. Channell and Dr. Uzi Nur.

BIOCHEMICAL INVESTIGATIONS

Factors Affecting Oxygen Evolution from Swiss Chard Chloroplasts

David C. Fork

It has been known for about eighty years that chloroplasts suspended in sucrose solutions retain a limited capacity to evolve O_2 upon illumination. Since Hill's discovery in 1937 that substantial quantities of O_2 could be produced from chloroplasts supplied with an appropriate hydrogen acceptor, there has been a multitude of publications on the subject. Study of the endogenous O_2 evolution from remaining traces of the natural Hill oxidant has been largely neglected. Recently, however, de Kouchkovsky has studied the endogenous O_2 evolution from isolated maize chloroplasts. In the present work a study was made of the endogenous O_2-evolving capacity of Swiss chard chloroplasts and of various factors affecting O_2 evolution and consumption.

It is possible to study the following different processes by measurements of O_2 exchange in chloroplasts: (1) O_2 production by light from an endogenous substrate without added reagents; (2) the regeneration by a dark process of the substrate used for O_2 evolution; (3) the photochemical regeneration of this material; (4) the production of O_2 from added substrates such as ferricyanide;

(5) the increase in O_2 consumption caused by light.

It appeared worth while to examine factors affecting O_2 evolution of chloroplasts before and after Hill oxidants had been added. Furthermore, a comparison of the action spectra for O_2 evolution by chloroplasts before and after addition of a Hill oxidant might be expected to reveal important differences in view of current findings of separate functions for chlorophyll *a* and accessory pigment systems.

Chloroplast preparation. Swiss chard (*Beta vulgaris* L. var. *cicla*) used in these experiments was grown in the garden at Stanford. Mature leaves were picked as needed, rinsed with distilled water, and chilled before use. All the steps in the preparation of the chloroplasts were performed in a cold room at 3 to 4°C in dim green light. Leaf blades free of large midribs were ground in a solution containing 0.4 *M* sucrose, 0.01 *M* NaCl, and 0.05 *M* K_2HPO_4-KH_2PO_4 buffer (*p*H 6.9). The slurry from the ground leaves was strained through 8 layers of cheesecloth and centrifuged at 200 × *g* for 2 minutes. The supernatant was centrifuged at 1000 × *g* for 8 minutes to sediment the whole chloroplasts. For making fragments, the chloroplasts were washed by resuspending them in grinding medium and centrifuging again at 1000 × *g* for 8 minutes. Chloroplast fragments were obtained by osmotic rupture of washed whole chloroplasts which were resuspended in 0.01 *M* NaCl and 0.05 *M* phosphate buffer for 10 minutes. Centrifugation at 18,800 × *g* for 15 minutes sedimented these fragments.

Measurement of oxygen exchange. O_2 changes in chloroplast preparations were measured by a platinum electrode covered by a thin film of Teflon, as described in another section. The Teflon film permitted O_2 to diffuse through it but protected the electrode surface from the effect of substances in the chloroplast preparation.

The solution used to grind leaves for isolating the chloroplasts was employed as the "standard" circulating solution. The experiments described here were conducted at room temperature (19° to 21°C) with unwashed whole chloroplasts.

The time course of oxygen evolution at 650 mμ. Figure 9 shows curves for O_2 production upon repeated exposures of whole chloroplasts to the same intensity of 650-mμ light under anaerobic and aerobic conditions. The chloroplasts were suspended in the "standard" circulating solution described above (no added Hill oxidant). Under anaerobic conditions (part *A*, fig. 9) the curves for O_2 production show a high initial rate (O_2 production spike). The high rate of O_2 production decreases rapidly in the light, reaching steady-state net O_2 evolution after about 3 minutes. Exposure to the same 650-mμ beam 3 minutes after the initial exposure gives rise to a lower O_2 spike than that observed initially. A dark interval of 10 minutes, however, serves to regenerate an O_2-producing capacity nearly equal to the original.

The steady-state rate is presumed to correspond to the equality between the rate of substrate utilization by light and its re-formation by both a thermal and a separate photochemical process.

Figure 9, part *B*, shows the effect of exposing these chloroplasts to the same intensity of 650-mμ light under aerobic conditions. Upon illumination, the O_2 production spike is seen again. However, the rate of O_2 production declines in the light, reaching a steady-state net O_2 uptake after about 3 minutes. The chloroplasts show an O_2 gulp when the light is turned off. The former dark base line is attained again after about 3 minutes in the dark.

The decline in rate of O_2 production during the exposure is much less steep with aerobic conditions, which suggests that the re-formation of the substrate is faster with O_2. The fall below the dark base line is evidence for a photooxidation process running concurrently with the O_2 production. The gulp is caused by the photooxidation process continuing longer than the O_2 evolution after the light is

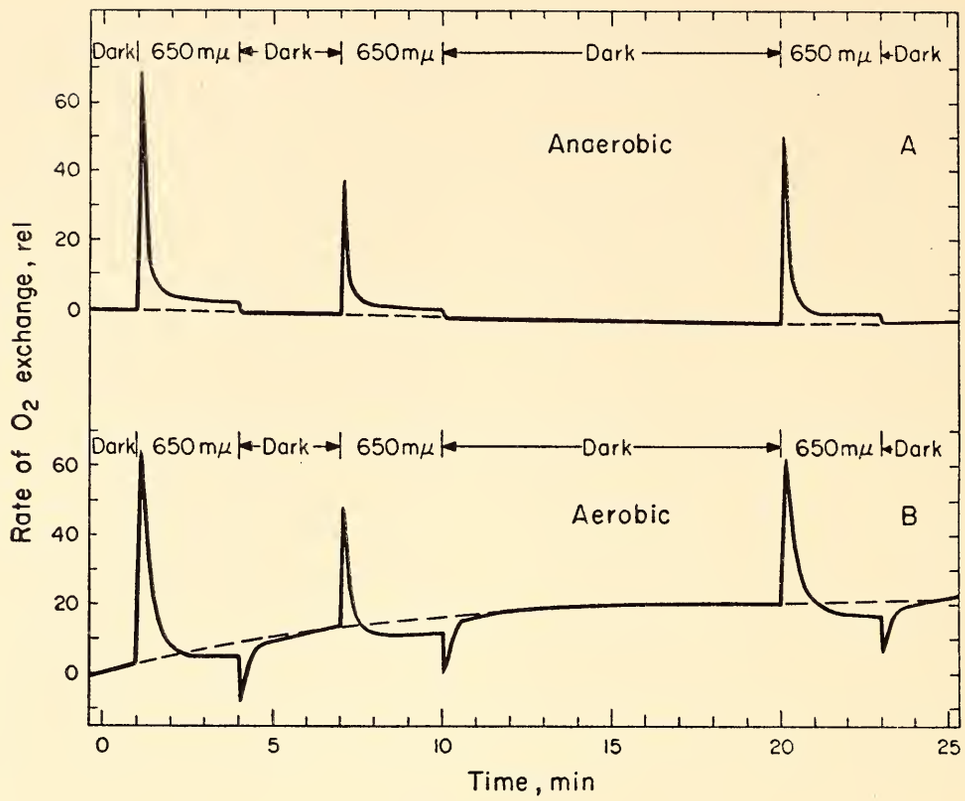

Fig. 9. O_2 exchange with illuminated Swiss chard chloroplasts under anaerobic (*A*) and aerobic (*B*) conditions. A dark interval of 17 minutes separated the last exposure of part *A* from the first exposure in part *B*. During this time air instead of purified nitrogen (99.995 per cent N_2) was bubbled through the gas exchanger. Intensity of the 650-mμ beam = 803 ergs cm^{-2} sec^{-1}.

turned off. This means that an oxidizable product is made by light, so that the "photooxidation" process may be a photochemical production of reducing power rather than a strict photooxidation. This reducing power may be the reduced form of the substrate used for O_2 production.

The effects described above for whole chloroplasts could also be observed for chloroplast fragments. Whole chloroplasts, as well as chloroplast fragments, retained their ability to evolve O_2 for many hours, sometimes even after being left on the electrode overnight in the dark.

The recovery of the oxygen-evolving capacity in the dark. The dark build-up of a product which brings about increased O_2 production was measured anaerobically by exposing the chloroplasts to 650-mμ light for 4 minutes to deplete this product. The light was then turned off. At increasing intervals afterward, the 650-mμ light was turned on just long enough (about 5 seconds) for the spike of O_2 production to reach its highest point and was then turned off. This "flash" sampled the amount of substrate reformed but without depleting it appreciably. Figure 10 shows that the O_2 production spike has recovered half its original height after about 1.5 minutes in the dark under anaerobic conditions. Figure 10 shows, furthermore, that the build-up continues for a considerable time in the dark, and that the maximum build-up had not been attained after 16 minutes.

The action spectrum for oxygen evolution. The action spectrum for the production of the O_2 spike was measured anaerobically for chloroplasts suspended in the "standard" circulating solution. For this purpose a 650-mμ reference beam was turned on, as described above, just long

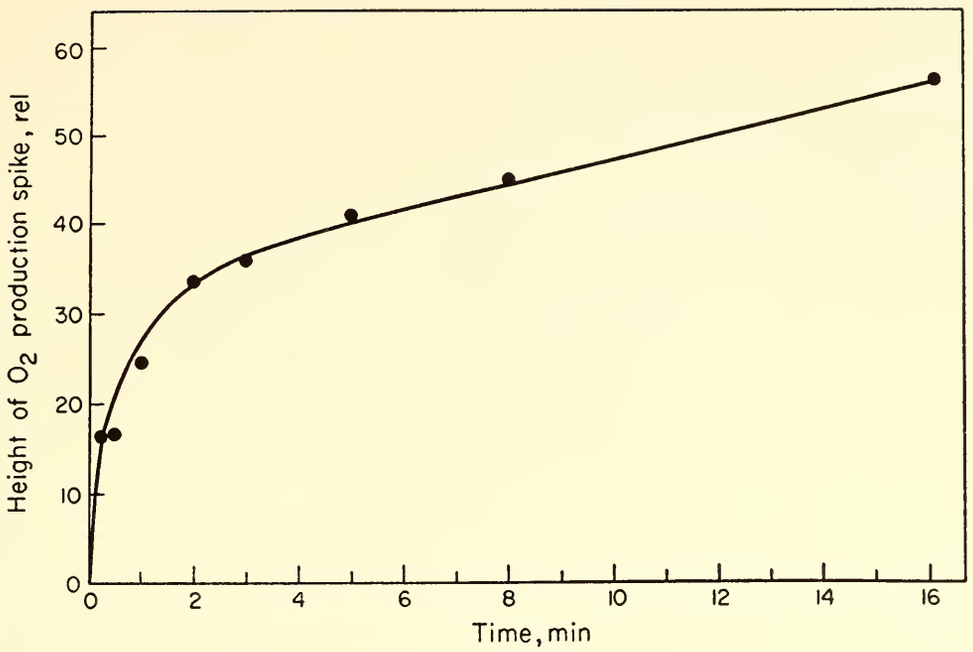

Fig. 10. Relative heights of O_2-production spikes from 650-mμ light flashed at various dark intervals after a previous 4-minute exposure to this 650-mμ beam. The light flashes were on just long enough to permit the spike to reach its highest value. The height of the O_2 production spike produced by the initial 4-minute exposure was 62 units. The intensity of the 650-mμ beam was 803 ergs cm^{-2} sec^{-1}. Gas phase, N_2.

enough to allow the maximum rise in the O_2 spike, about 5 to 10 seconds. This was repeated after 1-minute dark intervals until a constant response was attained. A similar flash exposure of a measuring wavelength from the monochromator was given, again just long enough for the peak rate to be reached, and was then turned off. After a 1-minute dark interval this measuring flash was followed by exposures to flashes of the 650-mμ adapting light and 1-minute dark intervals until a constant response was again attained, whereupon the chloroplasts were exposed to equal quanta of another wavelength for the next measurement. The ratio of the peak height of the response to the measuring beam to the preceding 650-mμ peak height was used to plot the action spectrum. The action spectrum for endogenous O_2 evolution in figure 11 shows peaks at about 650 and 480 mμ. These peaks correspond to regions of maximum absorption by chlorophyll *b*. A shoulder can be seen around 680 mμ which would correspond to chlorophyll *a* absorption. A check of the O_2 production spike versus 650-mμ intensity showed that, at 650 mμ and at the intensity used for the action spectrum measurements, the response fell on the linear region of the curve.

It is clear that illuminated chloroplasts are able to evolve O_2 even though no Hill oxidants are provided. The components responsible for the O_2 evolution must be bound to the chloroplasts, since a continual dialysis of water-soluble substances takes place into the circulating medium which passes over the chloroplasts.

This endogenous evolution of O_2 apparently results largely from the functioning of chlorophyll *b*, since peaks at 650 and 480 mμ in the action spectrum for the O_2-production spike were measured using chloroplasts without an added Hill oxidant. The shoulder in this action spectrum at 680 mμ indicates that chlorophyll *a* may be functioning to a limited extent also.

The 670-mμ form of chlorophyll *a* is believed to be a part of the accessory pigment system. Presumably this is the form of chlorophyll *a* active in the present system. The wavelength shift of the

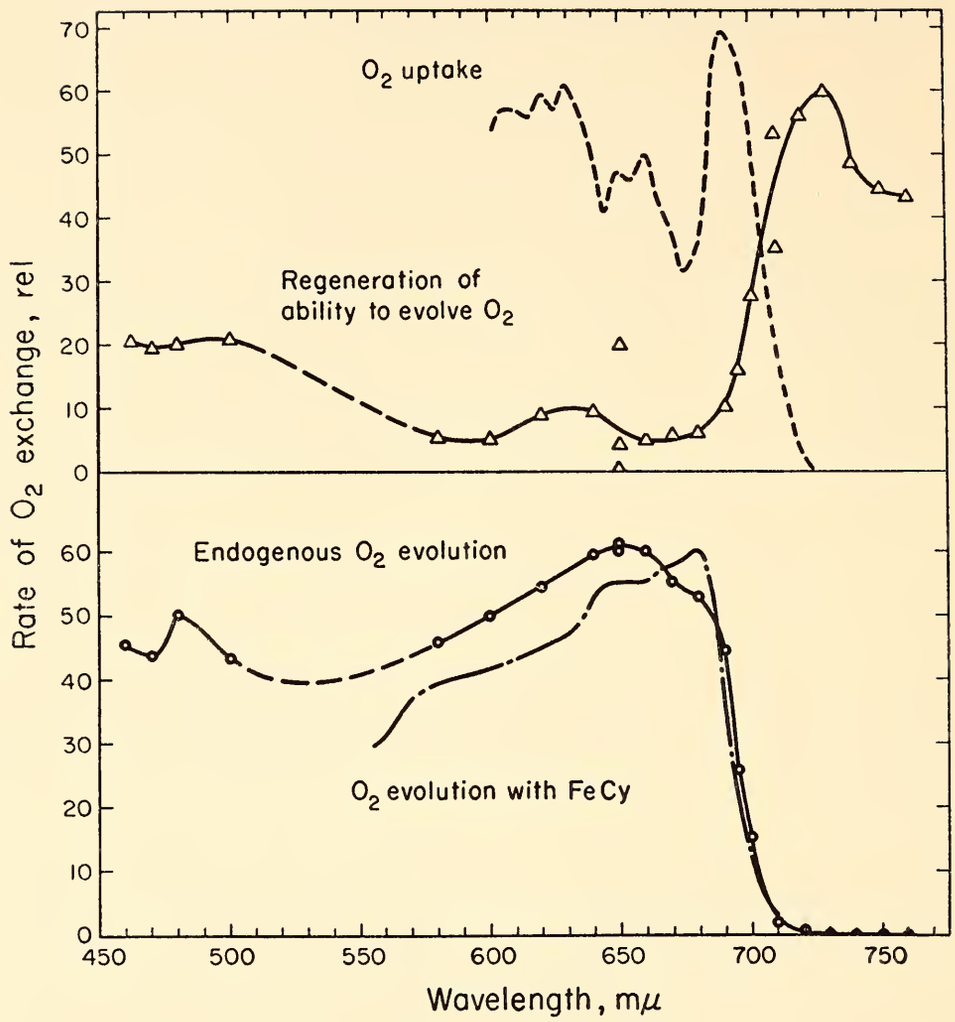

Fig. 11. Action spectra for various effects of light on O_2 exchange by isolated chloroplasts.

Endogenous O_2 evolution: The maximum rate of O_2 evolution from briefly illuminated chloroplasts without added Hill reagents. Gas phase, N_2. Temperature, 19.5°C. Measured for equal incident quanta at each wavelength above 580 mμ and corrected to this value below 580 mμ where less energy was available. Intensity at 650 mμ = 477 ergs cm^{-2} sec^{-1}.

Regeneration of ability to evolve O_2: The effect of 3-minute preillumination by various wavelengths on the O_2 evolution by 650 mμ.

O_2 uptake: The O_2 consumption induced photochemically by various wavelengths in chloroplasts whose O_2-evolving ability was poisoned with 2.7 × 10^{-5} *M* DCMU. Automatic recording with constant incident quanta per second. Gas phase, air.

O_2 evolution with FeCy: The effect of different wavelengths on O_2 evolution from chloroplasts with added ferricyanide: the Hill reaction. Automatic recording with constant incident quanta per second. At 675 mμ the intensity was 365 ergs cm^{-2} sec^{-1}. Gas phase, N_2.

shoulder to 680 mμ can be caused by the addition of the effect of the long-wave side of the 650 band to the effect of the 670 band.

The proximity of the O_2-evolving step in photosynthesis to the accessory pigment system has been postulated recently by Witt and co-workers, by Losada et al., and by Duysens' group. Witt's scheme, derived from studies on absorption changes, suggests that electrons from water reduce an unknown substance, X (plastoquinone?), and that the accessory pigment system is closely connected to this reduction. The oxidation of this reduced compound (X^-), in turn, depends on the activation of chlorophyll *a* whereby electrons, removed from X^-, ulti-

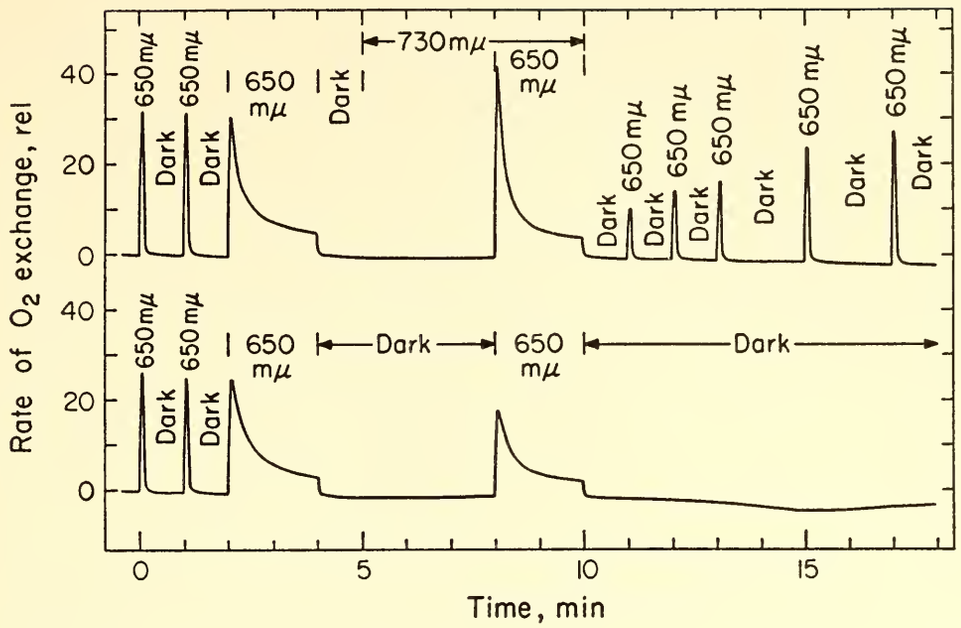

Fig. 12. The effect of 730-mμ light on the 650-mμ O_2-production spike in Swiss chard chloroplasts suspended in the solution described in the text. The lower part of the figure is a dark control. See text for details. Intensity of 650-mμ beam = 803 ergs cm^{-2} sec^{-1}; of the 730-mμ beam = 459 ergs cm^{-2} sec^{-1}. Gas phase, N_2.

mately reduce TPN (or an artificial electron acceptor such as potassium ferricyanide).

Effect of light on the recovery of oxygen-evolving capacity. The O_2 production brought about by the 650-mμ beam was influenced by a previous light exposure. To study this effect, flash exposures to 650-mμ light were given an anaerobic preparation at 1-minute intervals until a constant response was attained as shown in figure 12, upper left. A 2-minute exposure to the 650-mμ beam was then given. This exposure was followed by a 1-minute dark interval, whereupon a far-red light, 730 mμ, was turned on for 3 minutes. After 3 minutes of the far-red exposure the 650-mμ beam was superimposed on it. The effectiveness of 730 mμ in increasing the 650-mμ O_2 spike can be expressed as the height of the 650-mμ O_2 spike after an exposure to 730 mμ divided by the height of the 650-mμ O_2 spike before an exposure to 730 mμ (in this case 1.37). A control, with an equivalent dark period placed between the 650-mμ exposures, lower part of figure 12, gives a ratio of 0.77.

A similar time-course curve for O_2 production and enhancement of the height of the spike is observed when a 480-mμ beam is substituted for 650 mμ.

Figure 13 shows the increase in the 650-mμ O_2-evolution spike brought about by a previous exposure to 730 mμ for varying times. Since the build-up by 730-mμ light is relatively slow, a simultaneous exposure to 730-mμ and to 650-mμ light does not give noticeable enhancement. The effect of the intensity of 730-mμ light on the increase of the 650-mμ O_2 spike was approximately linear up to 500 ergs cm^{-2} sec^{-1}. At higher intensities there is an appreciable amount of oxygen evolution sustained in 730-mμ light and a corresponding drop in its effectiveness in causing increased O_2 production from 650 mμ.

The action spectrum for the light-induced recovery. The action spectrum for the effectiveness of light in regenerating the material used up by O_2 production from a 650-mμ beam was plotted from other data in the same set of measurements used for the endogenous O_2 production. A point on the ordinate of the curve in

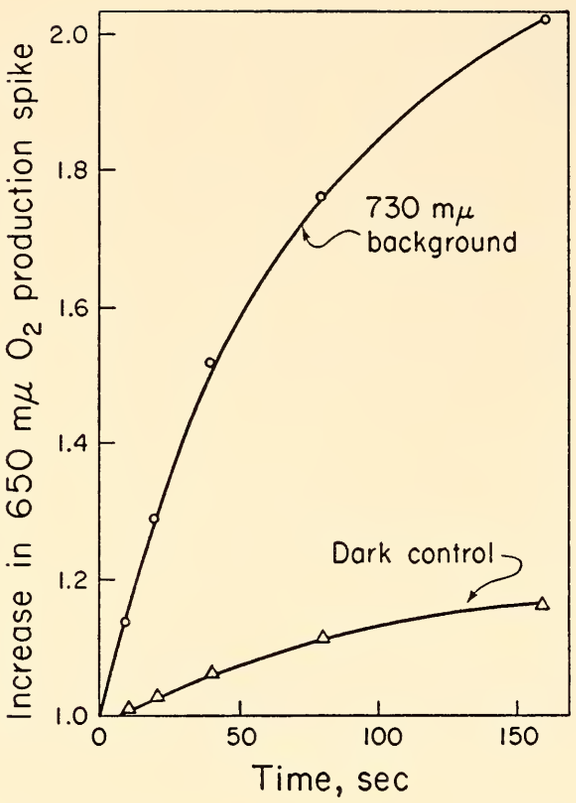

Fig. 13. The effect of 730-mμ exposure time on the O_2-production spike brought about by a 650-mμ light superimposed on 730 mμ. After 730-mμ light had been on for the time indicated, a 650-mμ beam was turned on long enough for the spike to reach its highest value. Both beams were then turned off. The ordinate is expressed as the ratio of the height of the 650-mμ O_2 spike after a 730-mμ exposure (or dark period for dark control) to the height of the 650-mμ O_2 spike before a 730-mμ exposure. The intensity of the 730-mμ beam was 570 ergs cm^{-2} sec^{-1}; of the 650-mμ beam, 803 ergs cm^{-2} sec^{-1}. Temperature, 20°C. Gas phase, N_2.

figure 11 labeled "Regeneration of ability to evolve O_2" is proportional to the ratio: height of the O_2-production spike from the standard 650-mμ exposure given 1 minute after a previous exposure to monochromatic light divided by the height of the O_2-production spike from the standard 650-mμ exposure. The red peak in this action spectrum occurs around 730 mμ. Other measurements of this action spectrum gave peaks at 713, 720, 725, and 729 mμ. Blue-green light can also bring about a response similar to that of far-red light. A dark control for this action spectrum, with a dark period substituted for a monochromatic exposure, gave a ratio of increased 650-mμ O_2 production of 1.03.

The far-red peak around 730 mμ in the action spectrum suggests that O_2 production might be influenced by a phytochrome system. However, the phytochrome system seems not to be involved, since the time-course curve of O_2 evolution at 480 mμ (not absorbed by the reversible phytochrome system) also shows a decline similar to that from 650-mμ light in the rate of O_2 evolution during the exposure.

At present, it would seem reasonable to attribute the stimulation of O_2 production by far-red to a light reaction which causes the reoxidation of the unknown reduced component whose oxidized form is the substrate for O_2 evolution.

A low-intensity photooxidative process and its action spectrum. Oxygen production by these chloroplasts was inhibited by DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, kindly supplied by Dr. H. J. Thome of E. I. du Pont de Nemours & Company, Wilmington, Delaware. Figure 14 shows the effect of adding this herbicide to chloroplasts. The 650-mμ light was again turned on 36 seconds after addition of the DCMU. The O_2 spike is smaller than before, and a rapid drop in O_2 evolution is seen during the 650-mμ exposure. A smaller O_2 gulp is also seen when the light is turned off. Subsequent exposures to 650-mμ light result in a net uptake of O_2. The light-dependent O_2 uptake becomes constant in magnitude about 5 minutes after poisoning, and the O_2 gulp disappears.

While the light is on, O_2 is produced from a substrate that is rapidly used up. At the same time a respiratory stimulation is induced by the formation of a product of the light reaction. When the light is turned off the respiratory stimulation persists until this photoproduct is used up, thus giving the O_2 gulp. DCMU poisons the O_2 evolution but not the photostimulated respiration, which can

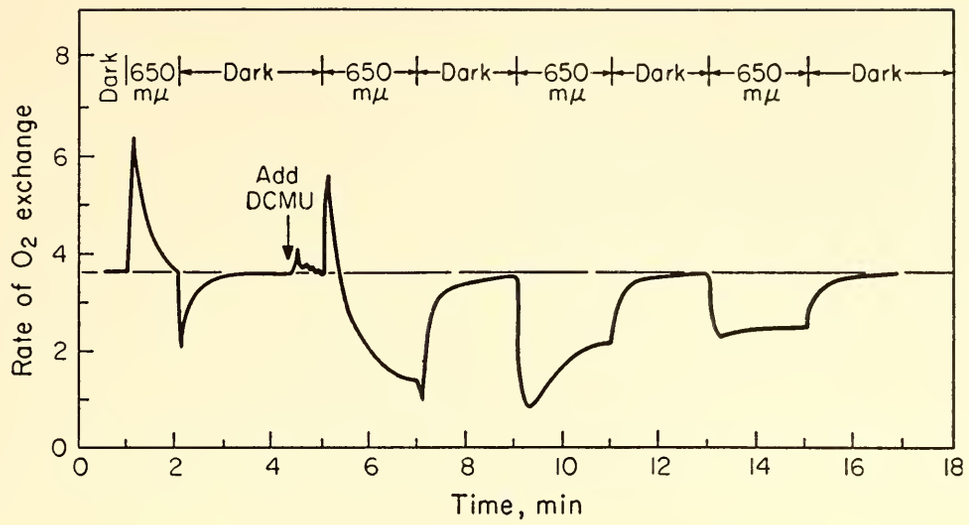

Fig. 14. Swiss chard chloroplasts exposed to 650-mμ light before and after the addition of DCMU. The final concentration of the DCMU (added dissolved in 0.2 ml 95 per cent ethanol) was 2.7×10^{-5} *M*. The same intensity 650-mμ beam (803 ergs cm^{-2} sec^{-1}) was used for all exposures. Gas phase, air.

now be measured without interference by O_2 evolution.

The action spectrum for O_2 uptake was determined for this chloroplast suspension by automatic recording using equal numbers of incident quanta. Figure 11 shows that the peak in the red region for this effect, labeled "O_2 uptake," occurs at 690 mμ. O_2 uptake as a function of intensity of 690 mμ is shown in figure 15. Since the curve is half saturated at about 250 ergs cm^{-2} sec^{-1} the action spectrum

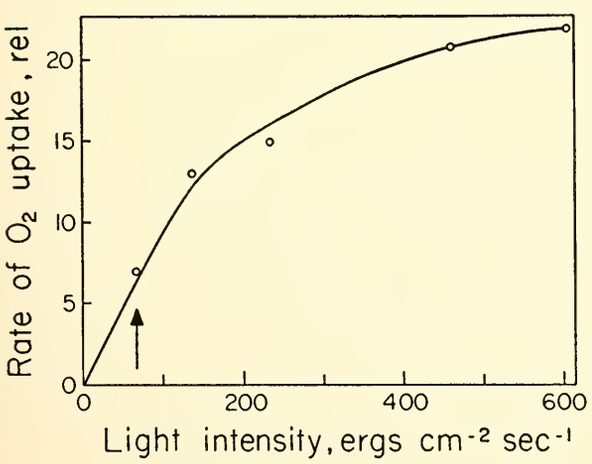

Fig. 15. Rate of O_2 uptake as a function of 690-mμ light intensity for the chloroplasts suspension treated with DCMU. The intensity marked by the arrow was used for the action spectrum recording.

was determined by using a quantum flux of 67.2 ergs cm^{-2} sec^{-1}, as shown by the position of the arrow. Upon the addition of trichloroacetic acid (TCA) to the circulating solution to a final concentration of 1.2 per cent, the chloroplasts turned olive-brown and O_2 uptake was abolished at the light intensity used for the action spectrum. A large O_2 uptake remained, however, when the TCA-treated chloroplasts were exposed to bright white light. It may be a result of photooxidation reactions similar to those described by Franck and French. Whereas the present photooxidation process is measurable at low intensity (i.e., with a quantum yield roughly comparable to that of photosynthesis), it is believed to be like that described as respiratory stimulation in *Porphyridium* (*Year Book 60*, pp. 351–357).

This uptake of O_2, in contrast to the uptake observed by Mehler after ethanol-catalase had been added as a "trap" for the H_2O_2, did not depend on the functioning of the O_2-evolving system. Green fragments of red algal chloroplasts which no longer evolved O_2 after phycobilin pigments had been leached out likewise showed a light-dependent uptake of O_2 (*Year Book 60*, pp. 369–370).

The effect of potassium ferricyanide on oxygen production. The O_2 production of chloroplasts to which no Hill oxidant had been supplied was compared with the O_2 production after the addition of potassium ferricyanide. The result obtained is shown in figure 16. The left part of the figure shows O_2 exchanges obtained when only the "standard" circulating solution was used. The first exposure to 650 mμ gave a time course for O_2 production very similar to the time course described earlier. The ability of a previous mμ no longer stimulates O_2 production in the 650-mμ beam. In some experiments with ferricyanide the time course for O_2 production exhibited a long-term induction effect with a protracted O_2-production spike. In these cases, however, steady-state O_2 production was attained after about 3 minutes in the light.

The action spectrum for oxygen evolution with potassium ferricyanide. The action spectrum for the evolution of O_2 after the addition of ferricyanide could be readily obtained, again by means of procedures

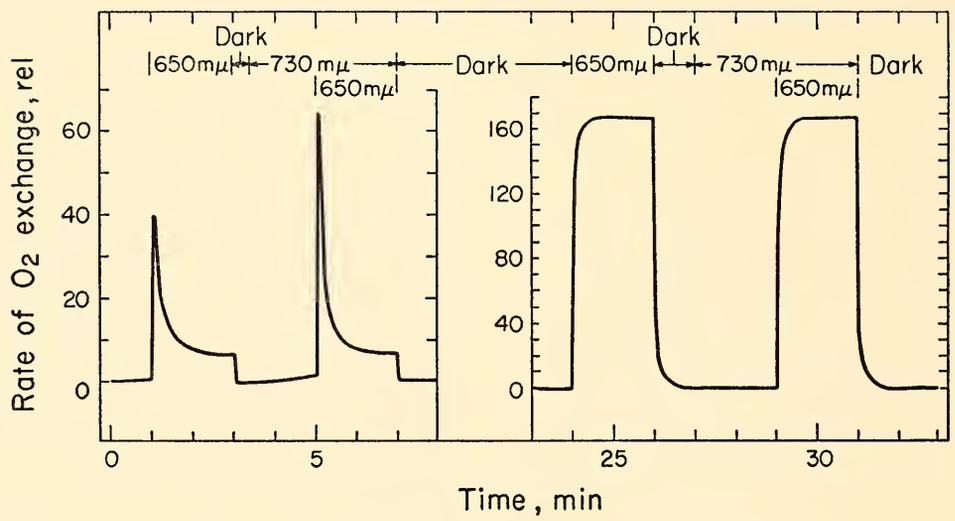

Fig. 16. The effect of potassium ferricyanide on the time-course curves of O_2 evolution and on the relative rate of O_2 evolution in the steady state. Potassium ferricyanide was added to the circulating solution during the 17-minute dark interval. Gas phase, N_2. The intensity of the 650-mμ beam used was 803 ergs cm^{-2} sec^{-1}; of the 730-mμ beam, 459 ergs cm^{-2} sec^{-1}.

730-mμ exposure to increase the 650-mμ O_2 spike is also seen. During a dark interval of 17 minutes potassium ferricyanide was added to the circulating solution to a final concentration of 4.3×10^{-3} M. A subsequent exposure to the same 650-mμ beam produced a steady-state rate of O_2 evolution about 25 times higher than that obtained previously. Moreover, the time course of O_2 evolution at 650 mμ in the presence of ferricyanide remains at a high level. A previous or concurrent exposure to 730 for the automatic recording of action spectra with equal incident quanta. The resulting action spectrum of figure 11 is labeled "O_2 evolution with FeCy." Its main peak was at 678 mμ, and there was a broad shoulder in the 640- to 650-mμ region. A check of the O_2 production as a function of intensity of 675-mμ light showed that the action spectrum was determined well within the linear region of the saturation curve. This action spectrum is similar to a "normal" action spectrum for O_2 evolution by green plants

like that of Haxo and Blinks for *Ulva taeniata.*

A Teflon-Covered Electrode Assembly

David C. Fork

The bare platinum electrode in use for several years was remodeled to separate the experimental material from the platinum by a thin Teflon membrane. With the present system reagents like ferricyanide that would influence the electrode behavior can now be used. The ¼-mil Teflon is permeable to oxygen but not to ions. Since it is an electrical insulator the reference electrode is also placed under the Teflon. This system has the added advantage of avoiding the passage of current through the sample. The present assembly, like the one it replaces, gives a relative measure of differences of rate in oxygen exchange between the sample in the light and in the dark. $Ag/Ag_2O/(0.5\ N\ KOH)$ under a plastic film as used by Clark and by

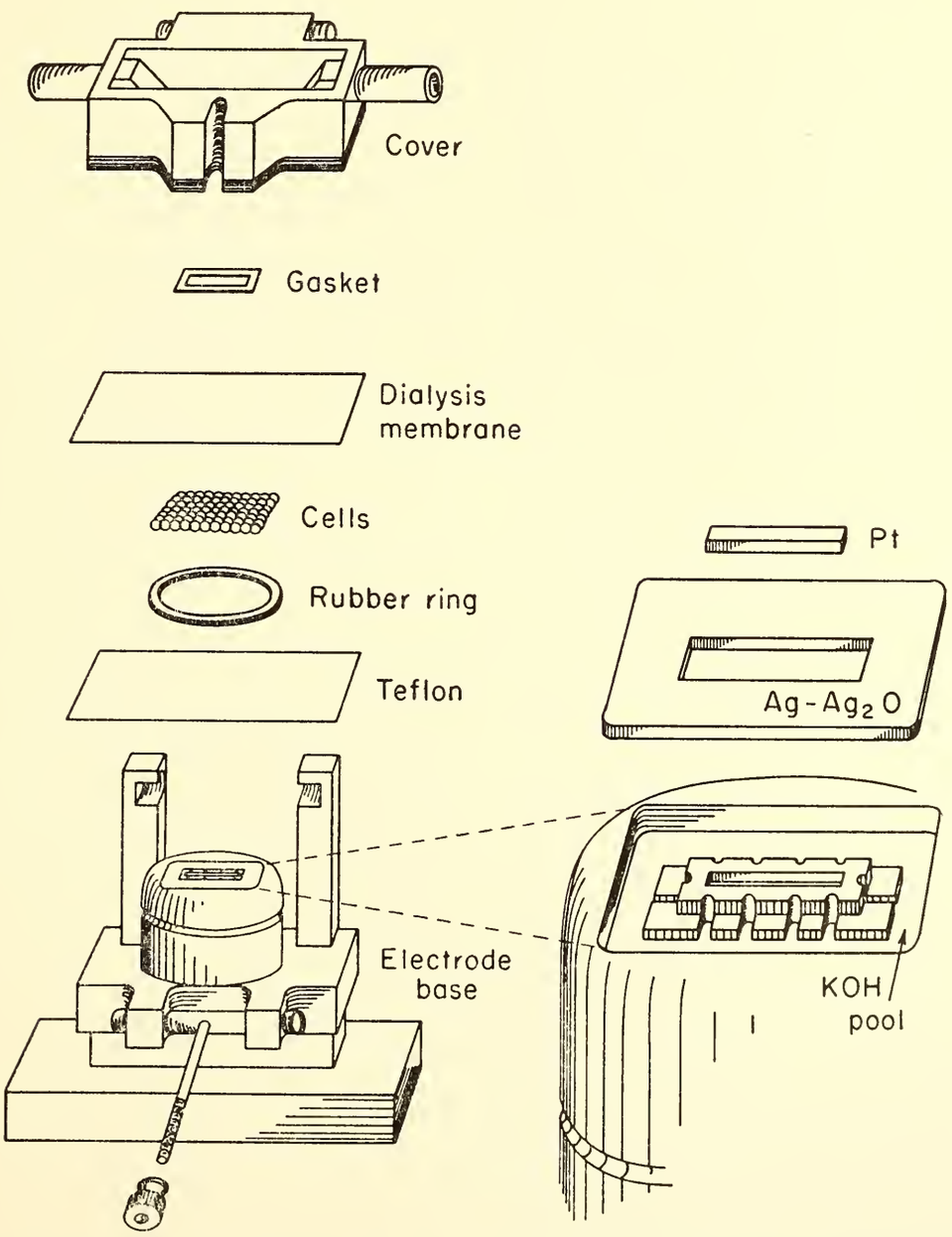

Fig. 17. The Teflon-covered Pt electrode for measuring the rate of O_2 production by chloroplasts.

Carritt and Kanwisher was adopted for a reference electrode.

An expanded view of the electrode assembly and the relation of its component parts is shown in figure 17. The base of clear Lucite was designed so that it could be positioned reproducibly under the beam from a monochromator. The platinum electrode, 1 by 15.3 mm, was set flush into the top surface of the Lucite. A rectangular Ag/Ag_2O reference electrode, 22.6 by 28.2 mm, with the center cut out was made from pure silver 0.8 mm thick, and was also mounted flush with the top of the Lucite base and with the platinum electrode. The Lucite was grooved underneath the Ag/Ag_2O reference electrode to make a pool for the KOH. The area of the Ag/Ag_2O reference electrode was about 75 times that of the Pt electrode. The Pt and the Ag/Ag_2O electrodes were covered with a 6.4-μ-thick sheet of Teflon film held down over the electrodes by a rubber ring. A thin film of KOH over the surface of the Pt and Ag/Ag_2O electrodes was trapped under the Teflon. The KOH pool and the KOH under the Teflon film were connected through channels cut away at intervals around the Pt electrode as shown in the insert of figure 17. Leads, soldered to the under sides of the Pt and Ag/Ag_2O electrodes, were brought out under the level of the rubber ring and were sealed in place with beeswax. The leads were attached to binding posts on the electrode base.

Chloroplasts (or cells) were spread in a thin layer on the surface of the Teflon above the Pt electrode and then covered with a piece of moistened dialysis membrane held in place by a rubber gasket and a cover. Circulating fluid entered through one end of the cover, flowed across the surface of the dialysis membrane, and passed out the other end. A modified flowing system suggested originally by Professor Jack Myers was used, so that a solution at constant O_2 tension was passed over the electrode at a steady rate. The electrode thus gave measurements of the rate of O_2 production by chloroplast preparations.

The fluid circulating system and gas exchange system for small volumes of solution is shown in figure 18. A Lucite centrifugal pump similar to that described in another report with a hold-up volume of about 3 ml gave a fluid flow of about 410 ml/minute past the electrode. The total volume of circulating fluid required to fill this system was 50 ml. The gas exchanger was made of Pyrex tubing 15 cm long and 4 cm in diameter and was about half filled with sections of small glass tubing, which served to increase the surface area for gas exchange and also prevented gas bubbles from entering the centrifugal pump. If nitrogen was substituted for air as the gas phase, a new stable dark base line was established after about 8 minutes.

The Ag_2O coating of the electrode was formed originally by polarizing the Pt electrode at -0.8 volt with reference to the Ag electrode and leaving it in the air for a day. The resulting Ag/Ag_2O reference electrode was then ready for use. Since the Teflon membrane is permeable to CO_2 in the atmosphere, the electrode, when not in use, was kept in a desiccator over NaOH pellets to prevent CO_2 from neutralizing the KOH in the electrode.

When in use, the Pt electrode was polarized at -0.8 volt by a circuit with 1.35-volt Mallory (RM-42RT) mercury batteries which also powered a balancing circuit. The voltage across a 100-ohm resistor in series with the electrode was measured by a Beckman model 14 chopper amplifier and a Varian recorder.

The electrode without the cover showed a light response and caused the recorder pen to go in the same direction as that caused by O_2 production. This signal apparently resulted from a light reaction at the Ag/Ag_2O reference electrode. When the gasket and cover were in place, the Ag/Ag_2O electrode was shaded and there was no longer a response to light with the highest intensities used in these experiments.

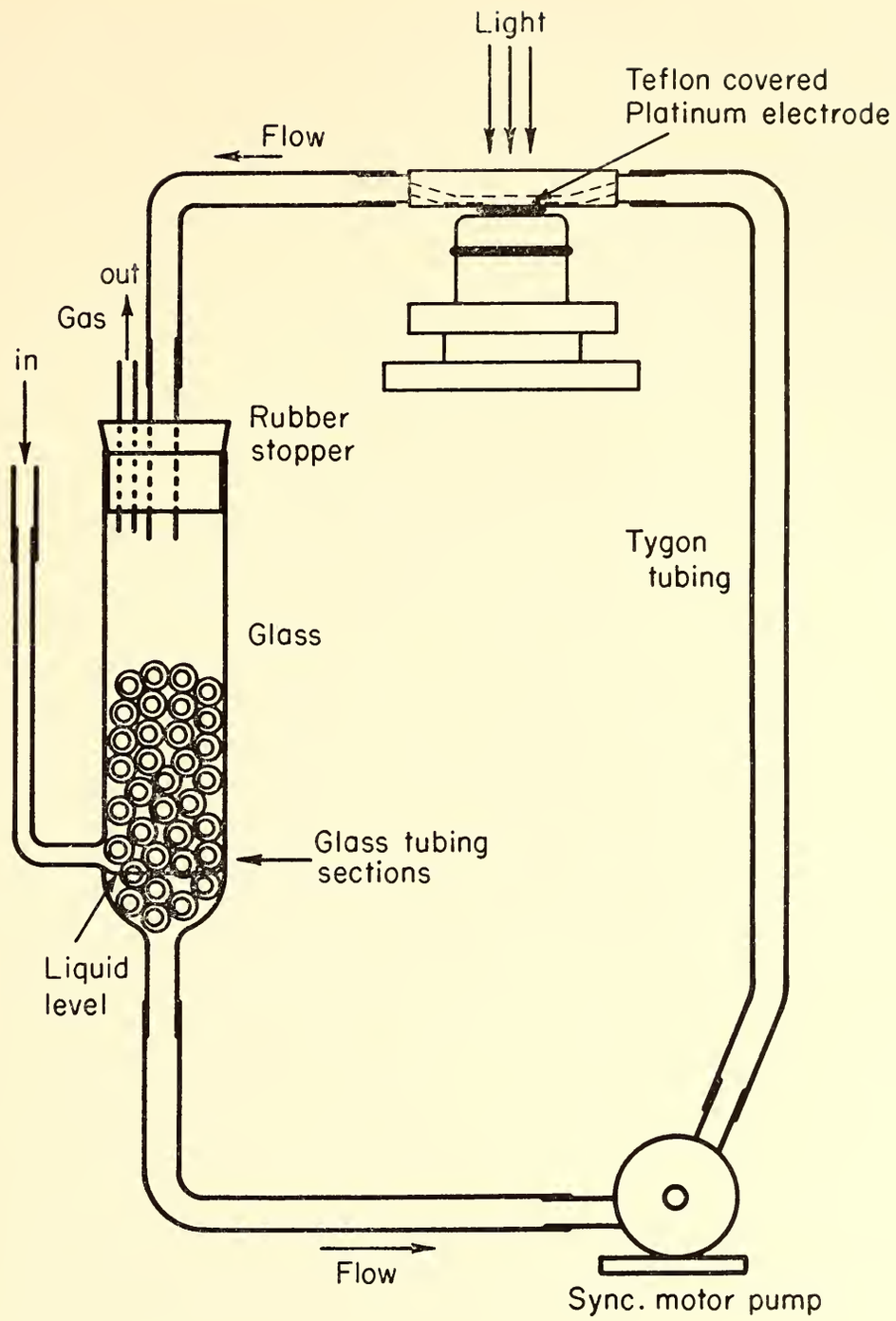

Fig. 18. The circulating and gas exchange system with the Teflon-covered Pt electrode.

Relations between the Two Photochemical Reactions of Photosynthesis

C. S. French

The two-pigment nature of photosynthesis is an aspect of the process particularly suitable for experimental investigation now. Several different types of model schemes have been proposed to explain the various manifestations of the two pigment systems. We therefore need simple experiments to distinguish between various models of this part of the photosynthetic process. Two types of kinetic experiments appear to be helpful in evaluating the relative merits of these models.

For practical reasons both types of experiments, O_2 evolution from paired flashes of two colors and the time course of the decay in O_2 evolution rate after a light period, are measured with the same preparations. Although both are in their early stages, the colored flash measurements have so far given clearer results than the studies on decay curves for O_2 evolution. Some illustrations of these experiments are presented as a report of work in progress rather than as a completed study.

Experiments with two light flashes different in color.[3] The two-pigment nature of the photosynthetic process is clearly shown by the enhancement found by Emerson when both pigment systems are illuminated together. A major question about the enhancement phenomenon is whether one specific pigment system helps the other one or whether the enhancement is mutual. That is, does the yield from each pigment system increase when the other one is also activated? Steady-state rate measurements during concurrent illumination by both wavelengths do not distinguish between the effects of the two beams, whereas illumination by the two colored beams separated in time should give results bearing on this question.

Several years ago Myers found that it was possible to alternate the light beams with periods of a few seconds and still observe enhancement when the continuous rate of O_2 evolution was measured. The present work is an attempt to measure the O_2 from single flashes or pairs of flashes rather than from a continuous series of alternate exposures.

In principle, if one pigment produces material necessary for the action of the other pigment it should be possible to tell the order in which the pigments must act. Thus if two consecutive flashes of different colors, one favoring each pigment, are given, and the total O_2 evolution is observed, a greater effect might result with one sequence than with the other. Furthermore, by giving variable dark times between the two flashes, intermediary nonphotochemical reactions may be observed that are related to the activation of certain pigments but not of others.

The measurements were made with a Teflon-covered electrode similar to that described by Fork elsewhere in this report except that the electrode system Au/(0.5 *M* $KHCO_3$, 0.5 *M* KCl)/AgCl was used under the Teflon. A thin layer of algae over the Teflon was held down by a cellophane membrane under tension. Above the cellophane, the algal growth medium was circulated as described by Fork.

The response time of this electrode covered with Teflon and cellophane was measured by injecting a small amount of deaerated water into a stirred bottle of air-equilibrated water that was continuously pumped through the electrode assembly. One-half the total response was achieved in 3 seconds. Presumably the response to illumination of cells placed directly over the Teflon-covered electrode would be shorter than the response of this complete system. A similar electrode assembly with a piece of fine nylon stocking to hold more electrolyte between the Teflon and the oxygen electrode was also tested. This assembly had a half-response time of 12 seconds. The bare Pt electrode previously used should be the best of all for speed of response, but its response speed has not yet been tested by this means.

The light beams came either from a monochromator with supplementary stray light filters or from tungsten lamps with interference filters. The rest of the equipment has been described in the reports of the last three years. The servomotor shutters opened or closed within 0.03 second as measured with a photocell and oscilloscope. For this work the shutters were controlled by a pair of motor-driven timers.

The time course for changes in rate of

[3] Some preliminary experiments with colored light flashes were made in collaboration with Dr. Fork and with Dr. Brown.

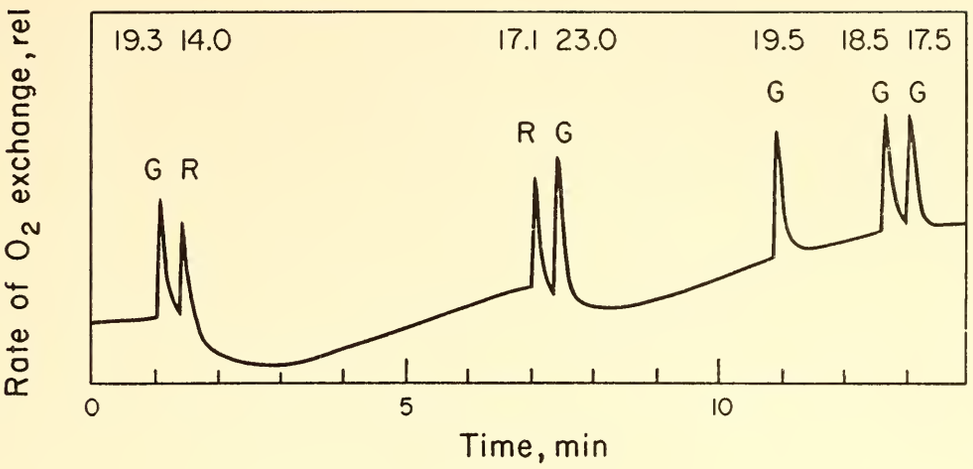

Fig. 19. The rate of O_2 exchange produced by 3-second flashes of green light, 570 mμ absorbed mainly by phycoerythrin, and of red light, 680 mμ absorbed mainly by chlorophyll *a*, in *Porphyridium* at 20°C. The peak heights are given above each spike. A red flash before green enhances the O_2 production from green. A green flash before red, however, instead of enhancing the O_2 evolution by red, reduces it slightly.

O_2 exchange from paired flashes is illustrated in figure 19, which also shows the peak rates produced when the flashes were given in various sequences.

It appears that material made by red light remains in the cells long enough to enhance a succeeding green flash. For the reversed order, however, green before red, or both together for these short times, there was no such enhancement. There is, in fact, a *depression* of the response to red light following a green flash. The effect is small, but it has been found repeatedly.

An attempt was made to see whether a longer exposure to green light absorbed by phycoerythrin in *Porphyridium* would produce material remaining long enough to give enhancement of a red flash. The peak height of a 10-second red flash before the green exposure was 10.8. Twenty seconds after the 1.5-minute green exposure the response to red was 10.1, again showing a small decline rather than an enhancement. The same red flash was also given while the cells were exposed to green light, both at the peak of the initial spike of the green-light time-course curve and again after the steady-state rate of photosynthesis had been reached. The responses to red light were 27.8 and 25.8, respectively. This shows strong enhancement of the red flash by continuous green but little difference at the two times, which were about 13 and 120 seconds after the start of the green exposure.

The peak heights reported are a measure of the speed with which photosynthesis gets started, that is, a decrease of the induction period, rather than a measure of the steady-state rate or of the total O_2 evolved. A number of records were made with a faster paper speed and the areas under the curves were measured with a planimeter to give the relative total amounts of O_2 produced by the pairs of flashes. For the total O_2 per pair of flashes, the results were qualitatively similar but less clear than the effects shown by the peak heights. The area measurements involve some uncertainty in deciding when the final base line is reached, and the peak measurements become hard to interpret except when the overlap in time is reduced by a dark period between the flashes, as in figure 19.

Following the observations of Whittingham and of others that a dark period between two flashes has an influence on the yield of the second flash, we varied the time between the start of the red

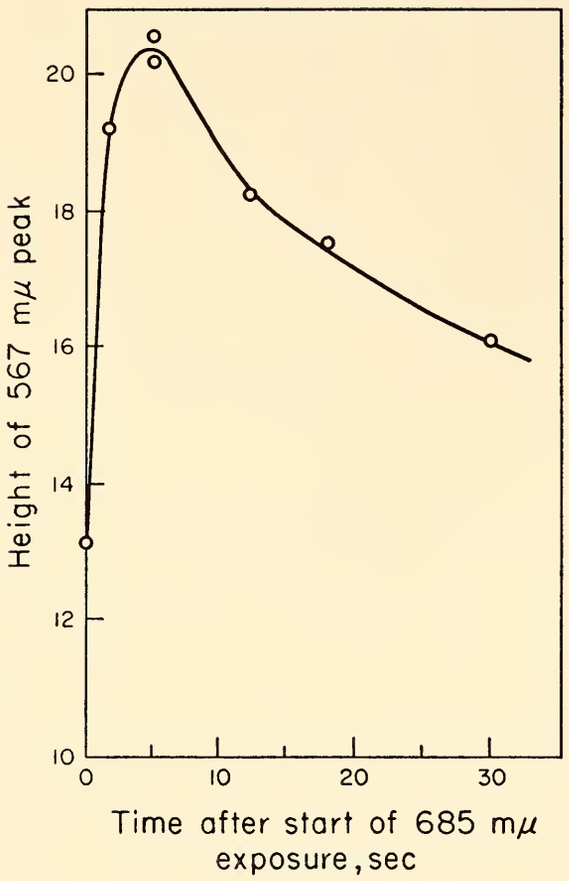

Fig. 20. The maximum rates of O_2 evolution in *Porphyridium* by a green flash given at various times after the start of a weak red flash. Red light: 685 mμ, 5 sec; green light: 567 mμ, 2 sec. The peak height from red alone was only 2.4 units; that from green alone, 13.7 units.

flash and that of the green flash. The results are shown in figure 20. The first point at $t = 0$ of figure 20 shows the result when both flashes are started together; for the second point the green was put on 2 seconds after the start of the 5-second red; and for the third point, shown in duplicate, the green was given immediately after the red, that is, 5 seconds later than the starting time. The dropping part of the curve clearly shows the rate of disappearance of the red product that makes the green light more effective. An approximate half-life of 18 seconds for the red-light product is found from the data of figure 20.

In summary, the experiments with two colors of flashes have shown that a product of chlorophyll *a* activity remains long enough to enhance a succeeding flash of light absorbed by the accessory pigment. The product of the accessory pigment's action, however, does not remain long enough to enhance a succeeding red flash.

The flash times used, 1 to 10 seconds, were not short enough to detect products with half-lives less than a few seconds. An extension to shorter times of the experiments with paired flashes of different colors may be undertaken in the future.

Experiments on the time course of oxygen exchange following a light period. Another way to get at the interrelation between the two pigment systems of photosynthesis is to investigate the kinetics of the slowly decaying O_2-evolution process after the photosynthetic light has been turned off. Certain model schemes predict that the curve relating rate of O_2 evolution with time after a light period should be first order, and that its shape should not be influenced by the color of the light previously used to drive the photosynthetic reaction. However, the model reaction scheme we used last year predicts that the decay curve should be second order and that its shape should vary with the ratio of the light absorbed by the two pigment systems.

These concepts are so simple and clear that the resolution of the question by experiment might have been an easy matter except for a complication, which is itself wavelength-dependent. The difficulty is the production by light of material that greatly increases the consumption of O_2 for a short time. This "respiratory stimulation," perhaps better called "increased oxygen consumption," is at its maximum when the light is turned off. It then declines rapidly during the period of interest for the study of the lingering O_2 evolution following a light period. The magnitude of the respiratory stimulation, which obscures the changes in O_2-evolution rate, is very dependent on the previous treatment of the cells. It

may be possible to design experiments in such a way as to reduce this source of confusion. Fortunately, various species of algae show the respiratory stimulation to very different degrees and with very different half-lives.

At present this approach to the comparison of different theories for two pigment mechanisms appears to have some potential value as a test for the relative merits of contrasting concepts of the mechanism of photosynthesis. Appropriate organisms and favorable conditions for their use to this end are being sought. A few measurements of the time course of the O_2-evolution rate after light exposure under different conditions will be discussed here.

The process of respiratory stimulation by light shows so clearly in *Porphyridium* because its magnitude and its half-life of about 1.1 minutes make its presence obvious on a time scale convenient for measurements of the time course of photosynthesis. If the half-life of the intermediate carrying the increased O_2 consumption had been an order of magnitude higher or lower it would not have been as evident in the records.

A much more rapid process of this nature has recently been observed in a green alga, *Scenedesmus*. Here the respiratory stimulation shows only as a brief dip in the otherwise orderly decay of O_2 evolution after a light exposure. That this irregularity, frequently observed in various laboratories, is due to the increased O_2 consumption by light becomes clear from the following experiment.

The time course of O_2 exchange was followed for a 20-second exposure to 650 mμ absorbed mainly by chlorophyll *b*. This was done both with and without continuous background light of a wavelength absorbed more by chlorophyll *a*. This background light of 690 mμ speeds up the rate of decay of the lingering O_2 evolution so much that the respiratory stimulation becomes recognizable as such by a short-time drop below the previous

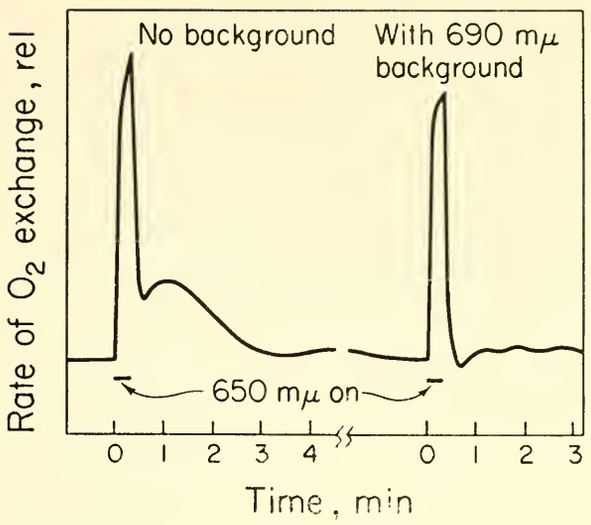

Fig. 21. The rate of O_2 exchange from a 20-second exposure at 650 mμ in *Scenedesmus*. A background light of 690 mμ increases the rate of decay of the lingering O_2 evolution so much that the opposing light-stimulated O_2 uptake becomes evident.

base line. That is, an actual net O_2 uptake was briefly observed. This effect is illustrated in figure 21, which makes it apparent that the second maximum of the normal decay curve is not due to an actual increase in rate of O_2 evolution.

A converse experiment was also per-

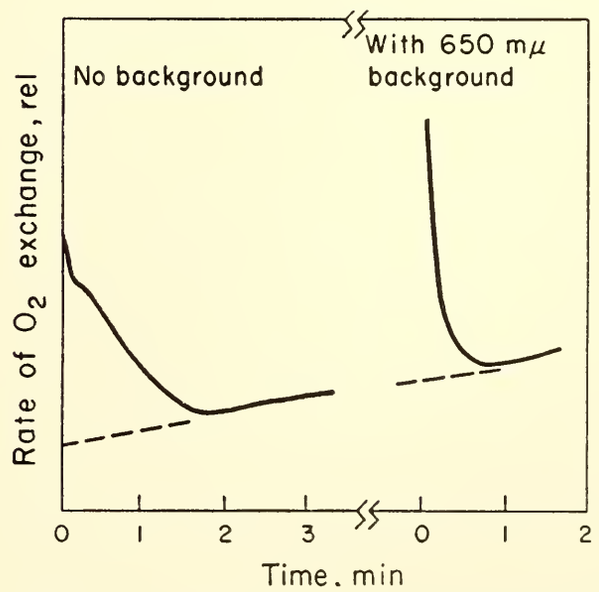

Fig. 22. The decline in rate of O_2 evolution in *Scenedesmus* after a 2-minute exposure to 700 mμ. With 650-mμ background the decline is much steeper.

formed, but in a somewhat different way. Measurements were made of the time course of the decay in rate of O_2 evolution by *Scenedesmus* after an exposure of 2 minutes to 700-mμ light. These curves are shown in figure 22. At the start of each record the 700-mμ light was turned off. The sloping base line is given by the dotted lines.

The decay of rate of O_2 evolution after the 700-mμ exposure is greatly increased by the presence of 650-mμ background light as seen from the slopes of these decay curves. In the curve measured without background light the small, rapid, initial drop is attributed to stimulated O_2 uptake which is finished long before the O_2-exchange curve reaches its base line. In the second curve this is obscured by the very rapid decay induced by the 650-mμ background.

Some attempts have been made at fitting similar data with positive first-order decay curves, corrected for the stimulated O_2 uptake by negative first-order curves with smaller time constants. So far no adequate fits have been achieved. Whether second-order curves for the decay as predicted by one of the models with first-order corrections will give better fits remains to be seen.

In any event, it is clear that the rate of decay of O_2 evolution is greatly speeded up by background light acting on either pigment system.

Enhancement and Photostimulated Oxygen Consumption in *Porphyridium*

J. S. Brown

Some clarification of the differences between the two primary photochemical reactions in photosynthesis driven by different pigments has come from experiments with the red alga *Porphyridium cruentum* (*Year Book 60*, p. 351). We have continued these experiments to see what role, if any, the physiology of the alga plays in the magnitude of photosynthesis, enhancement, and photostimulation of O_2 consumption as measured by the polarographic technique. We express enhancement as the ratio of the rate of O_2 evolution when two wavelengths of light are given simultaneously to the sum of the rates from the same two light beams separately. This avoids any assumption about the mechanism of the effect. *Porphyridium* under optimal conditions yields much higher enhancement ratios than green algae.

The enhancement ratio found is extremely sensitive to the physiological condition of the alga. Apparently all the growth conditions such as temperature, nutrition, and light must be kept rigidly constant in order to obtain reproducible enhancement values with different cultures of the same species.

In addition, the rate of photosynthesis at a single wavelength and intensity may change as the algae adapt to the conditions of measurement. Once this adaptation period of about 4 hours has passed, photosynthesis and enhancement shown by a particular cell preparation will remain constant for a day or two. Whether the rate of photosynthesis rises or falls during the adaptation period probably depends on the initial physiological state of the alga and the color and intensity of the light used for the measurements. The enhancement ratios are considerably larger at low than at high photosynthetic rates.

Another point of interest is the ratio of intensities of the two light beams required in order to yield maximum enhancement. We have confirmed with *Porphyridium* the earlier work of Myers and French with *Chlorella* that, to obtain maximum enhancement ratios, the photosynthetic rate from light absorbed mostly by accessory pigment should be two to three times the rate from light absorbed by the long-wavelength chlorophyll. Whether the same optimum ratio of photosynthetic rates holds true for all pairs of wavelengths that yield any enhancement has not been determined.

Figure 23 shows two action spectra for

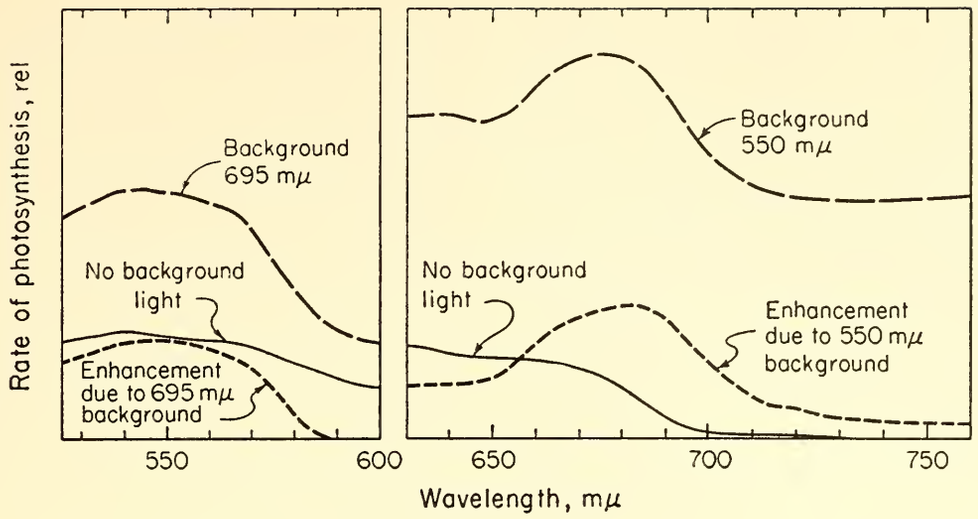

Fig. 23. Action spectra for rate of photosynthesis by *Porphyridium cruentum*. The upper curves were continuously recorded with a constant intensity of background light of 550 and 695 mμ, respectively. Enhancement was calculated by first subtracting the rate due to the background light alone from the upper curves and then determining the difference between the two action spectra at each wavelength. Small drifts with time of the base lines were also corrected in the calculated enhancement.

O_2 evolution from *Porphyridium* which had previously adapted to a steady-state rate of photosynthesis on the electrode. The solid curves give the rate of photosynthesis as the wavelength was slowly changed while the intensity in incident quanta per second was maintained constant. The upper curves represent enhanced photosynthesis, since the algae were receiving a constant intensity background light of 550 mμ as the red region was being traversed and were illuminated with background light of 695 mμ for the green region of the action spectrum. Although the difference between these two curves, with and without background light, has sometimes been assumed to be an action spectrum for enhancement, we do not believe it to be a true representation, for the following reason: the spectra were measured with a constant ratio of light intensity between the pairs of wavelengths, whereas we know that there is an optimum ratio between the rates of photosynthesis which should be kept constant when comparing effectiveness of different wavelength pairs. It should be possible to determine the approximate shape of the action spectrum for enhancement with a constant photosynthetic ratio from a family of curves as in figure 23, varying the intensity of the background light with each curve. From these data we could compare enhancement from different wavelength pairs but with the same ratio of the rates of photosynthesis from each wavelength given separately.

The wavelength-dependent light stimulation of O_2 uptake by *Porphyridium* was described in last year's report. It is observed as a large uptake of O_2 when the light is turned off. After particular wavelengths and intensities, the apparent increased rate of respiration may be as large as the previous photosynthetic rate and may have a half-life of 2 to 3 minutes. This photostimulated O_2 uptake is initially proportional to the light intensity but saturates at very low intensities. The maximum amount of photostimulated O_2 uptake has been found to vary with the growth conditions of the alga. For instance, initially, greater photostimulated O_2 consumption is shown by cells cultured with cool-white fluorescent lights than by cells cultured with tungsten lamps. It is also greatest when the cells are first placed on the electrode, and then it decreases gradually in time. By the

second day very little remains—a fortunate circumstance which makes feasible the continuous recording of an action spectrum for O_2 evolution. Numerous action spectra for the photostimulation of O_2 uptake have invariably shown a broad peak centered at 685 mμ. Since one of the forms of chlorophyll *a* observed in vivo also has an absorption maximum at 685 mμ, it is tempting to postulate that this form may be responsible for the formation of easily oxidizable material.

We have examined the absorption spectra of various *Porphyridium* cultures to determine whether the ratio of phycoerythrin to chlorophyll correlates with the amount of enhancement or photostimulated O_2 consumption shown by these cells. Our results corroborate those of Marcia Brody that the ratio of phycoerythrin to chlorophyll can be altered by changing the quality of the light with which the cells are grown. Different pigment ratios may account for initial differences in enhancement and in photostimulated O_2 uptake. However, this pigment ratio does not change during the 4 to 5 hours' measurement period and therefore cannot account for the changes occurring during this time.

Derivative absorption spectra of the red band of chlorophyll *a* in *Porphyridium* reveal the presence of the usual two forms of chlorophyll *a* with maxima at about 670 and 683 mμ, but the ratio of these forms does not seem to vary with the different growth conditions.

Although the photostimulation of O_2 consumption probably is induced via a specific pigment, C_a685, the magnitude of the response may depend on the size of the pool of intermediates. In turn this pool size may be limited also by the kind of alga being observed and by its physiological state.

All these observations support the contention that enhanced O_2 evolution is not the result of the direct physical interaction of two pigments but rather occurs because of the optimum production of intermediates which themselves result from the light activation of each of the two pigment systems

We believe that throughout most of the spectrum the pigment ratios are such that intermediates necessary for O_2 production are produced in near-optimum amounts. But at certain wavelengths—notably around 700 mμ with green algae and in the whole red region with red algae—photosynthesis is limited by too little of the intermediates produced by the accessory pigments and therefore is enhanced by adding light absorbed by this pigment system.

Physical Separation of Pigment Complexes from *Euglena*

J. S. Brown

Study of the chlorophyll complexes of aging *Euglena* has progressed during the past year. When a dense culture of *Euglena* cells is allowed to age in the dark cold room, the chlorophyll *a* absorption peak shifts toward longer wavelengths. The shift is attributable to a simultaneous increase of absorption at 710 mμ and a decrease of absorption at 695 mμ.

Last year we reported that the component absorbing at 710 mμ (P_a710) was in the chloroplast and could be separated by differential centrifugation from a component absorbing at 685 mμ (C_a685). Now we find that when the cells are aged for a longer period of time, four and a half weeks, the brownish-green P_a710 is mostly in the cytoplasm and can be separated to a large extent by centrifugation from the green chloroplast pigments which have absorption bands at 670, 685, and 695 mμ. P_a710 together with a high proportion of absorption which we attribute to C_a670 remains in the supernatant. These two absorption bands are probably due to pigments attached to the same particle, since they sediment together when centrifuged at very high speed.

Ether extraction and ascending paper chromatography of the cytoplasmic fraction were carried out in collaboration with Dr. James H. C. Smith. The results

strongly indicate that the pigment absorbing at 710 mμ is pheophorbide *a* or a closely related compound. The question still remains whether the organism first forms pheophytin *a* in the chloroplasts and then excretes it into the cytoplasm as a pheophorbide complex or forms the pheophorbide directly from C_a695.

Electron Paramagnetic Resonance Studies on *Chlamydomonas reinhardi*

Ellen C. Weaver

It is possible to make observations of the free electrons in living material by means of electron paramagnetic resonance (EPR) spectroscopy. The method is intrinsically harmless to the system being observed, and the modern instruments can detect electron levels of the order of 10^{11} for a 1-gauss line width in aqueous medium. It is a well established fact that chlorophyll-containing material has a higher level of unpaired electrons when it is illuminated than when it is in the dark, suggesting that at least some phase of photosynthesis proceeds by single-electron transfers. Although there has been general agreement on the gross observations among several groups using this relatively new (since 1956) technique on photosynthetic material, there has been no convincing identification of the substances responsible for the signal or any rigorous demonstration that the electron resonance has any direct connection with photosynthesis.

The original aim of the present study was to determine whether or not the resonance was associated with chlorophyll. It was soon found that the signal was difficult to reproduce, necessitating precise control of all experimental variables. It was logical to extend the study to include a determination of the effect of a few variations in the cell's environment on the electron resonance signal. This has led us to a tentative identification of one of the substances responsible for the signals and to a better understanding of the behavior of the phenomenon. The work to be described was started in Zurich and continued at the Department of Plant Biology this past year. EPR spectroscopy, already in wide use by chemists, physicists, geologists, and others, seems certain to become an important tool in the study of photosynthesis.

Materials and methods. The instrument for these studies was kindly made available by Varian Associates. The experiments deal entirely with intact, living cells of a green fresh-water alga, *Chlamydomonas reinhardi*. Cultures that had lost the typical wild-type ability to form chlorophyll in the dark were obtained from the Cambridge Collection of Algae and Protozoa. Later work was therefore done with a different wild type (21g2) obtained from Dr. Ruth Sager, but there was no obvious difference in the pigments or behavior of these stocks when they were grown in the light. Mutants were derived from the Cambridge stocks by ultraviolet irradiation, only one of which (no. 100) is pertinent to the present discussion. Cultures were grown in liquid shake culture, bubbled with 5 per cent CO_2 in air, and illuminated by combined fluorescent and incandescent light. Temperature was not accurately controlled but was maintained at approximately 25°C with a water bath. The medium contained only mineral nutrients, including trace amounts of some seven transition ions. The only nutritional element that was varied was manganese, which was 2×10^{-6} *M* unless otherwise specified.

A Varian model V4502 electron resonance spectrometer equipped with 100-kc/sec field modulation was used. Samples were contained in a flattened quartz cuvette, 1 cm wide, with an internal thickness of about 0.25 mm. The sensitive volume was approximately 0.01 ml. The sample was illuminated through a slotted window in the cavity. Light from a 500-watt projection lamp passed through a 7.5-cm water bath and was focused on the slots of the cavity. Light

intensity was regulated with a Variac. Nearly monochromatic light was provided by means of interference filters, with half-widths varying from 10 to 13 mμ, plus Corning glass cutoff filters to eliminate the harmonics. The relative intensity of light from all the filters in the optical system was measured for a series of lamp voltages with a bolometer.

Ratios of chlorophyll *a* to chlorophyll *b* were obtained, with the help of Dr. Jeanette S. Brown, by means of the derivative recording spectrophotometer. Percentage light transmission of the samples in the cuvette for one of the electron resonance experiments was measured by Dr. French.

Determinations of *g* values were made by Dr. John Maling, of the Biophysics Laboratory at Stanford University, by a frequency counting method. The *g* value expresses the ratio of the magnetic field and the microwave frequency at the point where the resonance is a maximum. Both field and frequency must be determined with great accuracy, since all free-radical *g* values lie close to the value of the free "conduction" electron, which is 2.0023. In EPR spectroscopy the *g* value provides a measure of the location for a given resonance in the magnetic field much as does the wavelength of an absorption maximum in optical spectroscopy.

Cells to be used for resonance measurements were harvested from a 4- to 5-day-old culture. They were centrifuged in the cold for 8 minutes at 3000 rpm, resuspended in an aqueous medium free of manganese, and centrifuged a second time at the same speed. They were then diluted as necessary and pipetted into the cuvette, which was placed in the cavity of the spectrometer and adjusted to lie precisely at the node of the electric field and at the corresponding maximum of the magnetic field. Since it is intrinsically easier to vary the magnetic field than the microwave frequency, the microwave frequency is held stationary and the field is swept. The resultant resonance is usually recorded as the first derivative on a chart. A Moseley X-Y recorder proved to be particularly convenient for this purpose, as successive signals can be superimposed. The time constants of rise and decay rates were determined by means of a Sanborn recorder.

Results and discussion. Two distinctly different light-induced resonances can be observed: one seen only when the cells are illuminated, and another which persists in the absence of illumination. The former is centered at $g = 2.0025$, is 8.3 gauss wide from peak to peak, and disappears with a half-time of a fraction of a second when the light is turned off, hence is termed the *R* (rapid-decaying) signal. The other is centered at $g = 2.0046$, is about 20 gauss wide, takes time of the order of hours to disappear in the absence of light, and so is designated the *S* (slow-decaying) signal. Figure 24 is an example of these two signals as observed in wild-type *Chlamydomonas*.

Our first task was to determine whether or not the *R* signal was associated with chlorophyll, and the evidence bearing on this point follows. A mutant was isolated which was clear yellow whether it was grown in light or dark. An absorption spectrum of the extracted pigment revealed no trace of chlorophyll. There was a slight resonance from a dense suspension of these cells but no observable increment of the signal with illumination. The two Cambridge cultures, 32A and 32B, formed no chlorophyll when they were grown in the dark and yielded the same results: no detectable light-induced signal from the dark-grown (yellow) material. In addition, it was shown that an aliquot of the same culture, when allowed to green for 24 hours in the light, produced a normal signal.

If the *R* signal is ascribable to chlorophyll, an action spectrum for its amplitude should correspond to other action spectra for chlorophyll-mediated reactions. Amplitude refers solely to the vertical peak-to-peak distance of a given trace and should not be confused with

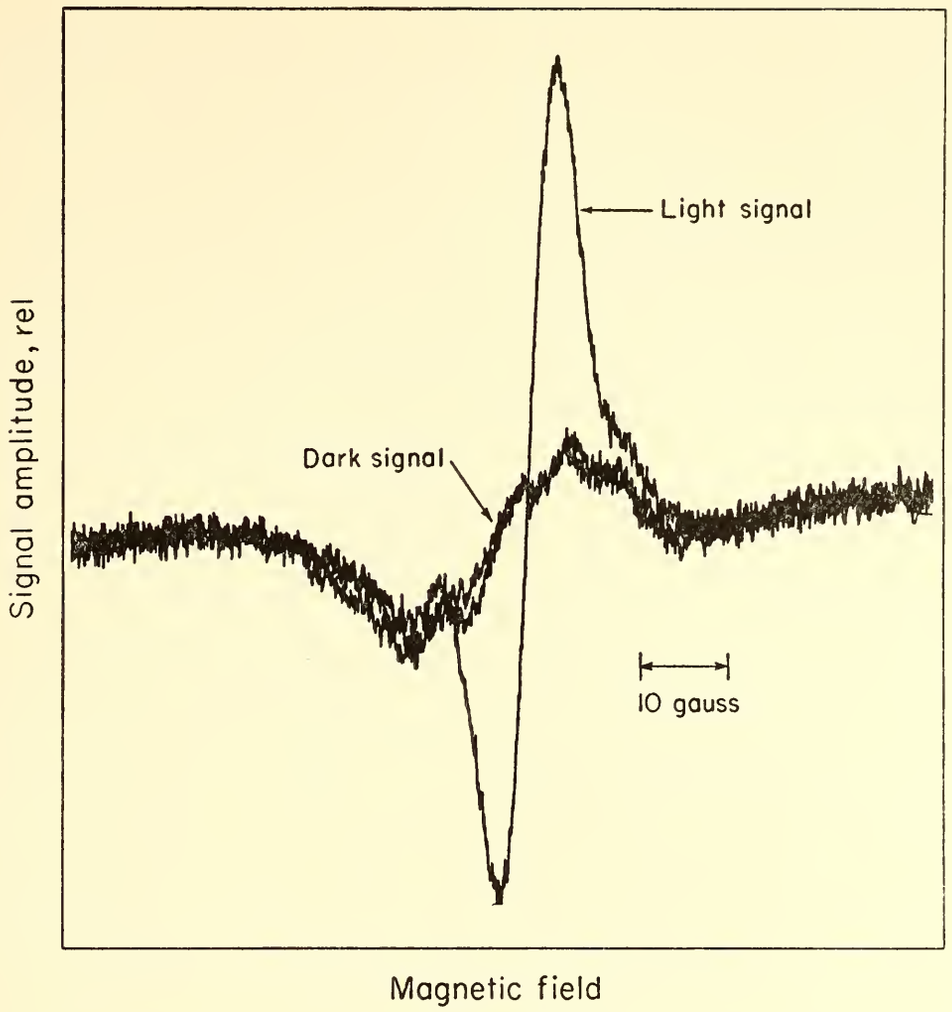

Fig. 24. EPR signals from *Chlamydomonas reinhardi* illuminated with red light, light signal, and in the absence of illumination, dark signal. The light *R* signal is superimposed on the persistent dark *S* signal, whose hyperfine structure is evident, owing to the use of a high gain and a low modulation (2.5 gauss peak to peak).

signal intensity, which is a measure of the number of spins being observed and can be calculated with accuracy only by precisely determining (1) the base line, (2) the width, and (3) the shape of the signal. The amplitude is a valid measure only when the other variables are known to be constant.

Our action spectra, as well as those reported by other groups working in this field, originally seemed to show that light with an average wavelength longer than that absorbed most strongly by chlorophyll *a* (about 680 mμ) was most effective in producing an *R* signal. Various explanations have been advanced to account for this, including one that ascribes the EPR signal to the "active centers" of chlorophyll that absorb most strongly at 700 mμ, like that of Beinert, Hoch, and Kok. However, we suspected it was simply a self-absorption phenomenon, and later we were able to show that, if the cell suspension was diluted sufficiently, 680-mμ light is indeed more effective than longer wavelengths in producing the narrow *R* signal. The degree to which material being observed can be diluted is limited by the signal-to-noise ratio of the resonance. The reduced precision was compensated for by making traces of the signal at each wavelength; by using several light levels, all well below saturation, for each wavelength; by randomizing the order in which measurements were made; by making frequent

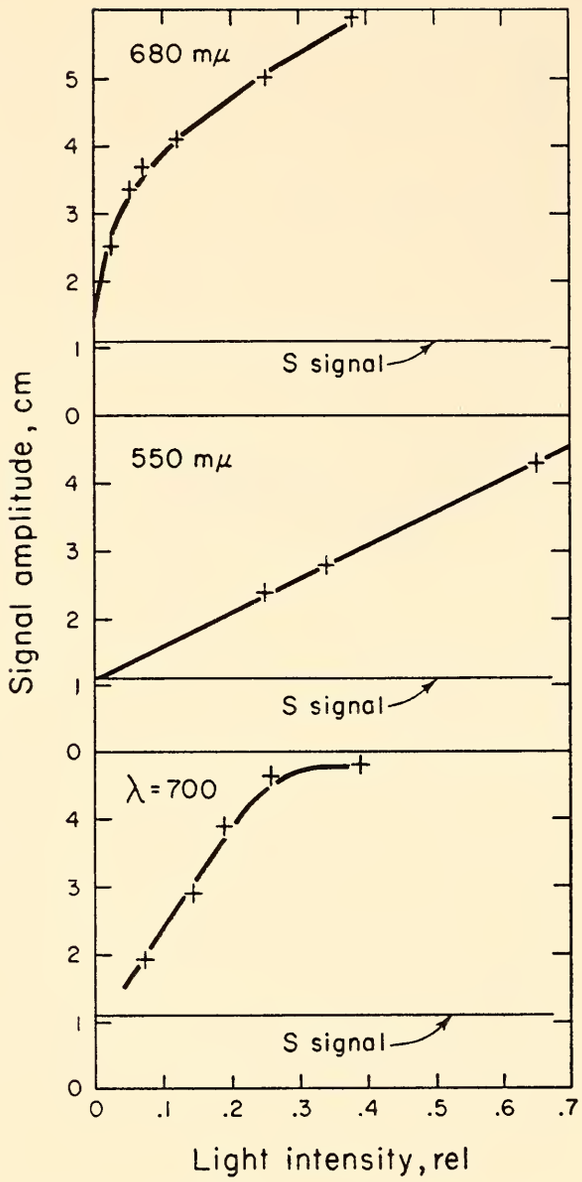

Fig. 25. Signal amplitude (vertical peak-to-peak distance) plotted as a function of light intensity for three different wavelengths. The *S* signal is constant, with an amplitude of 1.1 cm, and forms a base line for the *R* signal. The nonlinearity of the curve for 680 mμ indicates appreciable internal absorption.

checks of a standard filter and voltage combination to monitor the cells' response (which was normally reproducible over a period of several hours); and by making frequent checks of the signal in the absence of light.

With these data, it is possible to plot a light intensity versus signal amplitude curve for each wavelength (figure 25) and from this to plot an action spectrum for signal amplitude at a given light intensity for each wavelength. Figure 26 is an action spectrum from a cell suspension which transmitted about half of the incident light at 680 mμ in the cuvette used for EPR measurements. The maximum of the peak is far flatter than the absorption spectrum for chlorophyll, but in view of earlier findings it might be postulated that self-absorption is still playing a large part in the curve shape and that the peak would be sharpened by further dilution. The cell concentration for figures 25 and 26 was about 10^8/ml. Its percentage light transmission in the cuvette used for EPR measurements was as follows:

Wavelength	Transmission, %
650	46
680	45
694	58
730	90

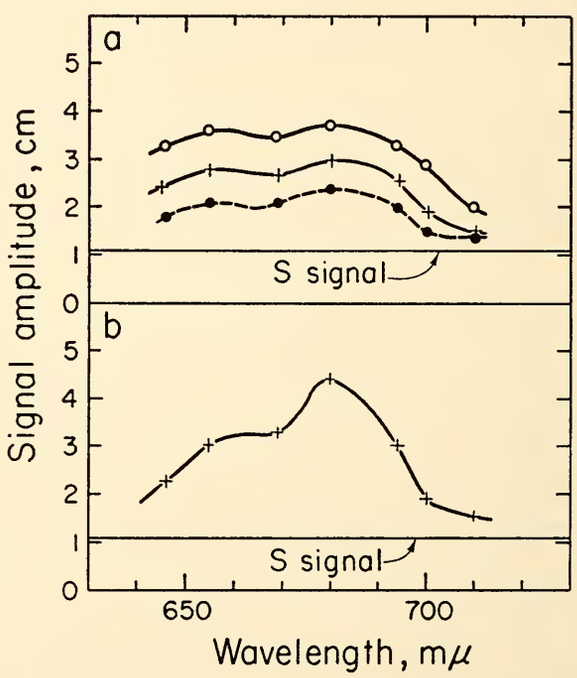

Fig. 26. (*a*) Action spectrum for production of EPR signal, derived from curves of the type illustrated in figure 25. Each of the three upper spectra represents the result for a different light intensity. These are flattened by the curvature of the plots shown in figure 25. (*b*) Action spectrum for production of the EPR signals obtained by back extrapolation of the same data to a very low intensity.

The nonlinearity of light intensity versus signal amplitude curves of figure 25 for strongly absorbed wavelengths indicates that self-absorption is playing an appreciable role. The extent of this effect can be roughly estimated by extrapolating the steepest part of the curve and plotting the points thus obtained. This has been done for figure 26*b*, with resultant sharpening of the peak at 680 mμ.

This estimation is strengthened by the shapes of the signal amplitude versus light intensity curves mentioned above. The curves for wavelengths strongly absorbed by chlorophyll have no linear portion, whereas those for other wavelengths do exhibit strict proportionality at low light levels, as shown in figure 25. There is also a corresponding variation in steepness of the curves.

Another experiment, by the same experimental techniques and with the same precautions as above, was performed to determine the maximum obtainable, or saturation, signal from each wavelength. It was found that the maximum signal amplitude was independent of wavelength, provided that the quantum energy was sufficient to excite an electron. Far-red light, 745 mμ, excites very nearly a maximum signal; 760-mμ light, even at rather high intensities, produced no detectable *R* signal (showing, incidentally, that the effect at 745 mμ is not a spurious one due to stray light). There was no obvious damage from 760-mμ light, since the response to shorter wavelengths was just as good after exposure as before. The shortest wavelength employed was 470 mμ. Since a dilute suspension saturates with a lower light level than a concentrated one, and the amount of light obtainable was limited, fairly dilute ones were used for this work. The efficiency of signal production is very low for wavelengths not strongly absorbed by chlorophyll. The action spectrum shows that light absorption by chlorophyll *a* leads to the production of the *R* signal. Whether the actual substance from which the free electrons are derived is chlorophyll *a* or some other material is not decided by the present experiments.

The tentative conclusion we have drawn from the foregoing results is that the *R* signal is ascribable to chlorophyll and that it arises as a consequence of the "primary" act of photosynthesis. This hypothesis is greatly strengthened by the finding in other laboratories, notably Calvin's, that the *R* signal can be produced at very low temperatures (−160°C) with no apparent increase in rise time of the resonance, indicating a purely physical rather than a chemical process. The *R* signal (or one that seems to be identical with it) has been reported not only in other algae and in chloroplasts of higher plants but also in methanol extracts of chlorophyll, dried chlorophyll films, and single crystals.

The amplitude of the *R* signal can be widely varied. Continuous or repeated illumination may bring about a reduction in *R*-signal amplitude, but eventually a steady state is reached in which signals are reproducible over a period of several hours. Freshly spun cells, which have been grown under optimal conditions and then suspended in a buffered medium, exhibit a rather small signal even at high white light intensities when they are in a physiologically healthy state. Presumably electrons are flowing smoothly in the well functioning system, and the steady-state level is rather low. If the cuvette is stored for an hour or two in the dark and again observed, it is found that both signals have disappeared. Under illumination, however, the *R* signal is induced and shows a very large amplitude, and subsequent tracing of the *S* signal reveals it to be similar to that originally observed. This "starvation effect" is illustrated in figure 27. Which of several factors is responsible for it is not known, but it is more pronounced when cells are grown in medium deficient in manganese or are suspended in distilled water rather than buffer. A similar enhancement of *R*-signal

amplitude can be demonstrated by treating the system with 10^{-5} *M* DCMU (3-[3,4-dichlorophenyl]-1,1-dimethyl urea), shown in figure 28. This compound acts very specifically to block the oxygen-evolving mechanism, with no other obvious toxic effects. Action spectra made with and without DCMU have the same maxima and minima, but the large signal amplitude produced by its use permits a greater accuracy of measurement.

Another way to block the oxygen-evolving mechanism of the cell is to limit manganese-ion concentration in the growing medium, since many studies have shown manganese to be an essential component of this system. Cells grown in medium having no manganese added (but in which no effort was made to exclude it, so traces were certainly present) grew slowly, were clumped, but produced ultimately a culture in which the proportions of chlorophyll *a* and *b* were similar to those in cells grown in normal medium, with perhaps a trace more chlorophyll *b*. The rate of oxygen production from these manganese-deficient cells was greatly reduced in comparison with cells grown in medium containing as little as 10^{-6} *M* Mn^{++}. However, they consistently gave rise to an enhanced *R* signal, in contrast to reports from at least two other laboratories.

It seems reasonable, then, to assume that, when the pathway of the electrons is in any way impeded, the net level of unpaired spins rises. Thus far we have only disturbed the mechanism for oxygen evolution, but it is hoped that eventually other pathways can be altered in specific and known ways and the effect on the electron resonance spectrum can be

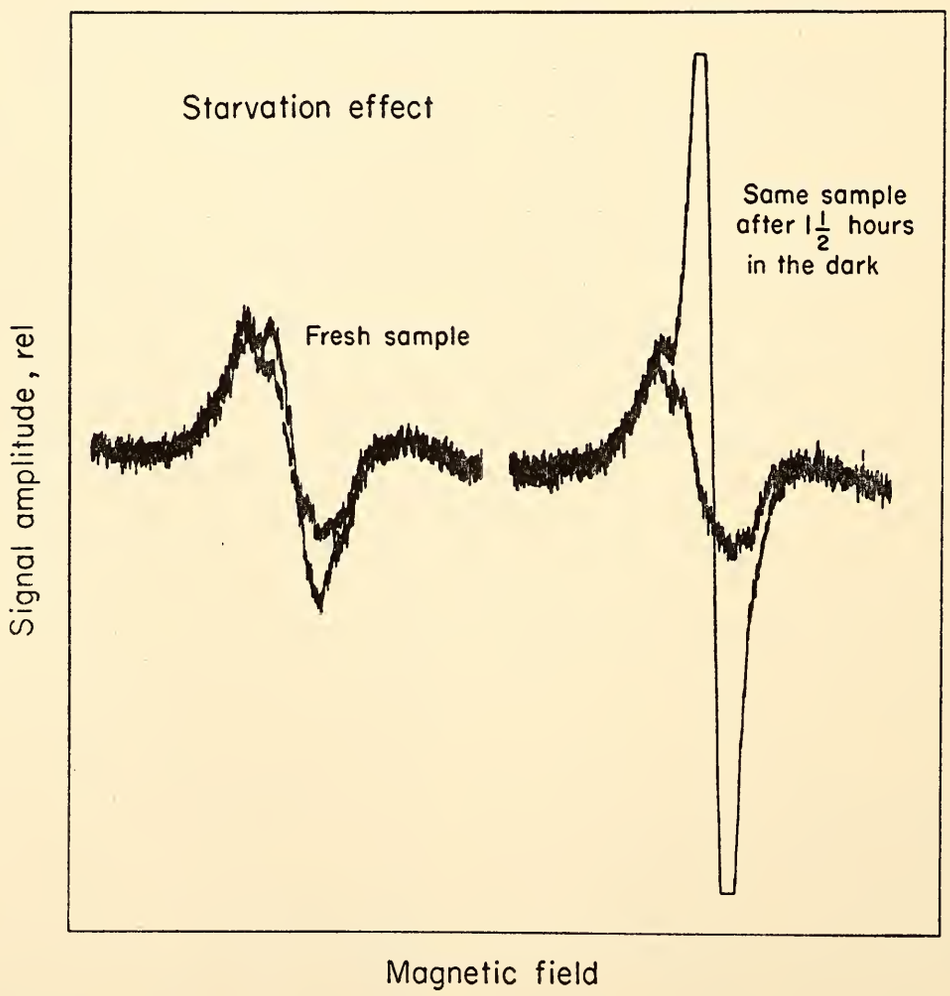

Fig. 27. The increase of the *R* signal induced by light after 1½ hours' previous storage in the dark.

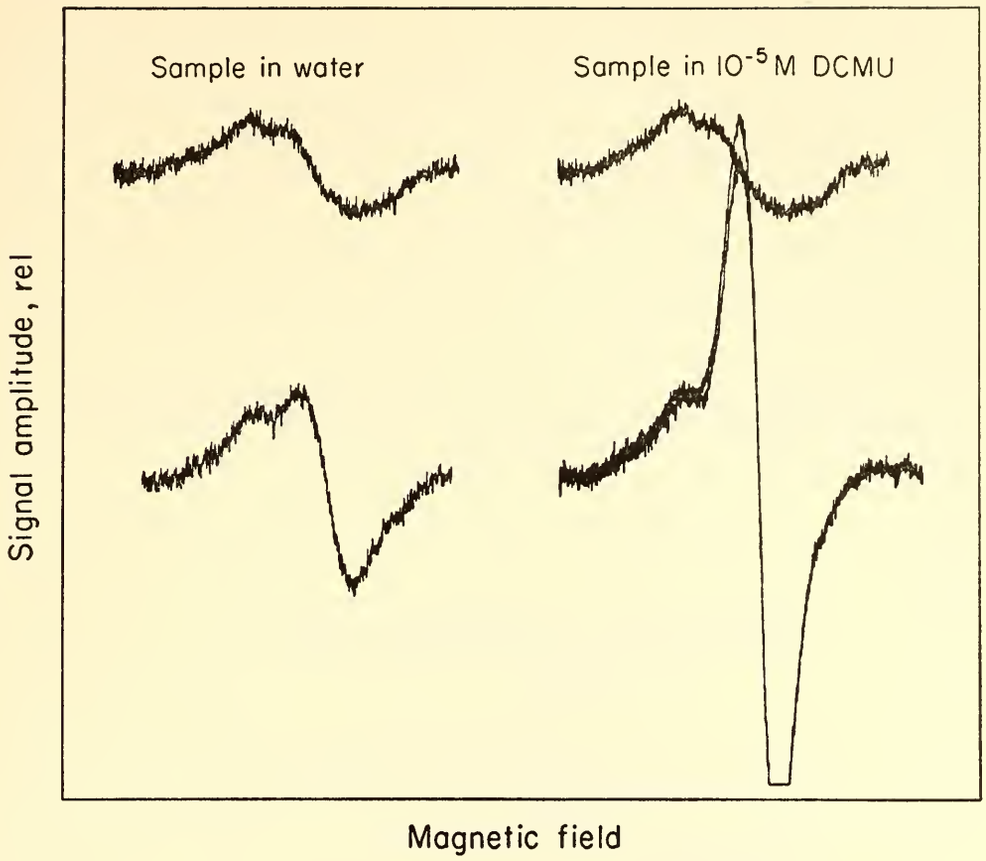

Fig. 28. Enhancement of R signal by 10^{-5} M DCMU (3-[3,4-dichlorophenyl]-1,1-dimethyl urea). Two aliquots of the same culture were used. *Upper curves:* S signal following a light exposure. *Lower curves:* $R + S$ signals during illumination.

observed. Obviously, the value of such studies will be greatly increased by concurrent biochemical and physiological studies.

Thus far we have been concerned mainly with the R signal, which is probably produced by chlorophyll. The S signal, however, is of great interest because it shows that the alga has some mechanism that maintains a free radical for an hour or more in the dark. Moreover, the signal is structured, as can be seen in figure 24, with six hyperfine peaks spaced about 5 gauss apart. This, together with the higher g value (2.0046), indicates an organic free radical, possibly a quinone. Other EPR observations include the fact that the S signal can be induced by both red and green light and is saturated by light levels an order of magnitude lower than those required to saturate the R signal, as shown in figure 29. Even stray light in a semidarkened room can suffice. For these reasons, no action spectrum has been made of the S signal.

One set of moment calculations, the double integral of the recorded traces, made on some twenty traces with and after light of various wavelengths and at various intensities with a fairly dilute suspension, showed that there were roughly ten times as many unpaired spins in the S signal as in the R signal alone. The S signal is not altered in appearance by "starvation" or DCMU. However, it was found that the manganese-deficient cultures had practically no S signal, as seen in figure 30. This is interesting in view of the fact that it has been reported that the purple bacteria, which do not evolve oxygen, are also lacking the typical broad, persistent resonance, which has been reported in a number of different aerobic organisms. The fact that DCMU does *not* abolish it indicates that the

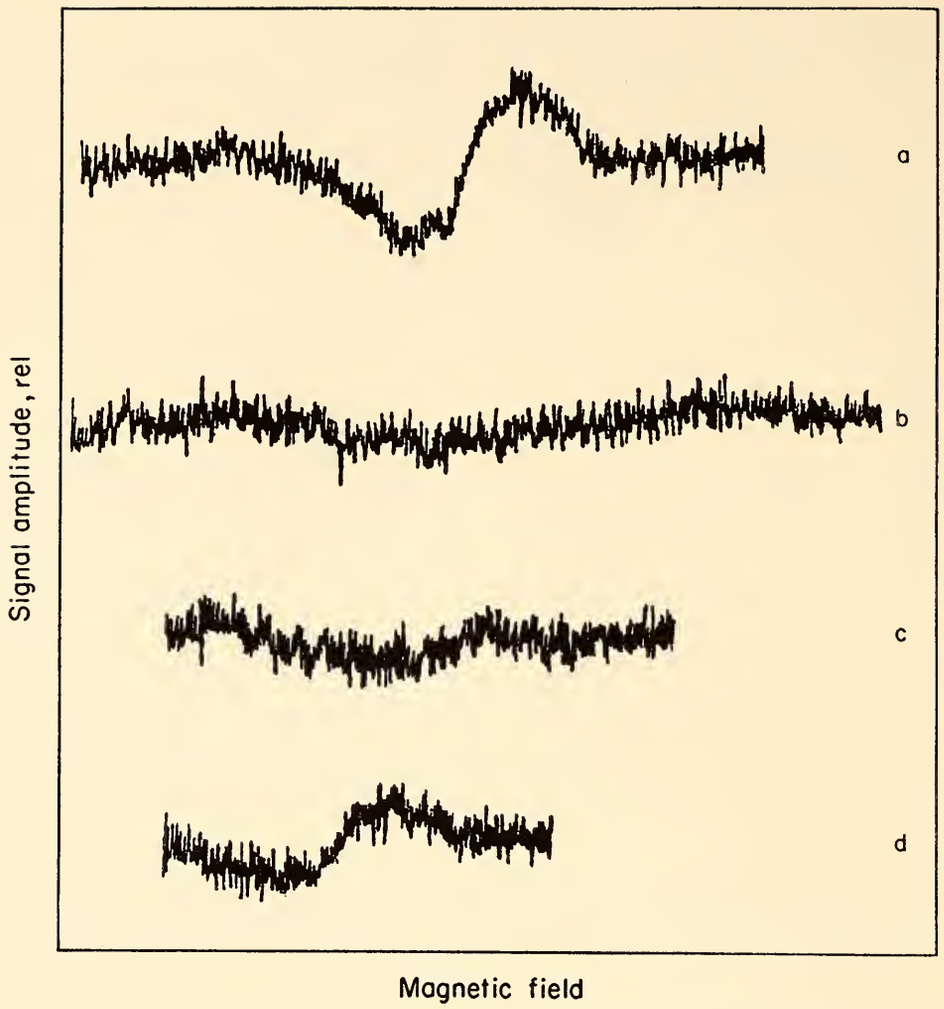

Fig. 29. The induction of the *S* signal by light and its decay. (*a*) *S* signal from freshly prepared cells. (*b*) Same preparation after 4 hours in total darkness. (*c*) Same as (*b*), but traced immediately after 30-second exposure to low level of illumination at 530 mμ. (*d*) After further exposure for 30 seconds to 694 mμ at a higher level of illumination. (Traces *b*, *c*, and *d* were made with a faster scanning rate than *a*, and so the signal appears narrower.)

resonance is not due simply to the process of oxygen evolution but to some substance vital to it. Experiments started in this laboratory some years ago have led Dr. Norman Bishop to the identification of a benzoquinone, Q-255, or plastoquinone, located in chloroplasts, which is a necessary and apparently universal factor in the oxygen-evolving mechanism of green plants. It seems very possible, but has not yet been demonstrated, that the reason plants deprived of manganese do not evolve oxygen is that they also lack plastoquinone.

Samples of purified crystalline plastoquinone have been obtained through the kindness of Dr. F. L. Crane of Purdue, Dr. Karl Folkers of Merck, and Dr. Bishop. Dr. Maling has been able to obtain a well resolved spectrum of the purified compound (fig. 31) as it is oxidized from the semiquinone to the quinone. The *g* value is 2.0044 $\pm$ 0.0001, which comes close to that calculated for the *S* signal. The spacing of the hyperfine lines is 2.1 gauss in the purified compound, less than half that observed for the *S* signal. Whether the binding of this compound and its close association with the chlorophyll-lipo-protein complex could result in broadening by a factor of 2 is unknown. This discrepancy, therefore, should not be considered proof that plastoquinone could not be responsible

for signal *S*. Plastoquinone remains a prime candidate on all other grounds, and it is hoped that more solid evidence can be offered.

A number of preliminary observations, not yet adequately confirmed, may be of some interest. Figure 32 presents three traces made with *Porphyridium cruentum*, a red alga studied in this laboratory (*Year Book 60*, pp. 351–362) with special reference to a two-wavelength model of photosynthesis. A typical light signal results from illumination with 680 mμ. However, when the suspension is illuminated with light of equal energy, but at a peak wavelength of 567 mμ, absorbed by phycoerythrin, there is no effect. This result supports the conclusion reached from the *Chlamydomonas* work that the primary resonance is due to chlorophyll, even though, in this case, phycoerythrin is more effective in photosynthesis. This is a most intriguing subject for further study with special reference to EPR

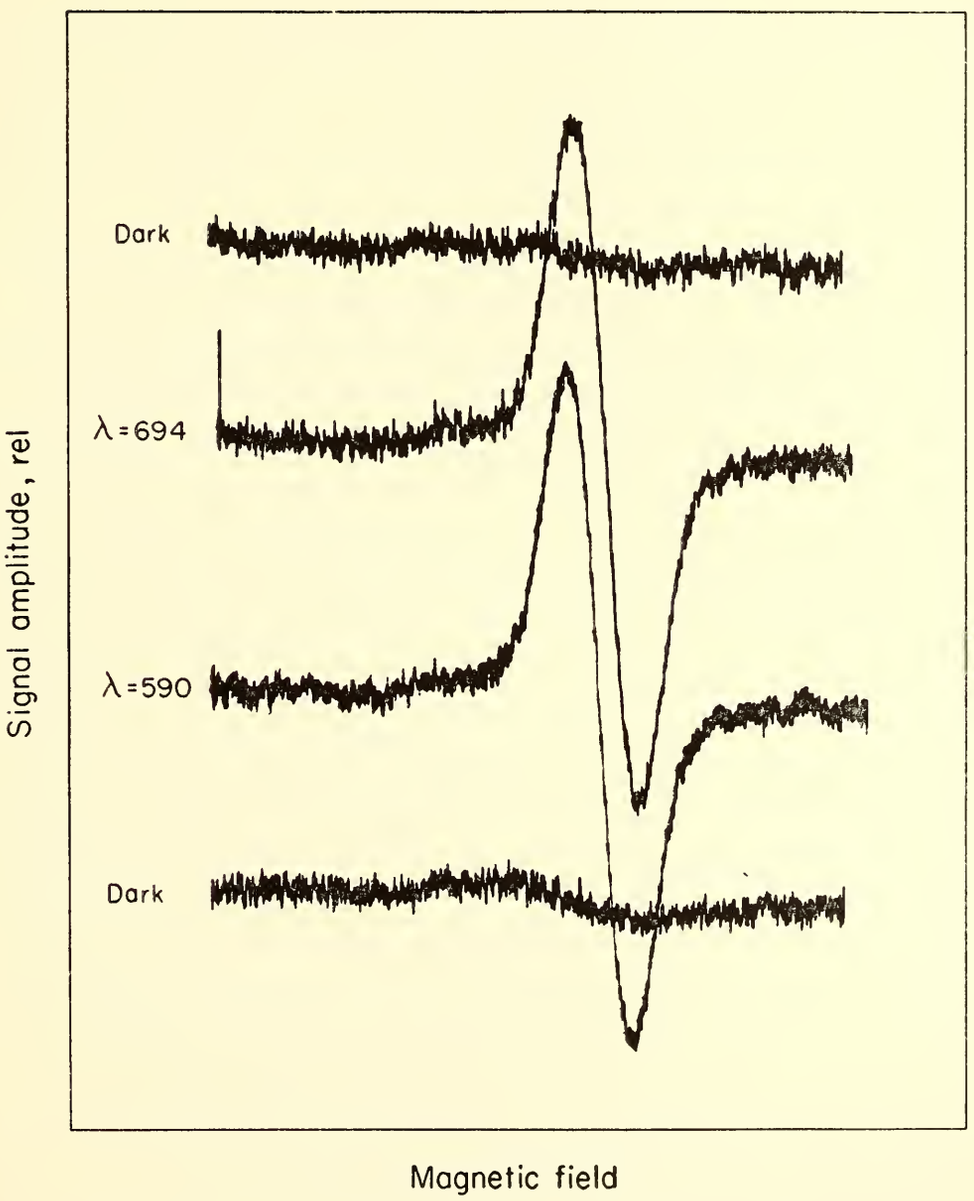

Fig. 30. The effect of manganese-deficient medium on the *S* signal. The top trace reveals a very slight resonance in the dark. Successive exposures to equal but saturating intensities of two wavelengths of light produced the second and third traces. The bottom trace, made immediately thereafter in the dark, shows almost no resonance. Instrumental conditions provided high sensitivity and are identical to those used for figures 27 through 29. A modulation of 5 gauss, peak to peak, was used. The dense cell concentrations in these four figures are all about 3×10^8/ml.

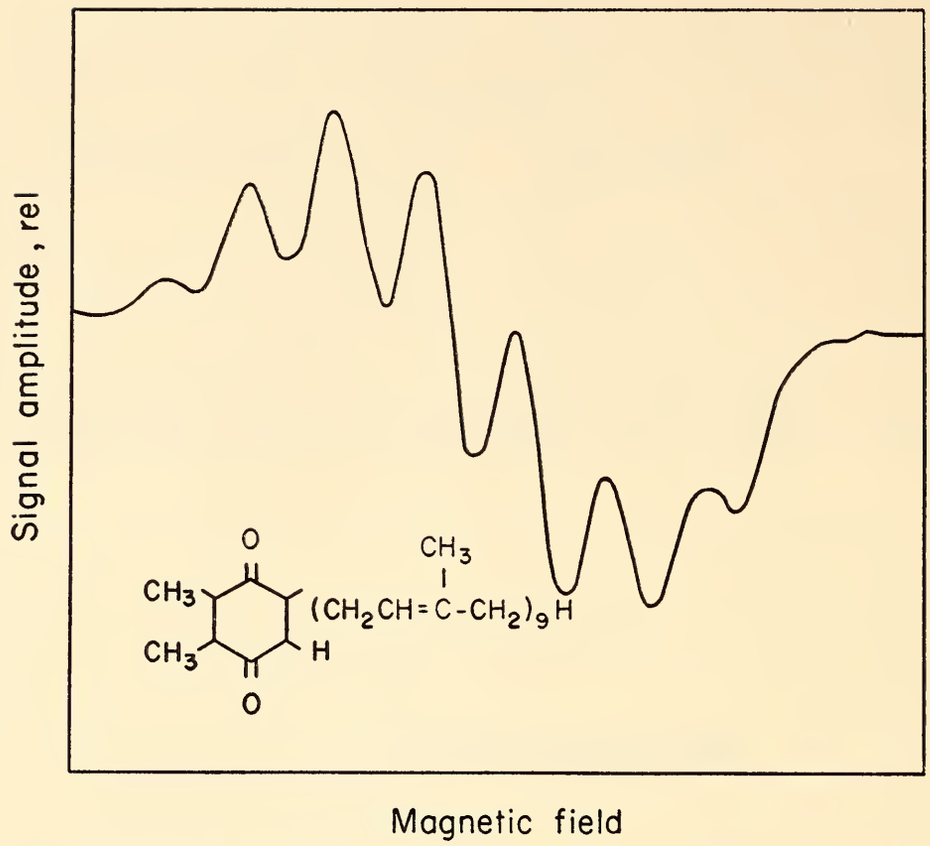

Fig. 31. The structure and EPR spectrum of plastoquinone. The ten hyperfine peaks are 2.1 gauss apart; the g value is 2.0044 $\pm$ 0.0001. (Courtesy of Dr. J. E. Maling.)

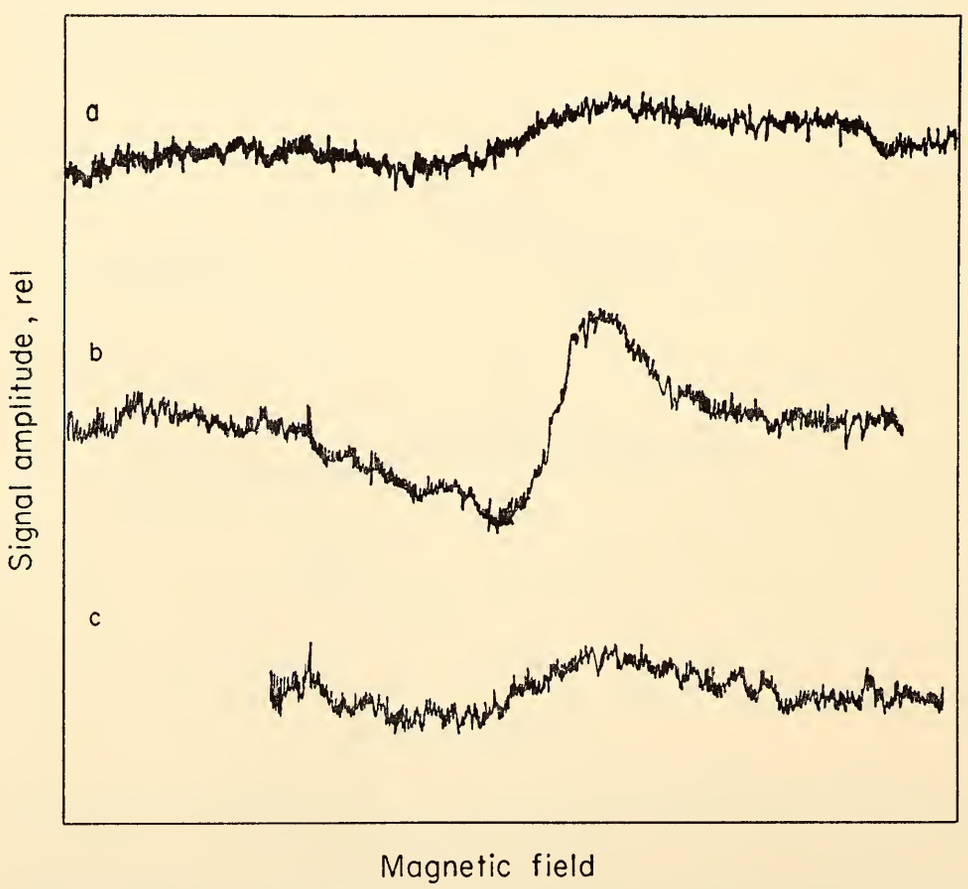

Fig. 32. EPR signals from *Porphyridium cruentum*. Trace (*a*) was made after the cells had been kept some hours in the dark. Trace (*b*) was made with red light, 680 mμ, and trace (*c*) was made a few minutes later with light of the same intensity but with a wavelength of 567 mμ.

experiments done with two wavelengths of light.

There have been many experiments on rise and decay times, but they have proved to be so variable that it is not possible to draw consistent conclusions. Records are obtained by setting the magnetic sweep on the point of maximum deflection, then turning the light on and off. It seems fairly safe to say, however, that both rise and decay times have two time constants: a fast response, followed by a slower one. The exact values vary with the number of spins observed, i.e., with density of cell suspension and with intensity and wavelength of the exciting light—the greater the number of spins, the faster the response. In DCMU-treated cultures, a minute or more was needed to attain maximum R signal amplitude, a correspondingly long decay time also being typical. Since the shape of the curves has not been shown to be exponential, the term $t_{1/2}$ cannot, strictly speaking, be used. By way of giving an order of magnitude, however, the time needed for an average signal to reach one-half of its maximum amplitude in untreated preparations is about 0.2 second. Figure 33 illustrates typical rise and decay curves. Properly controlled studies of the shapes and time constants of the build-up and disappearance of both R and S signals should yield valuable information on the classes of events involved in their formation.

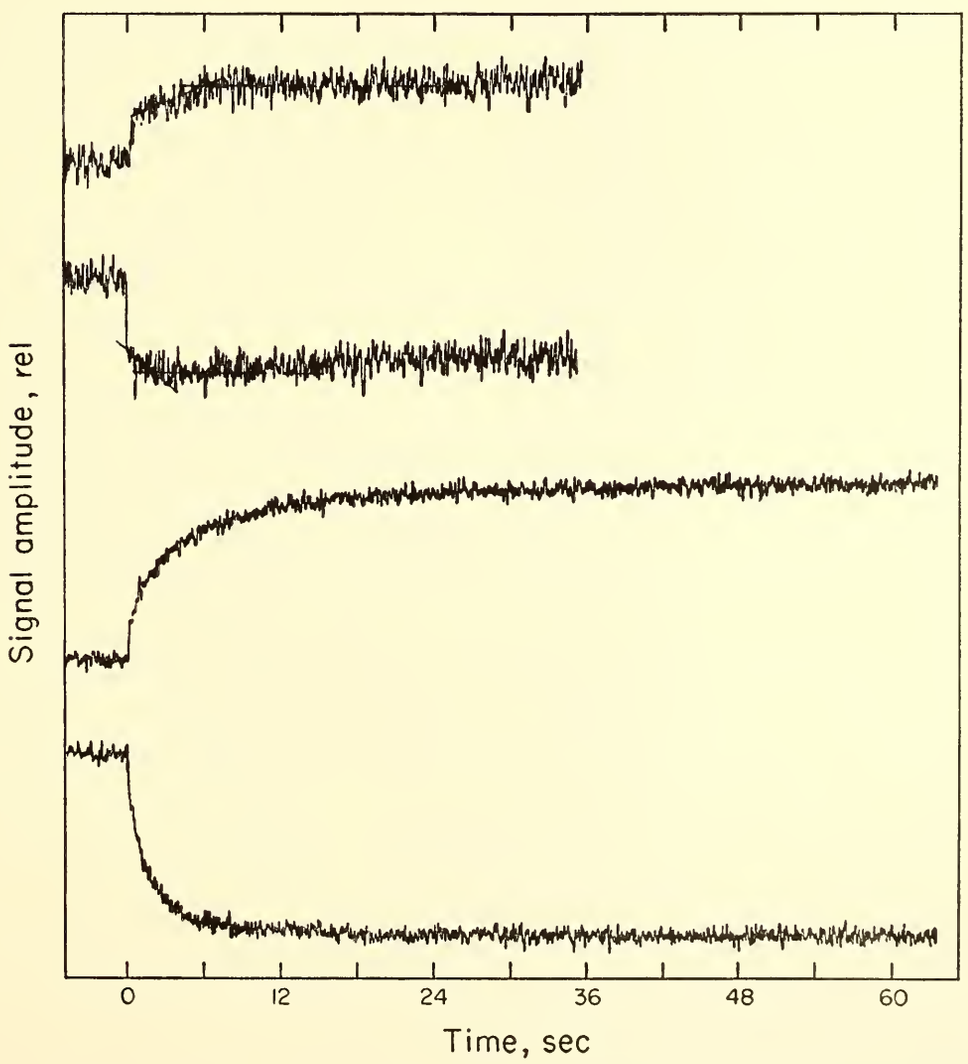

Fig. 33. (*a*) Rise and decay of R signal from cells suspended in water. Time required for signal to reach half its maximum amplitude is approximately 0.2 second. Decay takes place as fast as the recorder can move. (*b*) Rise and decay of R signal from cells suspended in 10^{-5} M DCMU. Times are 2.2 seconds for half of the maximum rise (not shown) and 2 seconds for half of the decay.

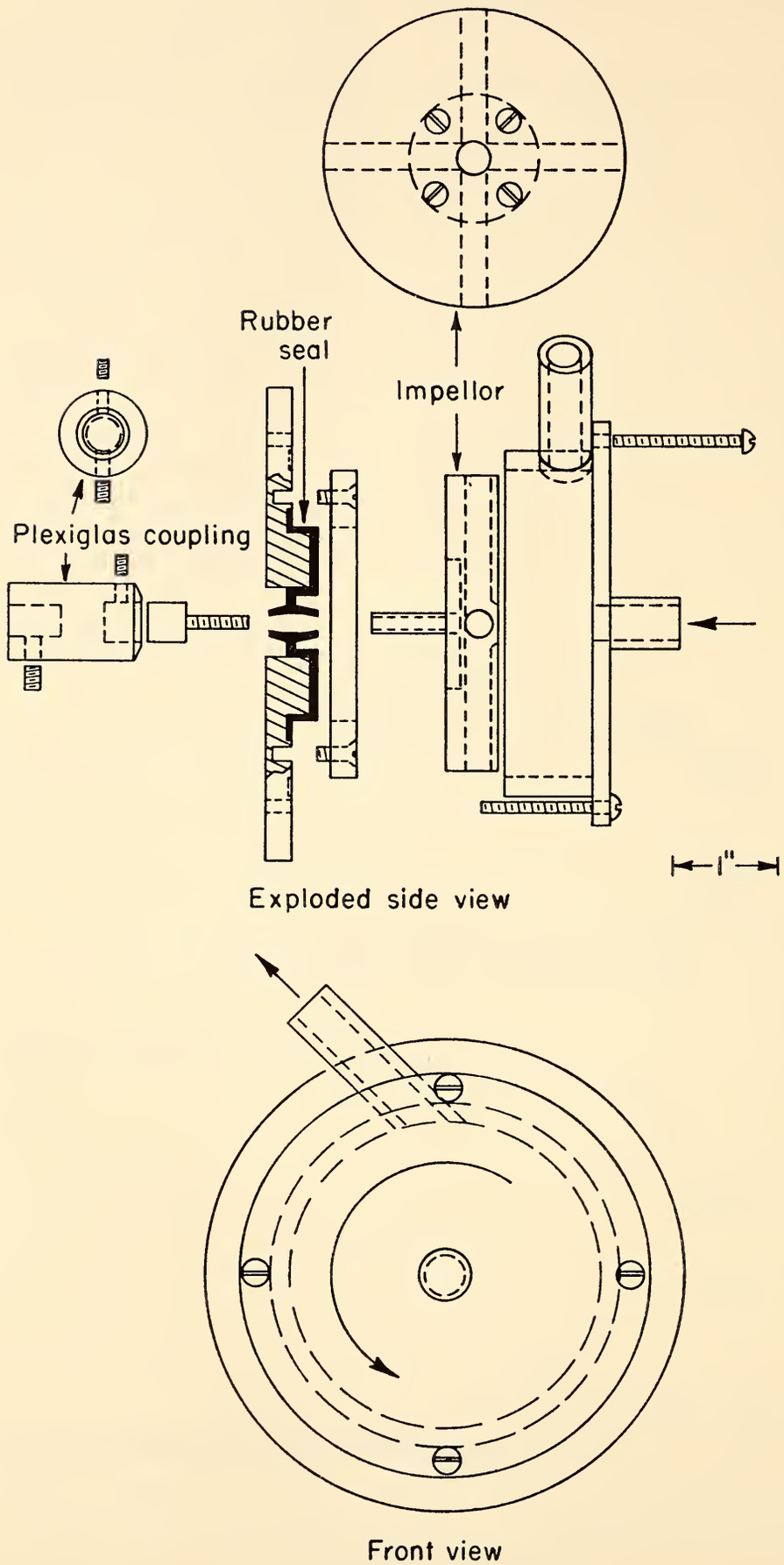

Fig. 34. A Plexiglas centrifugal liquid pump giving electrical isolation, a constant flow rate, and a small internal volume.

We have no direct evidence on the relationship between the two signals. Commoner and co-workers suggested in 1957 that electrons from the *R* signal proceeded to the *S* signal, from there to disappear so slowly that their passage could not be traced. We have not found any evidence to contradict this hypothesis, and several other studies of chlorophyll (or chlorophyll-like) and quinone systems have demonstrated that single-electron transfers do take place between them. We have presented evidence from an intact photosynthetic organism to support the idea that chlorophyll is the source of one free electron, and that plastoquinone is the site of another; we have been able to demonstrate some correlation of the behavior of these two signals with evolution of oxygen by photosynthesis. It is hoped that further work on these and other materials will yield results that will help solve some of the unknowns of photosynthesis.

An Electrically Isolated Transparent Liquid Pump

R. W. Hart

The need for a thermally and electrically isolated pump, adaptable to diverse applications for pumping liquids, led to the design and fabrication of one constructed mainly of Plexiglas. Although it was built primarily for circulating solutions over the oxygen electrode for photosynthesis measurements, it seems to have many potential uses.

The inert transparent Plexiglas allows contamination to be detected and permits continuous observation of the pumping action. An 1850-rpm synchronous motor gives quiet, vibration-free operation with a constant flow rate.

The construction is shown in figure 34. A centrifugal impellor with a low hold-up volume was machined from Plexiglas. Four holes spaced 90° apart were drilled from the circumference to the center axis, intersecting an inlet hole drilled along the axis. The impellor shaft of stainless steel is threaded into a stainless-steel disk mounted on the impellor. A long-life impellor seal of frictionless Hycar was obtained from Schaar and Company, 7300 West Montrose Avenue, Chicago 34, Illinois (model AR4360).

Several modifications in size and proportions of the basic design have been made with no apparent loss of versatility or performance. Figure 34 does not show the method of mounting motor and pump as a unit because of the varied motor designs available. The rotation of the impellor must be counterclockwise facing the motor shaft so that the motive force acts to tighten the screw supporting the impellor shaft. Electrical isolation is assured by a Plexiglas coupling on the motor shaft. The performance of one model is shown in table 10.

TABLE 10. Performance of a Pump Having a 2⅞-Inch-Diameter Impellor with ¼-Inch Holes

Head, in.	6	12	18	24	30	36	42
Flow rate, liters/min	6.60	6.10	6.05	5.75	5.40	5.00	4.60

SPEECHES

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Fork, D. C., Evidence for a 2-pigment mechanism in *Prophyridium cruentum*, AAAS, Lafayette, Indiana, August 1961.

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Contents

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INTRODUCTION

The year 1916 saw Frank R. Lillie, then in his forty-sixth year, at the peak of his career as investigator, teacher, and science administrator par excellence. Although he was known earlier for his studies on the physiology of cellular differentiation and cell lineage in annelids and mollusks, and much later for his definitive analysis of tissue interactions in the developing feather germ, Lillie's most lasting contributions, and those of greatest heuristic value, came in the middle of his scientific lifetime. It was in 1916 that he published in *Science* two "milestones," his first analysis of the freemartin and a definitive article summarizing his theory of the interactions of egg and sperm substances in fertilization.

In the fertilization article, Lillie wrote, referring to the history of the fertilization problem, "Possibly the results seem slight as a record of 265 years of continuous study of a single biological problem. But we read the history of science very superficially indeed if we fail to realize the constant interdependence of all scientific thought. There has probably been no time in the history of our particular subject when a greater amount of work on its problems would have caused a much more rapid advance. Scientific discovery is a truly epigenetic process in which the germs of thought develop in the total environment of knowledge. Investigation of particular problems cannot be accelerated beyond well-defined limits; progress in each depends on the movement of the whole of science."

Lillie's words might have been written yesterday, so aptly do they fit the current state of research in developmental biology; and, since, to a large degree, the activities of the Department of Embryology mirror and influence those of the field as a whole, they also represent the philosophy of the writer and the Department. It cannot be denied that from time to time thought has been given to molding the Department into one directed solely toward some major problem, emphasizing either one currently fashionable experimental system, say antibody formation or myogenesis, or one of the areas largely neglected of late, like neurogenesis; but each time the idea has been discarded in favor of the Department's traditional organization of a group of independent investigators whose interests range widely from biochemistry and microbiology to anatomy and physiology, with substantial overlapping in experience and approach. Such an organization might not be desirable in all fields, but in developmental biology today it appears to favor the generation and interchange of ideas; its flexibility permits expansion as needed for limited periods as advances on a given problem warrant it; and it helps ensure the vitality and sense of accomplishment of the Department, it being unlikely that all members of the group would be experiencing at the same time the temporary lulls or setbacks that are always a part of research. Someone is always ready to take the lead, setting the pace and providing an example and stimulus for the others.

During the past year it fell to a newly appointed staff member, Irwin R. Konigsberg, and two Fellows of Carnegie Institution of Washington, Donald D. Brown and Douglas Caston, to assume that role. Substantial progress is reported in the long-range programs of established staff members, especially those of David W. Bishop and Robert DeHaan, but none has generated the excitement of the Department's new ventures. Perhaps faithful readers of these reports will be pleasantly surprised, as the writer has been, at the year's achievements, for much less might have been expected in a year in which at least the first four months were disrupted by difficulties experienced in occupying the new building. The move from the former location

in the New Hunterian Building to 115 West University Parkway, which began on August 1, 1961, found the staff occupying a building in which construction was halted by a prolonged electricians' strike. Although a few experiments were conducted almost at once, it was November before serious work was possible. But those difficult months are almost forgotten as the building has already proved to be a place in which the staff can think and work effectively. William E. Haible's design provided a handsome and efficient structure, with an unusual combination of fine qualities, pleasing to both aesthetic and practical senses. It is not fitting, perhaps, to lay too much emphasis on the building itself, for after all it is just the setting of our continuing research story, but it is an accomplishment in which we can take pride, being a further, notable example of the rewards of cooperative effort, which the Institution has long stressed.

Apart from the new building, the year was one of eventful change. As was mentioned above, Irwin R. Konigsberg joined the research staff on July 1, 1961. A student of B. H. Willier, and former associate of Heinz Herrmann in the University of Colorado's Laboratory of Chemical Embryology, Konigsberg brought with him, from his most recent position in the National Institutes of Health's Gerontology Branch, a store of experience and a continuing interest in problems of muscle development. During the year he has made great strides in developing a system that would permit rigorous control of the cellular population and the extracellular environment when embryonic skeletal muscle cells were grown in dispersed cell cultures. He has demonstrated convincingly that cells in monolayer cultures prepared from suspensions of 11- to 12-day embryonic leg muscle undergo the characteristic changes of myogenesis: rapid proliferation of mononuclear cells, the formation of multinucleated myotubes by the fusion of these cells (a point on which Konigsberg had earlier provided some of the definitive evidence), and the progressive development of the cross-striated pattern (with the initiation of spontaneous contractions). He has now begun to probe more deeply into the mechanisms of these steps, as the body of the report will reveal.

It was noted in *Year Book 60* (p. 440) that frog embryos develop normally in media lacking magnesium until stage 21, when they die. As Donald D. Brown observed, however, the addition of magnesium before stage 20 allows normal development. At the time that report was written, the observation was an isolated one; it did not fit readily into any overall scheme. As so often happens in research, however, this seemingly isolated fact has now become an important piece in a much larger mosaic of findings now reported by Brown and his co-worker, a newly appointed Fellow, Douglas Caston. Early embryos contain a measurable but small population of cytoplasmic ribosomes. These have been isolated and quantitated by an isotope dilution technique coupled with standard homogenization and differential centrifugation methods. There is little change in ribosomal content until near the end of morphogenesis after stage 18, when there is a striking rapid appearance of these particles. At about this time the embryo requires exogenous magnesium ions, which can be shown to preserve the integrity of ribosomes in the aggregated form. Hence the death of embryos due to magnesium deficiency is the consequence of the failure of a key step in the development of the machinery for protein synthesis. Further details of this intensive study of ribosomal development will be found in the pages to follow.

Gerald L. Carlson, Given Foundation–National Research Council Fellow in Academic Medicine, continued his study, in collaboration with Bishop, of the nature of the testicular antigen in induced aspermatogenesis. At the same time, however, he has made important progress in analyzing the serum amylase activity

of the frog, *Rana pipiens*; amylase is among the enzymes that change rapidly at metamorphosis, to which much attention is now being devoted.

Another enzyme in this group is acid deoxyribonuclease (DNase II), which John R. Coleman observed (*Year Book 60*, pp. 400–404) to increase concomitantly with resorption of the tail. Coleman, who left at the end of 1961 to begin a period of further training and collaboration with Heinz Herrmann at the University of Connecticut, has extended these studies, the findings of which now make it clear that DNase II begins to rise shortly before extensive tail resorption begins.

Two visiting investigators devoted their time to exploratory forays into the application of immunochemical techniques to problems of development. As a Fellow of Carnegie Institution of Washington, Michael Abercrombie, recently appointed Jodrell Professor of Zoology, University College, London, was concerned with techniques that might advance knowledge of the composition of the cell surface. Arthur LaVelle, of the Department of Anatomy, University of Illinois College of Medicine, centered his attention on the antigenic properties of substances extracted from brain.

Another aspect of the interplay of embryology and immunology occupied Bertie F. Argyris of Syracuse University, a Fellow of the U. S. Public Health Service who enjoyed a productive period of research concerned with mechanisms of acquired tolerance to skin homografts in mice. Thomas S. Argyris, a Fellow of the National Science Foundation, devoted his stay in the Department to the development of biochemical techniques for the eventual identification and measurement of tissue-specific growth-promoting substances.

Two visiting investigators, Ronan O'Rahilly of the Department of Anatomy, Wayne State University, recently appointed Director of the Department of Anatomy at St. Louis University, and Peter H. S. Silver, Department of Anatomy, Middlesex Hospital Medical School, London, examined the development of the eye. O'Rahilly, Special Fellow of the U. S. Public Health Service, began a comprehensive study of the developing human eye, while Silver, National Institutes of Health International Postdoctoral Traveling Fellow, working in part in collaboration with John Papaconstantinou of the University of Connecticut, began an analysis of lens induction, emphasizing techniques of experimental morphology as a background for biochemical and immunochemical approaches.

In December J. W. S. Harris returned to his post at London Hospital Medical College, after a productive stay during which he completed a substantial part of a study of the vascular pattern of the human uterus with placenta in situ.

In the fall of 1961, Ian Wilson, a recent graduate in zoology at University College of North Wales, and recipient of the Thomas Henry Huxley Award of the Zoological Society of London for 1961, arrived to spend a year as a Fellow of Carnegie Institution of Washington working in consultation with Bent Böving on factors effecting the orientation, spacing, and siting of the blastocyst in the mouse.

Chinami Takata, formerly associated with T. Yamada at Nagoya University, and most recently a member of the Department of Anatomy, Tokyo University, took up a one-year appointment in the Department in September 1961 to work in consultation with James D. Ebert on factors affecting the lability of the chorioallantoic membrane.

In addition to the visiting scientists already named, nearly fifty investigators from eighteen countries shared in the activities of the Department. Among them, to name only a few with continuing programs, were George W. Bartelmez, G. W. Corner, Jr., Anatole S. Dekaban, Arentje Dekker, Martin W. Donner, W. Richard Ferguson, and Sheila J. Moody.

As always, the list of visiting investigators and their activities is impressive;

yet such a listing tells only a part of the story. The visitors do contribute vitally to the Department, directly and indirectly, but of far greater moment is the question whether a visit adds measurably to a man's ability as an investigator and teacher when he returns to his home laboratory. Has he found new direction or meaning for his research? Has the opportunity for reflection, away from the usual distractions of his own laboratory and classroom, led to a searching reexamination of his program?

Such questions can be answered objectively only with difficulty—if at all. But the writer can state his own impression that the Department's emphasis on opportunities for visiting investigators is contributing effectively to the number of well trained students of development at a time when the demand far exceeds the supply.

Before concluding this recital of arrivals and departures, one more departure must be recorded, that of the senior member of the research staff, R. K. Burns, who retired on June 30, 1962. It is hardly necessary to recount even the highlights in his distinguished career; his achievements, described in these reports over the last twenty-two years, and in numerous publications, speak for themselves. It is a pleasure to report that he has accepted an appointment as Professor of Biology at his alma mater, Bridgewater College, where he will teach embryology and continue his investigations of sex differentiation.

CELLULAR REGULATORY MECHANISMS

Development of Machinery for Protein Synthesis

Striking changes occur in metabolic activities of eggs after fertilization. The degree of change is particularly impressive in amphibians like the frog, *Rana pipiens*, since thousands of mature eggs remain in the ovary over the winter with little change in their metabolism. If the gravid female is injected with pituitary glands she ovulates in about two days, and if the eggs are then fertilized development begins, resulting in a swimming larva in six days. The only exogenous requirements during this period are several inorganic ions. New nucleic acids, proteins, and carbohydrates are synthesized from material stored within the eggs.

The following experiments were begun by Donald Brown and Douglas Caston to study components of protein synthesis during embryogenesis with the hope of elucidating the steps that regulate the amount of protein formed in early stages. In particular, this report correlates the amount, time of appearance, and composition of ribosomes and ribosome-like RNA with other cellular constituents during early stages of development.

Materials and methods. Fertilized eggs developed in a modification of Holtfreter's salt solution containing NaCl, KCl, $CaCl_2$, and $MgCl_2$; the medium described by Holtfreter contains $NaHCO_3$ but no $MgCl_2$. Staging experiments were performed using embryos from the same female. At the appropriate time the jelly was removed manually and the embryos were frozen at −70°C until a complete series could be processed together. Usually 50 embryos were taken each time. Embryos were homogenized in 0.01 *M* tris HCl (trishydroxyaminomethane) buffer, *p*H 7.3, containing 0.001 *M* $MgCl_2$ (TM). Homogenates were divided into three fractions. The first, termed "pellet," is the part of the homogenate sedimented at 12,000*g* in a Servall centrifuge. This fraction includes lysosomes and mitochondria as well as all heavier particles like yolk platelets, melanin granules, and nuclei. The Servall supernatant was centrifuged at 105,000*g* for 2 hours in a Spinco model L ultracentrifuge, and the resulting soluble supernatant was the

second fraction. The Spinco pellet was rehomogenized in a small volume of TM; this suspension was centrifuged for 20 minutes at 12,000*g*, and the supernatant was saved as the third or "ribosomal" fraction.

RNA, DNA, and protein were measured routinely by the orcinol, diphenylamine (Burton modification), and Lowry methods, respectively.

To obtain purified RNA, material was homogenized in a suspension containing sodium chloride and sodium lauryl sulfate at *p*H 5.0 and then extracted with phenol. After centrifugation, nucleic acids in the aqueous phase were precipitated twice with ethanol and finally dissolved for column chromatography. These preparations had very low levels of DNA. The high-molecular-weight RNA was fractionated on a 2 by 2 cm column of Celite containing methylated bovine serum albumin according to the method of Mandell and Hershey. The nucleic acids were eluted with a linear gradient of NaCl increasing from 0.2 *M* to 1.0 *M*, 5 ml/tube for 300 ml. Elution patterns were determined by reading the eluate from each tube in a spectrophotometer at 260 millimicrons. The peak tubes were pooled, dialyzed against distilled H_2O, and concentrated in vacuo. Base compositions were determined according to the method of Wyatt.

Radioactive ribosomes were prepared by intraperitoneal injection of large *R. pipiens* tadpoles with high specific activity orotic acid-C^{14}. Ribosomes were isolated by the same technique described above between 30 and 50 hours after injection.

Density-gradient centrifugation was performed according to the method of Roberts and Britten. Horse spleen ferritin, a product of Pentex Corporation, and its antiserum (rabbit) were generous gifts of Zoltan Ovary.

Cytoplasmic Ribosomes

When "ribosomes" prepared from ovarian eggs were compared with purified liver particles several distinct differences were apparent at once. Sucrose density-gradient studies show that the major component of this fraction from eggs moves more slowly in the centrifugal field than its counterpart isolated from liver. More striking still is the insensitivity of the egg particle to magnesium ions. The ribosomes from adult liver are RNA-protein subunits, held together by magnesium. Removal of magnesium by versene causes disaggregation of the normal 80 to 100 S particles from frog liver or whole tadpoles into 2 to 4 S pieces. However, only a minor fraction of the egg preparation is solubilized by versene treatment, the major part remaining unchanged. Figure 1, plate 1 (at the end of the report), shows sedimentation patterns taken with schlieren optics of particles contained in the "ribosomal" fractions of frog liver, whole tadpole, and body cavity eggs. Note the marked coloration (yellow) associated with the major egg peak. The smaller slower-moving peak in the liver preparation is also colored, but has the same S value as the egg particle, and is presumably identical. Subsequent purification of the minor liver peak after treatment with versene and recentrifugation confirms the similarity. Another striking difference between the major component of the liver "ribosomal" fraction and that of the egg was their behavior to high concentrations of equivalent cations. Ribosomes are further aggregated at concentrations of Mg^{++}, Ba^{++}, or Ca^{++} exceeding 0.01–0.02 *M* and can then be sedimented at very low centrifugal speeds. Figure 2 compares the response of these two preparations (egg and liver) to incubation for 45 minutes at 0°C in different concentrations of $BaCl_2$. After incubation the preparations were centrifuged at 1500*g*, and the supernatants were decanted and read in a spectrophotometer. Figure 2 plots percentage of original optical density remaining in solution and clearly demonstrates the marked difference between egg and liver particles. Identical curves were

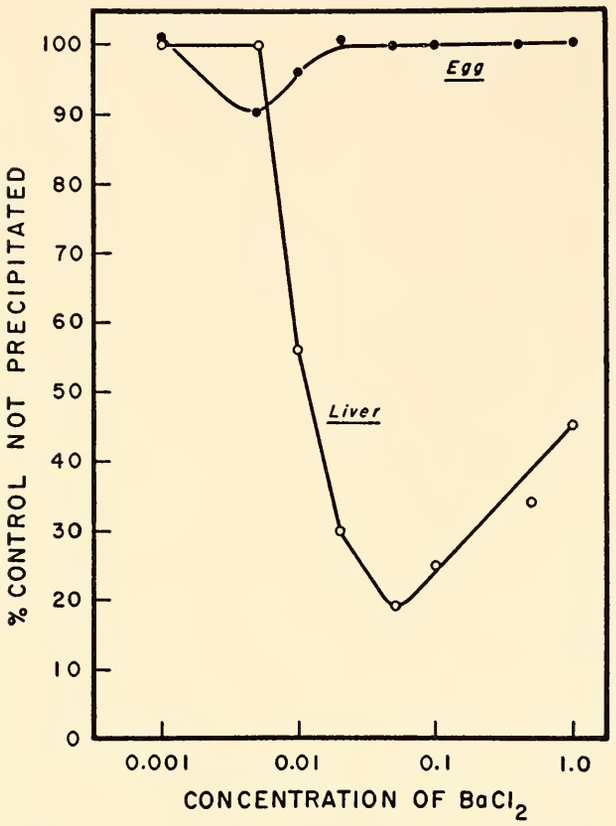

Fig. 2. Precipitation of "ribosomal" fraction of ovarian eggs and liver by different molar concentrations of $BaCl_2$.

obtained using Mg^{++} or Ca^{++} instead of Ba^{++}.

At this point in the investigation the problems were clearly outlined. If the particles present in the egg were not ribosomes, were they in fact related in some way to ribosomes, perhaps functionally or as ribosomal precursors? If not, what were these particles, and, of greater interest, what was the concentration of typical cytoplasmic ribosomes in the egg? Subsequent investigations unequivocally identified the major component of the egg "ribosomal" fraction as the iron storage protein complex, ferritin. We will next describe the studies supporting this conclusion and will then proceed with a description of analyses of frog eggs and early tadpoles for ribosomes, ribosomal RNA, and other biochemical parameters.

Identification of Ferritin in the Egg

After the preparation of the "ribosomal" fraction from mature ovarian eggs, the egg particle was further purified by elution from a diethylaminoethyl cellulose column (DEAE) with NaCl. The ferritin was still particulate and could be recovered in the pellet after further centrifugation at 105,000*g* for 2 hours. Table 1 compares this preparation from eggs with a commercially obtained sample of horse spleen ferritin further purified in the same manner by elution from a DEAE column. The two substances had identical spectra and distinction coefficients, and both had a high iron content. It cannot be stated definitively at this time whether the small amount of orcinol reacting material associated with purified ferritin is RNA.

As a further proof the cross reaction of egg ferritin with antiserum to horse spleen ferritin was investigated. Upon combination of the two, a precipitate

TABLE 1. Comparison of Purified Egg Particles from "Ribosomal" Fraction and Horse Spleen Ferritin (Pentex)

	Egg	Horse
O.D. 260 mμ/mg protein/ml	8.8	9.0
mg iron*/mg protein	0.13	0.16
mg "RNA"†/mg protein	0.011	0.0012
O.C. ratios, mμ		
235/260	1.47	1.35
250/260	1.05	1.05
280/260	0.93	0.93
400/260	0.21	0.24

* Iron was measured by the *o*-phenanthroline method.

† "RNA" was measured by the orcinol reaction, which is not specific for RNA.

developed. As a more sensitive test the passive cutaneous anaphylaxis test developed by Ovary was performed using intracutaneous sensitization to horse spleen ferritin antiserum followed by intravenous challenge with egg ferritin mixed with Evans blue dye 5 hours later. Figure 3 (pl. 1) is a photograph of the resulting large blue areas surrounding sites of antibody injection. Serum from a control rabbit gave a completely negative response. Furthermore, if the antiserum was preincubated with horse spleen ferritin and then used to sensitize the guinea pig, no blue spot was formed after the challenge with egg ferritin. The tests further demonstrated the expected finding that the reaction of frog ferritin with horse ferritin antibody was considerably less intense than the homologous control, but no attempt was made to quantitate this difference. Subsequent experiments have shown that the entire complement of iron in the egg and early developmental stages is confined to the ferritin fraction, which contains about 1 μg of iron per embryo (8 μg of ferritin). Iron measurements have been carried to Shumway stage 20 (see illustration of stages in fig. 6), in which blood circulation has already begun. In spite of the presumed initiation of hemoglobin synthesis as early as stages 16–18, the bulk of iron remained bound in the ferritin fraction as late as stage 20.

Correlation of Death from Magnesium Starvation and Appearance of Cytoplasmic Ribosomes in Development

It was noted in *Year Book 60* that the defined media previously described by others for raising early *R. pipiens* embryos to feeding stages were inadequate. The deficiency could be remedied by the addition of 10^{-4} *M* $MgCl_2$. In the absence of magnesium, embryos develop normally to Shumway stages 21–23, after which they become immobile but remain alive for another 2 to 3 days. Development continues to some extent, including the enclosure of gills within the opercular fold, but growth in length is arrested completely after the onset of obvious symptoms. Death occurs between 48 and 72 hours after the signs of deficiency first appear. Furthermore, during this period the progression of death cannot be reversed by the addition of magnesium. Substitution of manganese is completely ineffective, even if provided from the beginning of development. Magnesium starvation can be speeded up or retarded by raising or lowering the temperature and is correlated directly with the Shumway stage and the length of the embryo. (Deficient embryos are always arrested at about 9 mm in length.)

Initial experiments demonstrated a striking correlation between the stage when ribosomes can first be detected and the onset of magnesium deficiency. Only a small fraction of total RNA can be ascribed to cytoplasmic ribosomes until between stages 21 and 23, when they appear suddenly and accumulate exponentially, doubling in amount approximately each day (at 21°C). Figures 4–6 compile values of RNA, protein, and DNA in the three fractions of homogenized embryos throughout the period of early embryogenesis. Two control series are plotted together with one series of embryos allowed to develop in the absence of magnesium. There is a similarity in the different parameters except for ribosomal RNA. At the time that cytoplasmic ribosomes begin to appear in quantity the symptoms of magnesium deficiency become apparent. It can be seen that there is some variation between series in levels of RNA, DNA, or protein per embryo, although the shapes of the curves are comparable. To remedy this discrepancy embryos were reared on the salt mixture containing magnesium to stage 22 (118 hours). Half of the embryos were then transferred to magnesium-deficient medium after careful washing with distilled water. Development was allowed to continue to stage 23 to 24 (189 hours), at which time the embryos were homogenized and the three

fractions analyzed for RNA, DNA, and protein.

Table 2 summarizes the values. Although total RNA is the same, the control ribosomal fraction contains about 6 times more RNA than the same fraction from magnesium-deficient embryos. Sucrose density-gradient studies were performed to compare the two "ribosomal" fractions. Figure 7 shows that, unlike the control, the ribosome fraction of the magnesium-starved embryos was devoid of 80 and 100 S particles. It can be concluded that at least a contributing cause of death from magnesium starvation is the inability to make cytoplasmic ribosomes at the very time when they normally accumulate at the most rapid rate. Since the ribonucleoprotein subunits

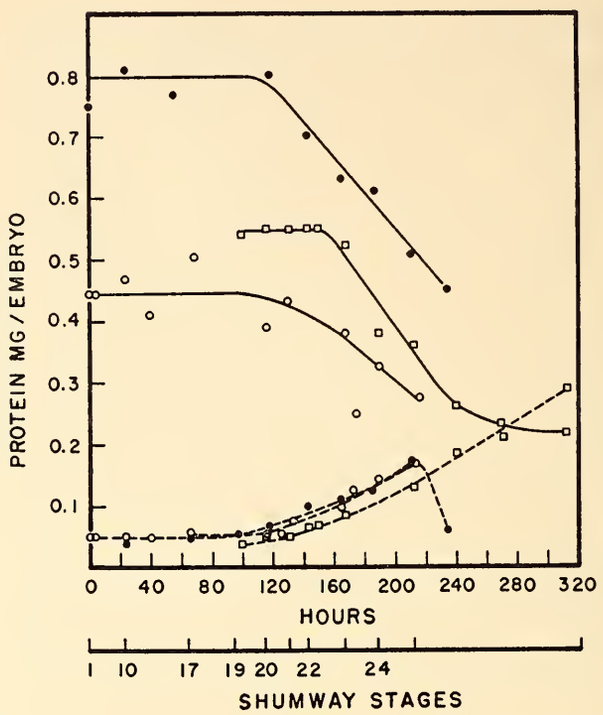

Fig. 5. "Pellet" (solid line) and soluble (dotted line) protein in the same control and magnesium-starved (closed circles) embryos. Symbols are the same as in figure 4.

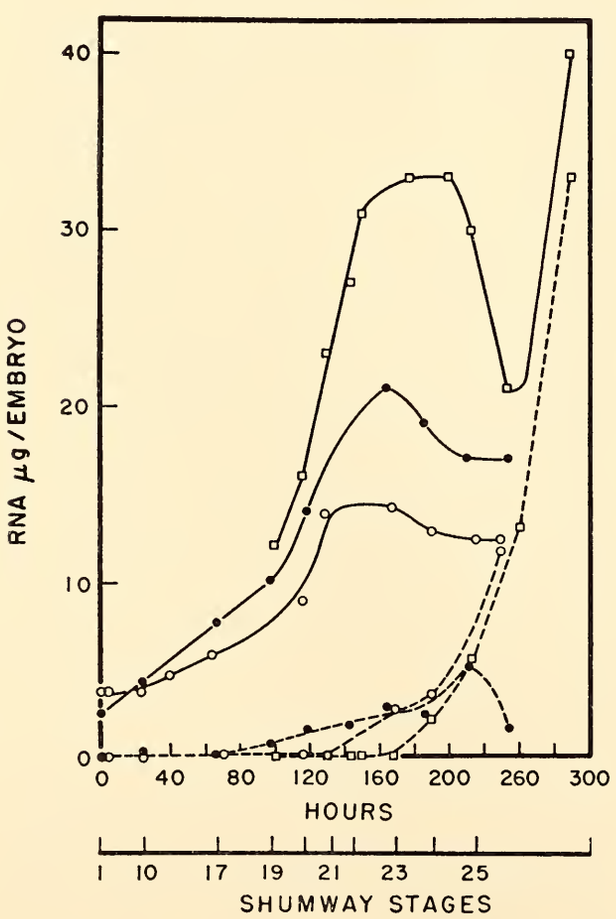

Fig. 4. "Pellet" (solid line) and "ribosomal" (dotted line) RNA at different stages of development. Two control series (open circles and open squares) are compared with one magnesium-starved series (closed circles).

are held together by Mg^{++} the reverse reasoning may be equally applicable; that is, binding of magnesium by increased quantities of ribosomes could effectively deplete the cells of the activity of many magnesium-dependent enzymes. (Ts'o found 3.7 moles of magnesium bound per 12 moles of bases in ribosomal RNA.)

Unlike adult tissues the egg contains most of its protein in an easily sedimented form which can be called structural or insoluble. Just before the dramatic appearance of ribosomes, soluble supernatant protein begins to rise and the insoluble fraction concomitantly drops. The drop is due to utilization of yolk. Magnesium-deficient embryos normally utilize yolk and form soluble protein before the onset of symptoms (cf. fig. 5). However, when death occurs the gut is still filled with yolk, and table 2 shows that magnesium-deficient embryos have a larger quantity of precipitable ("pellet") protein and less soluble protein than the control.

Binding Ribosomes

Results of staging experiments (fig. 4) required careful analysis to determine the quantity of ribosomes present in embryos from Shumway stages 1 to 21. The conventional homogenization and isolation techniques failed repeatedly to demonstrate more than 0.1 μg of "ribosomal" RNA per embryo, a small proportion of the total RNA present in the egg and early embryos. Homogenization with isotonic sucrose, phosphate buffers in different concentrations, different concentrations of Mg^{++}, and sodium deoxycholate at several *p*H values varying from 6.5 to 8.0 all failed to yield any significantly larger quantity of ribosomes. Prolonged centrifugation (105,000*g* for 15 hours) failed to sediment any macromolecule containing RNA.

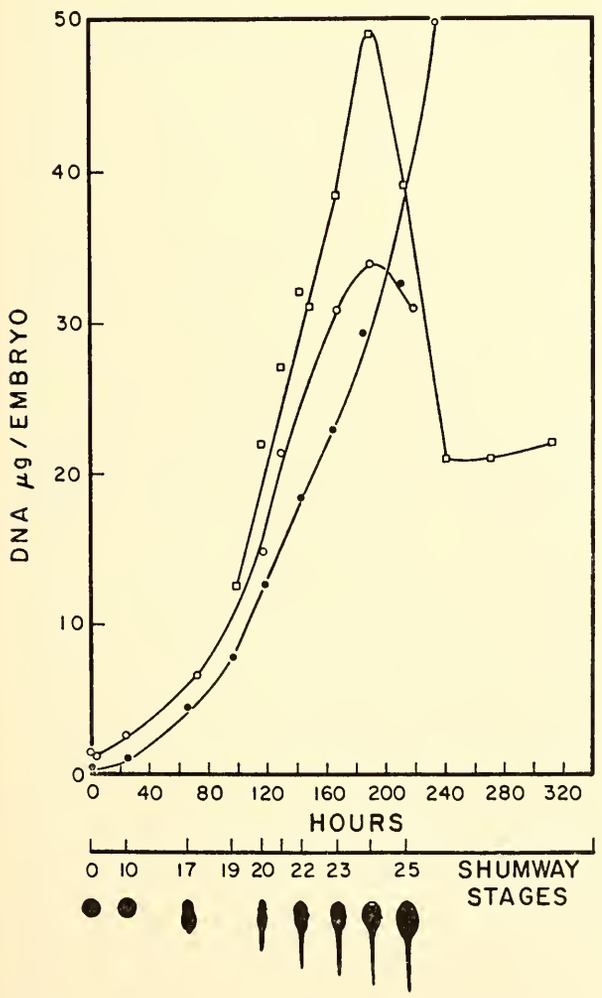

Fig. 6. "Pellet" DNA from the same three series as shown in figures 4 and 5.

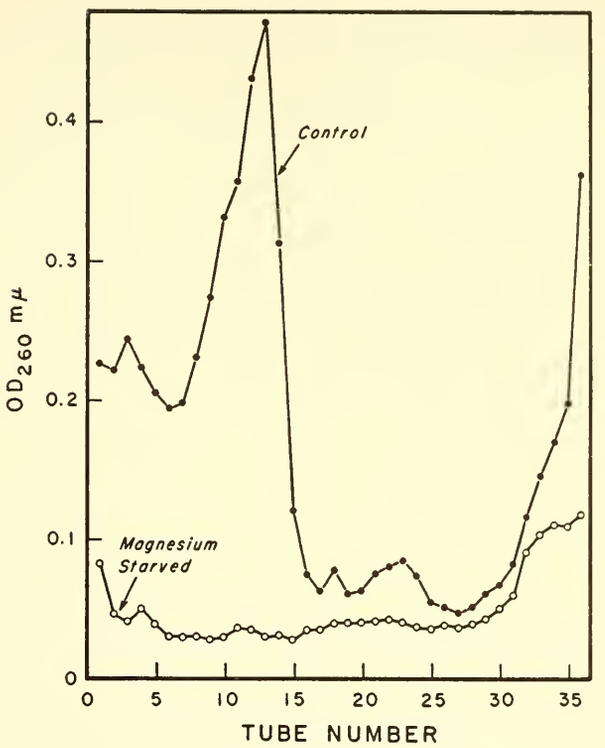

Fig. 7. Sucrose density-gradient centrifugation pattern of purified ribosomes from control and magnesium-starved stage 24 embryos (30 embryos each). The gradient decreases from tube 1 (bottom) to tube 36 (top).

Next the possibility was examined that ribosomes were indeed present but bound or destroyed in some way during homogenization. When liver and egg were homogenized together and the ribosomes were isolated, extremely poor yields of particles were obtained and under certain conditions all the liver ribosomes were lost. Subsequent investigations ruled out the action of a nuclease, since there was no apparent solubilization of the particles. Instead it could readily be demonstrated that large quantities of added ribosomes and in fact yeast RNA were bound by the low-speed egg pellet. To date no technique or medium has freed these bound particles. Figure 8 gives the results of a staging experiment in which homogenization was performed in the presence of added purified tadpole ribosomes labeled with orotic acid-C^{14}. It can be seen that none of the added radioactivity could be recovered in the ribosome fraction until stages 21–23, when both ribosomes (as

TABLE 2. Comparison of Control and Magnesium-Deficient Stage 24 Embryos (189 hours)

	Control, μg/embryo	Magnesium-Starved, μg/embryo
RNA		
Pellet	25.1	32.5
Supernatant	23.7	22.5
Ribosomes	8.7	1.5
Total	57.5	56.5
DNA	44.6	31.0
Protein		
Pellet	550	675
Supernatant	229	158
Total	779	833

measured by ribosomal RNA) and counts began to appear.

Although no specific medium increased the recovery of added counts during the early stages, two simple modifications of the homogenization technique resulted in much greater recovery of added radioactive ribosomes. These two procedures entailed homogenization in a very large volume (1:100 w/v) and centrifugation within 5 minutes after homogenizing the embryos. With these modifications as much as 50 per cent of added radioactive ribosomes was recovered. The technique made quantitation of endogenous ribosomes by an isotope dilution experiment possible.

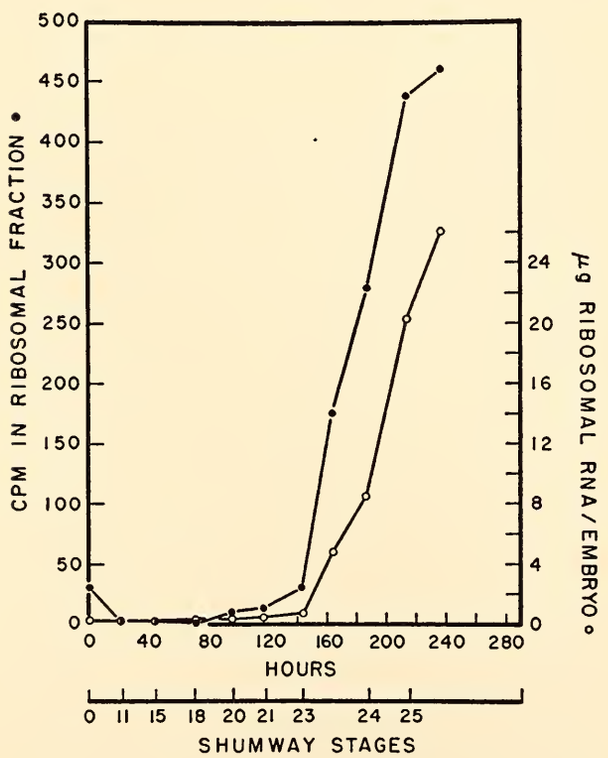

Fig. 8. CPM (closed circles) and μg RNA (open circles) in the ribosomal fraction at different developmental stages. Purified radioactive tadpole ribosomes labeled with orotic acid-2-C^{14} were added to the homogenization medium. The RNA values are corrected for the added radioactive RNA.

The principle of isotope dilution is as follows: It is assumed that any endogenous ribosomes are present in the free state in the egg but upon homogenization become bound to other particles in the egg (or embryo). When enough ribosomes are present under normal homogenizing conditions the binding sites become saturated and free ribosomes become detectable. If homogenization is carried out in the presence of a known amount of radioactive ribosomes of known specific activity (expressed as CPM/μg RNA), the ribosomes will freely and completely mix with the endogenous pool. When the purified ribosomal fraction is isolated, counted, and measured for RNA content the new specific activity can be calculated. From this value the endogenous ribosomal "pool" size can be determined. Figure 9 demonstrates the validity of this technique. Jelly was removed from ovulated eggs, and different numbers were

homogenized in the presence of the same amount of radioactive ribosomes. The 105,000g pellet was homogenized in 0.01 M versene and recentrifuged at 105,000g for 1 hour. This procedure removed substances other than ribosomal RNA which react with orcinol, thus ensuring an accurate RNA value for the specific-activity determinations. In this experiment it was found that the specific activity of the added labeled ribosomes was indeed diluted after reisolation. When the appropriate correction was made a linear curve resulted giving a value for endogenous ribosomal RNA of about 1.6 μg/embryo, at least 10 times previous values. With this technique another staging experiment was done

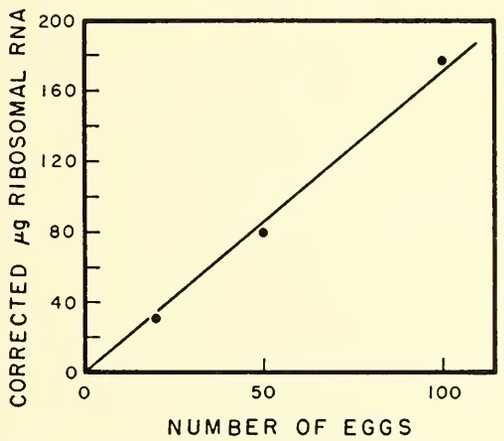

Fig. 9. Ribosomal RNA in ovulated unfertilized eggs corrected for binding by the isotope dilution technique.

(figs. 10, 11). Ribosomal RNA isolated by both the conventional and isotope dilution techniques is plotted. Although the base-line level of ribosomes is raised there still appears to be little increase in ribosomes before stage 20, the great bulk of the RNA being associated with the "pellet" fraction. Increase in supernatant protein can now be correlated directly in time with increase in ribosomes.

Using Kutsky's technique of labeling eggs by intraperitoneal injection of P^{32}

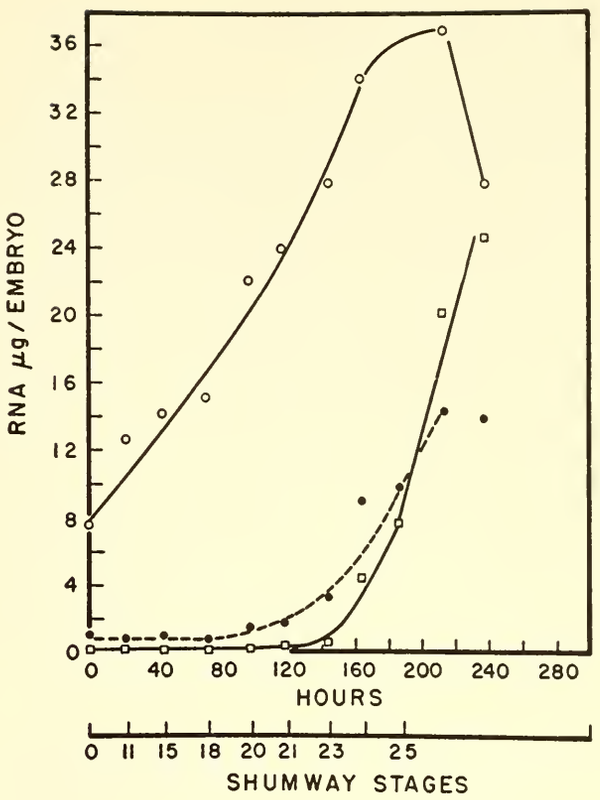

Fig. 10. Ribosomal and "pellet" RNA (open circles) from embryos at different stages of development. Values for ribosomal RNA are plotted before (squares) and after (closed circles) correction by the isotope dilution technique.

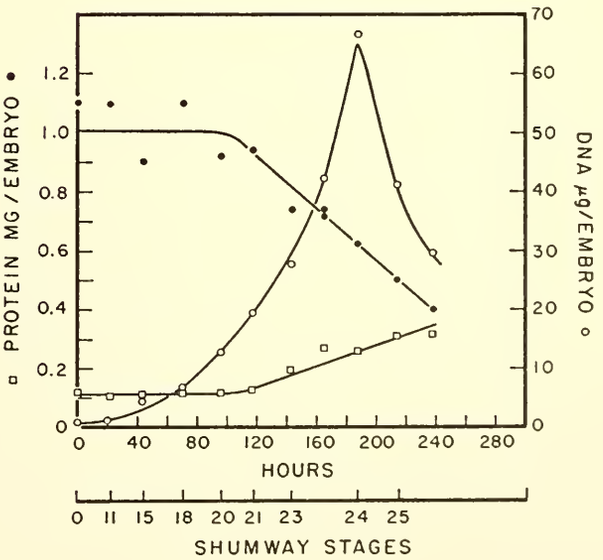

Fig. 11. DNA (open circles), pellet (closed circles), and soluble supernatant protein (squares) values for the staging experiment recorded in figure 10.

into the gravid female, it was possible to reverse the isotope dilution experiment and study the appearance of P^{32}-labeled ribosomes in the developing embryo. When radioactive embryos were homogenized in the presence of a large excess of added purified nonradioactive tadpole ribosomes only trace amounts of P^{32} were detectable in the ribosomal fraction until after Shumway stage 18. At this time there was a rapid appearance of radioactivity in the ribosomal fraction. Radioactive unfertilized eggs have the same low level of ribosomal radioactivity as long as 6 days after P^{32} injection. At 21°C the lag period preceding the appearance of radioactivity in the ribosomal fraction in a large tadpole is 30 to 40 hours.

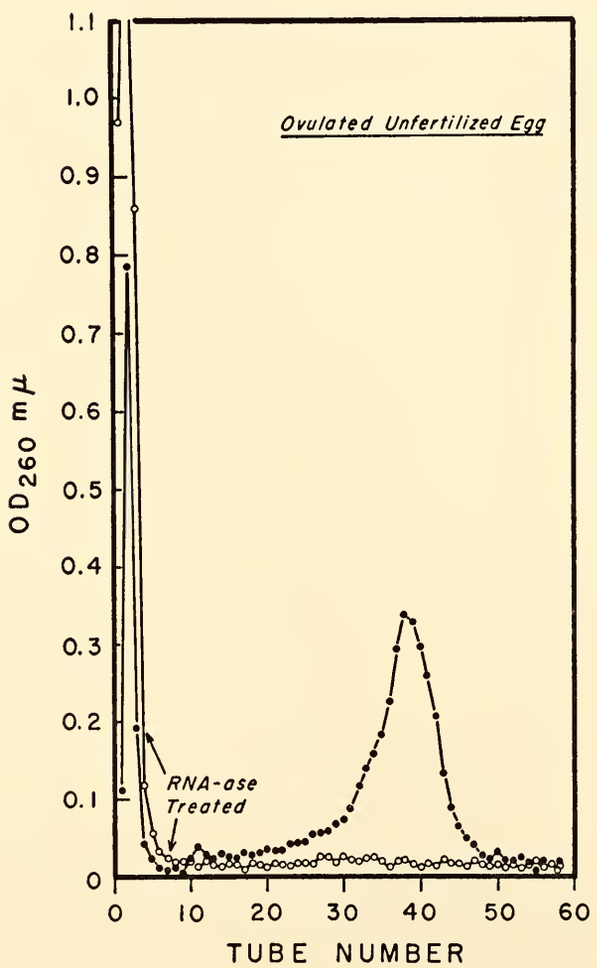

Fig. 12. Elution pattern of purified ovulated unfertilized egg nucleic acid before (closed circles) and after (open circles) ribonuclease digestion.

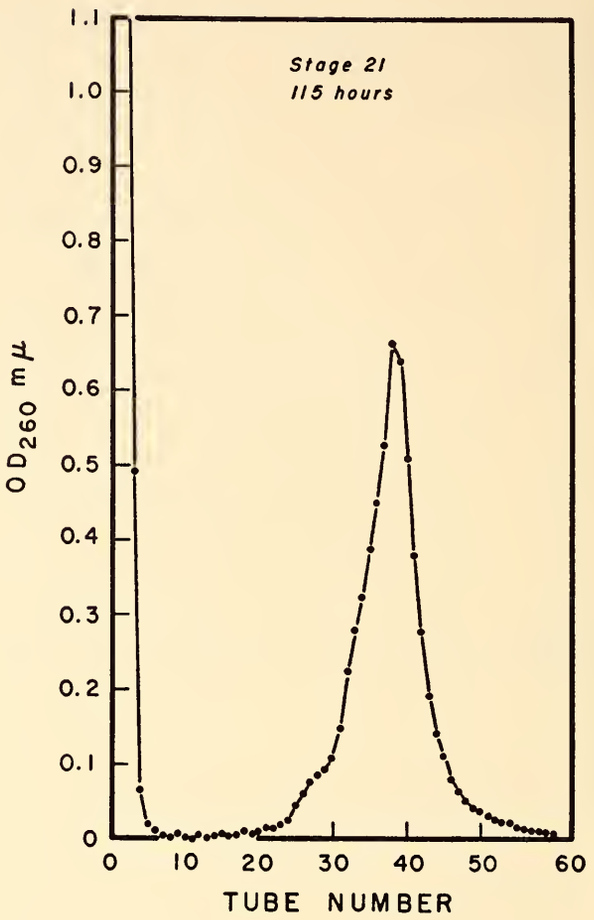

Fig. 13. Elution pattern of purified RNA from stage 21 embryos (115 hours at 21°C).

High-Molecular-Weight RNA in Development

In view of the low level of ribosomes in the frog's egg and early stages as well as the unusual characteristics of isolated RNA of these eggs described by Finamore and Volkin, techniques were developed to isolate undegraded high-molecular-weight RNA from early stages. This RNA has been compared with RNA isolated by the same technique from large whole tadpoles and adult frog liver. The features of the homogenizing medium are (*a*) the inclusion of sodium lauryl sulfate, without which subsequent phenol extraction is completely ineffective, and (*b*) acidic *p*H (5.0) which both augments phenol precipitation of protein and keeps the

extraction at a *p*H where ribonuclease, if present in these early stages, is inactive. The entire procedure is carried out at 0°C. Very little DNA is extracted by this method.

The methylated serum albumin column developed by Mandell and Hershey for DNA separates nucleic acids according to size. The column has been applied to RNA by McCarthy, Sueoka, and others and has been shown to separate soluble RNA from ribosomal RNA. Original ultracentrifuge studies of ribosomal RNA described two large sizes, 16 and 23 S. More recent studies imply that there is only one size, of about 30 S, and that the smaller pieces were the result of nuclease action. As techniques for isolation of RNA's have improved, "messenger" RNA has also been found to be of high molecular weight. Figure 12 shows a NaCl gradient elution pattern of ovulated egg RNA and demonstrates the presence of high-molecular-weight RNA. Subsequent stages are given in figures 13–15. A comparable fraction of RNA was always eluted at 0.7 *M* NaCl. Table 3 summarizes the similar if not identical base composition of this peak in the various preparations and further compares these results with the base composition of purified tadpole ribosomal RNA. It cannot as yet be determined what percentage of the high-molecular-weight RNA isolated by this technique is attributable to the small number of ribosomes present in the egg.

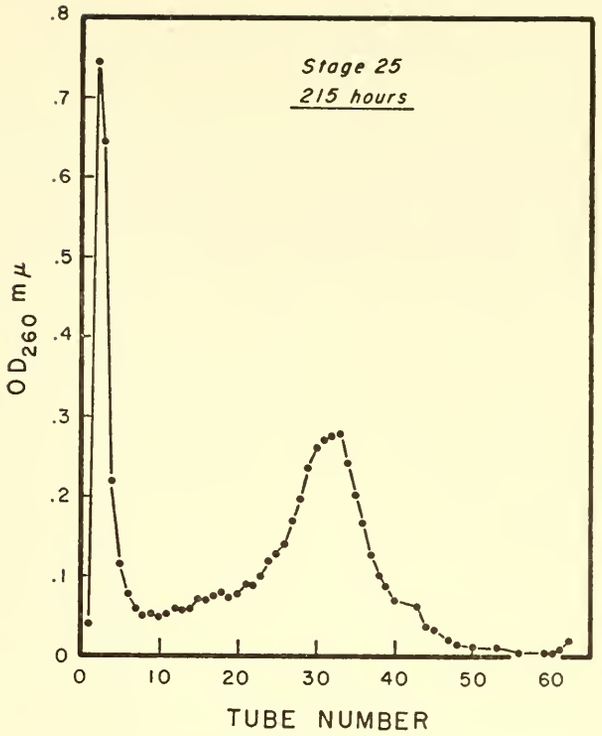

Fig. 14. Elution pattern of purified RNA from stage 25 embryos (215 hours at 21°C).

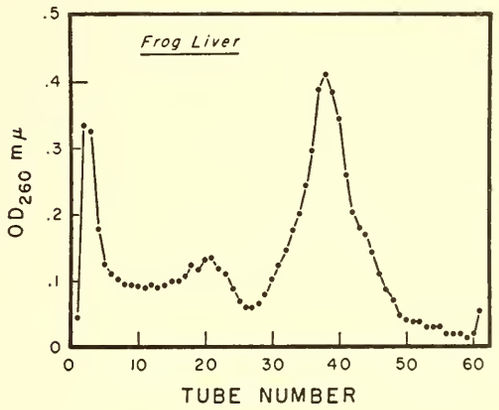

Fig. 15. Elution pattern of purified RNA from adult frog liver (0.5 gram wet weight).

TABLE 3. Base Composition of High-Molecular-Weight RNA Eluted at 0.7 *M* NaCl from Methylated Serum Albumin Column

Shumway Stage	Guanine	Adenine	Cytosine	Uracil
0 (unfertilized)	32	21	29	18
11 (gastrula)	36	18	29	17
21	36	20	27	17
25	34	19	29	18
Whole tadpole	32	19	28	21
Liver	35	21	27	17
Tadpole ribosomal RNA*	34	21	28	17

* RNA was isolated from 105,000*g* pellet by the phenol procedure. The ethanol precipitate was hydrolyzed directly and not chromatographed on the column.

Size and Localization of Synthesized Ribosomes after Stage 23

Roberts et al. have demonstrated by isotope studies that large bacterial ribosomes are constructed by aggregation of smaller ribonucleoprotein particles. When ribosomes accumulate in the cytoplasm of embryos after stages 21–23 they are already in the form of 80–100 S particles. This observation implies that these particles had been formed elsewhere, perhaps in the nucleus as suggested by several workers.

The question arose whether ribosomes appeared in all tissues of the developing embryo simultaneously. Twenty-five stage 24 embryos (189 hours) were divided into three parts: (*a*) tail; (*b*) contents of the abdominal cavity, the largest part of which was yolk-filled gut; and (*c*) the remainder of the embryo, which included the head and the dorsal part. The usual homogenization and fractionation was performed, and RNA, DNA, and protein contents were measured. Table 4 compares values of dissected embryos with values for whole embryos homogenized at the same time. The summation of the different parameters of dissected embryos is in good agreement with control values for whole embryo homogenates. In the final line ribosomal RNA/DNA ratio represents a measure of ribosomes per cell. There is a striking absence of ribosomal particles in the ventral or visceral fraction, implying that ribosome appearance here lags behind that in the tail, head, and dorsal parts of the embryo.

Discussion. In a developing system where all components during the critical early period of development are derived from stored materials it is not surprising that the egg should contain a completely different array of macromolecules both particulate and soluble from those present in a mature tissue. From one point of view the egg is a remarkably inert storage depot, consisting largely of temporary items that will be used for the formation of more permanent ones during development. This report has added the iron binding protein complex ferritin to the list of constituents in the frog egg.

Since early development of the frog (to feeding) does not require net increase in mass it can be considered a rearrangement of substances already present. The greatest part of this change from both a chemical and morphological point of view is structural. In fact, Brown and Caston have shown in experiments not reported here that in very early stages and in the unfertilized egg itself radioactive precursors introduced into the pool for protein synthesis are largely incorporated into "pellet" or structural protein. As development progresses the ratio of radioactivity found in "pellet" protein to

TABLE 4. Regional RNA and DNA Contents in Stage 24 Embryo (189 hours)

	Dissected Embryo,* μg				Whole Embryo, μg
	Dorsal	Visceral	Tail	Total	
RNA					
Pellet	12.3	13.7	3.2	29.2	25.1
Supernatant	17.2	5.3	6.2	28.7	23.7
Ribosomes	6.8	0.03	2.1	8.9	8.7
DNA	21.9	10.7	4.4	37.0	44.6
Ribosomal RNA/DNA	0.31	0.003	0.48	----	0.20

* See text for description of three dissected regions.

that incorporated into soluble protein shifts greatly, so that by stage 20 equal amounts are incorporated into each fraction. Similar results were found by Hultin in his studies on sea urchin development. Whether this classification of soluble and structural proteins based on centrifugal properties is of biological significance remains an important subject for future investigation. Staging experiments, illustrated in figures 4–6 and 10–11, lend further support to this distinction, since only a slight net increase in soluble protein occurs before stage 20. Then, directly concurrent with the appearance of cytoplasmic ribosomes, the amount of soluble protein increases rapidly. This correlation suggests to Brown and Caston that "soluble" protein may be formed by the classical sequence of events where final amino acid assembly occurs on "cytoplasmic" ribosomes whereas the "structural" or sedimentible protein so necessary for early morphogenesis is a product of another as yet undefined series of events not requiring the presence of typical ribosomes. There is very little evidence at present regarding the origin of "structural" protein such as that contained in mitochondria, cell wall, or the nucleus. The other known cellular system for protein synthesis is the similar if not identical sequence of events that occurs in the cell nucleus, partly elucidated by Allfrey, Mirsky, and their collaborators. The attractive possibility that "structural" protein is nuclear in origin remains to be proved. Staging experiments show that the great bulk of RNA before stage 20 is localized in parts of the cell that are not in communication with cytoplasmic ribosomes. Whether or not this RNA is nuclear, it parallels DNA appearance closely.

Ribosomal synthesis begins after much of organogenesis and morphogenesis is completed. Perhaps we can think of these ribosomes as being involved in the synthesis of proteins required for growth of the differentiated system rather than for the process of differentiation itself. With the rapid synthesis of these particles existing stores of Mg^{++} are rapidly bound, imposing a magnesium deficiency on the embryo. As would be expected such a deficient embryo completely stops growing but can in fact continue a certain amount of development even in a moribund state. Although few ribosomes can be demonstrated in the early embryo, all stages contain large-molecular-weight RNA having a base composition like that of ribosomal RNA. However, large-molecular-weight RNA isolated from ovulated, unfertilized eggs labeled 6 days previously with P^{32} is completely devoid of radioactivity (less than 0.01 per cent of the fraction isolated by the phenol extraction technique).

Summary. Early embryos contain a measurable but small population of cytoplasmic ribosomes. These have been isolated and quantitated by an isotope dilution technique coupled with standard homogenization and differential centrifugation methods. There is little change in ribosomal content until near the end of morphogenesis after Shumway stage 18, when there is a striking rapid appearance of these particles. Simultaneously, the embryo requires exogenous magnesium ion, which can be shown to retain the integrity of the ribosomes in the 80–100 S aggregated form. When ribosomes are formed there is a concomitant increase in "soluble protein" at the expense of precipitable protein (yolk). Ribosomes appear in the cytoplasm as "mature" particles 80–100 S in size and do not accumulate uniformly in all parts of the embryo, the visceral part being practically devoid of ribosomes at a time when tail and head parts have sizable amounts.

The pattern and base ratio of high-molecular-weight RNA throughout early development has been studied, revealing "ribosomal" RNA in all stages having comparable base composition.

Iron is stored in the egg bound to ferritin. The protein moiety has been unequivocally identified by its com-

parison with purified horse spleen ferritin by both chemical and immunological techniques.

Assay of Vertebrate Ribonucleic Acid for a Ribonucleic Acid Fraction Specifying Polypeptide Sequence

Since specific proteins are detected in different tissues, the regulation of protein synthesis in developmental processes by regulation of synthesis of specific RNA differentially coded from genetic material has become a popular hypothesis (e.g., Jacob and Monod, Leslie). With the description by Nirenberg and Matthaei of a cell-free system from *Escherichia coli* which catalyzes the incorporation of amino acids into polypeptide chains in sequences somehow specified by the sequence of nucleotides in ribonucleotide polymers, it became possible to test vertebrate tissues for the presence of similar RNA active in specifying protein structure. The assay of RNA active in coding for specific proteins would in turn permit a functional test of the idea of differential gene expression in development through differential elaboration of coded RNA.

In studies related to those of Brown and Caston, but conducted independently by G. L. Carlson, cell-free preparations from *E. coli* corresponding to the 30 S (30 × 10^3g supernatant) fraction of Nirenberg and Matthaei were treated with DNase and preincubated to inactivate the "template" RNA in extracts. RNA was prepared from chicken testes and frog liver by several phenol extraction procedures and incubated together with the 30 S fraction and supplements described by Nirenberg and Matthaei. Incorporation of C^{14}-arginine into the acid-insoluble protein of the 30 S fraction was not stimulated by any of the vertebrate RNA preparations, hence these negative experiments are not reported in detail. Efforts are being continued toward preparation of vertebrate RNA functional in this or a similar assay system.

Regulation of Antibody Formation

Acquired Tolerance to Skin Homografts in Mice

As an embryo develops into an adult, it acquires the capacity to recognize and reject foreign invaders. The rate of development of the immune system varies with the species, but, in general, it can be said that at birth, or shortly before or after birth, most animals become capable of an immune response and are able to distinguish "self" from "nonself." The phenomenon of actively acquired tolerance was first demonstrated experimentally by Billingham, Brent, and Medawar, who injected fetal or newborn mice with suspensions of homologous cells including lymphoid cells, and found that these mice, when challenged later, accepted skin homografts from mice of the same inbred strain as the original donor. The "stem cell" concept of acquired tolerance, which holds that the immune system of the recipient is affected specifically at a critical time during its maturation, is now being subjected to careful examination in several laboratories. It appears likely that a modification of the original hypothesis will be necessary, for evidence is now mounting to indicate that actively acquired tolerance and immunologic paralysis are closely related phenomena. The following experiments conducted by B. F. Argyris are concerned with the mechanisms of acquired tolerance to skin homografts in mice.

Newborn C_3H mice are injected intravenously with spleen cells from adult female mice of the CBA strain. Two months later the injected mice are test-grafted with CBA skin, and all accept homografts for at least 2 months. At times when the tolerant mice are bearing such successful homografts, donor (CBA) cells can be found in the lymphoid tissues of all tolerant C_3H mice (table 5). To detect the presence of these cells, the "chimera" test as described by Billingham and his associates is employed. Lymphoid cells from the C_3H chimeras

TABLE 5. Chimera Analysis of Fully, Partly, and Post-Tolerant C_3H Mice

Assay C_3H mice injected intraperitoneally with lymphoid cells of the tolerant animals and 6 days later test-grafted with CBA strain skin. Biopsies removed for histological analysis 7 days later.

Tolerance	No. Mice	Chimeras (presence of CBA strain cells)
Full	4	4
Partial	3	2
After 1 graft	5	5
After 2 grafts	7	1

are injected into C_3H assay mice. Six days later these assay mice are test-grafted with skin from a mouse of strain CBA. Histological analysis of 7-day biopsies of these skin grafts indicates a first- or second-set response, in turn suggesting the respective absence or presence of CBA strain cells in the C_3H tolerant mice.

To study the fate of skin homografts in older tolerant mice, a group of 109 C_3H mice which bore successful CBA skin grafts for at least 2 months has been set aside for long-term observations. As these mice grow older, an increasing number of them show signs of losing their tolerance. The loss of tolerance usually starts with hair loss on the graft and subsequent contraction. No scab is formed, and the end point is marked by a smooth scar. Graft rejection can be a prolonged process (average duration 62 days; range 1 day to 6 months). When the loss of tolerance is rapid, sudden scab formation takes place, starting on the periphery of the graft and spreading inward. At present 41 mice (37 per cent) have rejected their grafts between 2.5 and 10 months after grafting and 27 (25 per cent) are in the process of rejecting their grafts. This means that a total of 62 per cent of the once-tolerant mice are losing or have lost their tolerance. No clear correlation has been found between the number of cells injected at birth and the time of onset or completion of graft rejection, but a more detailed analysis of the quantitative aspects of this problem is in progress.

Fifteen tolerant mice, which rejected their skin homografts between 71 and 290 days after grafting (average 183 days), were regrafted on the contralateral side with CBA strain skin. Ten of the mice that rejected their first graft between 71 and 290 days (average 166 days) rejected their second graft in 11 to 32 days (average 19 days). This suggests that these mice have almost or completely recovered their immune reactivity to the cells to which they were once tolerant. None gave a second-set response. The remaining 5 regrafted mice, which rejected their first graft in 149 to 257 days (average 216 days), are still bearing their second graft at the time of writing (2–3 months after grafting). We may conclude therefore that the delayed loss of tolerance is not always accompanied by a complete recovery of the immune system of the host, differing in this respect from the immediate immune recovery of chickens after the loss of tolerance to homologous red blood cells or of mice after the loss of tolerance to a soluble protein, described by Mitchison and Torres, respectively.

Chimera analysis of a small number of mice during the process of graft contraction ("partly tolerant" mice) and shortly after the rejection of the first skin graft ("post-tolerant; 1 graft") indicates that donor cells are present even after the graft is rejected (table 5). At later stages after the loss of the skin graft and after the second graft has been rejected no donor cells can be detected in the post-tolerant mice (table 5). Since the sensitivity of detection of CBA cells in C_3H assay mice is of the order of 0.5

million CBA cells (table 6), we can conclude that fewer than 0.5 million CBA cells are present in these post-tolerant mice.

The delayed loss of tolerance and the disappearance of CBA cells in the tolerant C_3H mice suggest a reaction between the host and donor cells with final victory for the host. To determine whether there is histological evidence for such a host-versus-graft reaction, and, if so, when it develops in the tolerant host, a detailed histological analysis of the lymphoid tissues of tolerant mice has been undertaken. The strain combination selected (CBA→C_3H) is particularly advantageous, because the high incidence of acquired tolerance (100 per cent) after injection of standard doses of homologous lymphoid cells allows for the study of early stages in the development of acquired tolerance before test grafting becomes technically feasible. Moreover, the absence of runt disease, believed to be a graft-versus-host reaction, avoids additional complications. In these studies the spleen, thymus, and axillary and mesenteric lymph nodes were examined. During the early stages kidney and liver were also studied. Routine histological procedures were followed, and 5-micron sections were stained with methyl green/pyronin and hematoxylin/eosin. So far 63 tolerant, 7 partly tolerant, 16 post-tolerant, 72 control (nongrafted), 10 control (isografted), 13 control (homografted), and 10 control (injected with homologous cell suspension) mice have been examined. Although the study has not been completed the following account can be given with reasonable confidence.

TABLE 6. Sensitivity of Assay Test for Homologous Cells in the CBA ⇄ C_3H Strain Combination of Mice

Assay mice injected intraperitoneally with homologous lymphoid cells and 6 days later test-grafted with donor-type skin. Biopsies removed for histological analysis.

No. Homologous Cells Injected, $\times 10^6$	Strain Combination					
	CBA→C_3H			C_3H→CBA		
	No. Mice Tested	No. Mice with		No. Mice Tested	No. Mice with	
		1st-set response	2nd-set response		1st-set response	2nd-set response
10	5	0	5			
5	7	1	6	4	1	3
1	5	0	5	5	3	2
0.5	7	1	6	5	3	2
0.25	6	3	3			
0.125	4	3	1			

Horizontal arrows denote lower level of sensitivity.

Although the postnatal development of the lymphoid system has been described in detail for the guinea pig by Gyllensten, and in general seems to resemble that of the mouse, species and strain variations in the rate of maturation of the immune system warrant a short description of the events taking place in the lymphoid tissues of the postnatal C_3H mouse. At birth the C_3H spleen consists mainly of red pulp, which is densely packed with myelopoietic and erythropoietic elements, megakaryocytes and large pyroninophilic

cells. Lymphoid follicles are just beginning to form around a central artery. The lymph nodes are undifferentiated small nodules of reticulum cells. The liver shows a high rate of hemopoiesis, and the thymus is well developed, consisting of a cortex of actively dividing thymocytes and a less dense medulla. Since no unusual changes take place in the thymus except for a gradual involution in older mice, we will make no further mention of this organ. After 8 to 9 days myelopoiesis disappears from the splenic red pulp and the liver; the lymph nodes begin to differentiate; primary nodules form in the cortex, and cords begin to develop in the medulla. Around 12 to 14 days of age, few germinal centers develop in the splenic white pulp. Erythropoiesis disappears from the liver at this stage, and no further pronounced changes are seen in the liver. Mast cells appear in the medulla of the axillary lymph nodes. No difference is found between the control and experimental (tolerant) animals except for an occasional plasma cell in the cortex or medulla of the lymph nodes in the tolerant animals. These cells probably represent donor cells, reacting against the host antigens. This weak graft-versus-host reaction appears of no consequence to the host.

No differences in histological appearance can be detected between lymphoid tissues from normal (control) and tolerant (experimental) mice ranging in age from 2 weeks to 4 months. During this time the white pulp of the spleen expands, germinal centers increase in size, the density of the red pulp decreases, and the number of stored red blood cells in the red pulp increases. At 2 months the spleens of both control and experimental mice can be active, with extensive germinal centers and occasional small clusters of plasma cells. Germinal centers can also be found in the cortex of the lymph nodes at this time, and plasma cells are present in the medullary cords. The mesenteric node has many more plasma cells than the axillary node. Generally after 2 months the spleen becomes less active, germinal centers decrease in size, lymph nodes become less active, and very few plasma cells are found in the axillary lymph nodes of control mice. Mesenteric nodes, on the other hand, continue to contain many plasma cells.

Around 4 months of age (2 months after grafting) a difference in histological picture between control and experimental mice becomes apparent. The axillary (regional) lymph nodes of tolerant mice enlarge, the number and size of germinal centers increase, and many plasma cells can be found in the medullary cords of these nodes. At 7 months (the oldest tolerant mice studied so far) this difference between control and experimental mice is still evident. The cortex of the axillary lymph node may also show clusters of plasma cells, a phenomenon rarely observed in normal axillary lymph nodes. As before, the spleens of tolerant and of control mice present a similar picture. At this age the red pulp has decreased considerably in cellularity, the amount of erythropoiesis is reduced significantly, many red blood cells are stored in the red pulp, and a large amount of pigment is present.

To be certain that the enlargement of the regional lymph node in tolerant mice is not a nonspecific response to trauma of the skin grafting procedure, the effect of isografts on regional lymph nodes of control C_3H mice was studied. No enlargement or plasma cells are found in the regional lymph nodes of mice 1 or 2 months after the receipt of an isograft. The enlargement of the regional nodes in tolerant mice therefore appears to be a specific immunologic response to the graft.

In the partly tolerant mice, those killed at the time of graft contraction, the picture is similar to that in tolerant mice. No difference is observed in the spleen or mesenteric lymph node, but an immune response of the regional lymph node is apparent. A few post-tolerant mice have

been studied shortly after the contraction of their first graft. The histological picture of these animals resembles that of the preceding group. After the rejection of a second graft, however, the post-tolerant mice present an entirely different picture. As before, the regional node shows an immune response, but now the spleen is involved also, showing enlarged germinal centers, and clusters of plasma cells in both red and white pulp.

Before analyzing the results it might be well to point out that others have shown, and these observations have confirmed, that skin homografts elicit an immune response limited to the regional lymph node, whereas the injection of homologous cells is followed by an immune response on the part of both the spleen and lymph nodes. Although these studies are not complete, the results suggest that, in tolerant mice, a host-versus-graft reaction, which is histologically demonstrable, precedes external signs of graft rejection. This host-versus-graft reaction appears limited to the regional lymph node. Chimera analysis of lymphoid tissues at this time does show the presence of donor cells. The immunological reaction appears to be directed primarily against the donor skin graft and not against the donor lymph cells. During graft contraction, and shortly thereafter, the immune response is still limited to the regional lymph node. It is of interest that preliminary evidence suggests that at these times partly and post-tolerant mice are still chimeras. After the rejection of a second graft by post-tolerant mice, however, the immune response seems to involve both spleen and lymph nodes. Accordingly no donor cells can be found by the chimera test at this time. A summary of these findings is presented in table 7.

TABLE 7. Summary of Histological and Chimera Analysis in Tolerant Mice of Different Stages and in Control Mice

	State of Tolerance				Control Graft			
	Full	Partial (contracting)	1st Graft	2nd Graft	None	Iso-	Homo-	Homologous Cells
Chimera test	+	+	+	−				
Immune response								
Regional lymph node	+	+	+	+	−	−	+	+
Spleen	−	−	−	+	−	−	−	+

In summary the results suggest that:

1. Tolerance to skin homografts in the CBA→C_3H strain combination of mice is not permanent.

2. A host-versus-graft reaction against the skin homograft precedes that against the homologous lymphoid cells. This host-versus-graft reaction is evident in the regional lymph node before any external signs of graft rejection.

3. After rejection of the graft a state of "restricted" tolerance sets in, during which donor lymphoid cells are still present.

4. In the final stage of the delayed loss of tolerance, the donor lymphoid cells are rejected with a concomitant immune response in host spleen and lymph nodes.

To determine whether removal of lymphoid cells from a tolerant animal affects the animal's tolerant behavior, newborn C_3H mice were again injected with 5 million spleen cells from adult female CBA donor mice. At 2 months of age they were test-grafted with CBA strain skin, and all were tolerant for at least 2 months. At this time the lymphoid

tissues (bone marrow, lymph nodes, and spleen) from the tolerant mice were removed and transferred to lethally irradiated C_3H and CBA mice. The bone marrow was injected intravenously and the lymph node and spleen cells intraperitoneally. Three weeks later the irradiated recipient mice were test-grafted with C_3H, CBA, and C57BL/6 skin. Two months later they were killed and their lymphoid tissues were analyzed for the presence of C_3H and CBA cells by the "chimera" test. Control irradiated mice were injected with equivalent amounts of isologous lymphoid cells and similarly test-grafted. A second control group was irradiated but received no further treatment.

Table 8 contains the results of this experiment. The acceptance of CBA strain skin grafts of the X-irradiated CBA and C_3H recipients of "tolerant" tissues indicates that tolerant tissues (here, those of C_3H origin) remain tolerant in a different environment. The acceptance of C_3H strain skin grafts by the irradiated recipients of tolerant tissues suggests that the original donor cells (those of CBA origin), which were injected into the newborn C_3H mice as adult cells, have become tolerant of the host type (C_3H) antigens. Here, then, is an example of host-versus-graft as well as graft-versus-host tolerance. The persistence of this graft-versus-host tolerance in the irradiated CBA recipients suggests, furthermore, that the graft-versus-host tolerance is not the result of an immunological paralysis. If the CBA donor cells, upon injection into the newborn C_3H host, had been overwhelmed and paralyzed by the large number of host-type antigens present, they would have recovered their immunological reactivity to C_3H type antigens upon transfer to the irradiated CBA recipients, where the number of C_3H type antigens was greatly reduced. That no such recovery of the CBA cells occurred, and that they continued to tolerate C_3H skin grafts, suggest that a "true" tolerance was induced in these adult CBA donor cells. The rejection of C57BL/6 skin grafts by the irradiated recipients of "tolerant" tissues indicates that the acceptance of CBA and C_3H skin grafts by these animals is not due to a general immunological impairment but to a specific inhibition of an immune response.

Upon chimera analysis, it was found (table 9) that 5 out of 7 irradiated C_3H recipients of tolerant tissues harbored CBA cells, confirming that both the original host (C_3H) and donor (CBA) cells were transferred from the tolerant to the irradiated recipient mouse. Chi-

TABLE 8. Fate of Skin Grafts in Irradiated CBA and C_3H Recipient Mice Injected with Lymphoid Cells from Normal and Tolerant Donors

XR Recipient Strain	Type of Lymphoid Cells	No. Mice	Early Mortality	Fate of Skin Grafts					
				Donor CBA		Donor C_3H		Donor C57BL/6	
				No. Tested	Rej. (MST)	No. Tested	Rej. (MST)	No. Tested	Rej. (MST)
C_3H	Tolerant	12	3	9	1 (12)	8*	0	8	8 (12±0)
	Isologous	10	2	8	8 (15±)	3†	0	4	4 (12±0)
	None	9	8	—		—		—	
CBA	Tolerant	10	1	9	0	9	0	9	8 (12±0)
	Isologous	10	0	9	0	10‡	10 (15±1)	9	9 (12±0)
	None	5	4	—		—		—	

MST = mean survival time.
* One mouse died after rejection of the CBA graft.
† Only 3 out of the 8 mice in this group were tested with isografts.
‡ One mouse died after rejection of the C_3H graft.

TABLE 9. Chimera Analysis of C_3H and CBA X-Irradiated Recipients of "Tolerant" Lymphoid Tissues

X-Irradiated Recipients					
C_3H			CBA		
Individual Mouse No.	No. Cells Transferred in Analysis, $\times 10^6$	Chimera	Individual Mouse No.	No. Cells Transferred in Analysis, $\times 10^6$	Chimera
1	150	Yes	1	130	No
2	175	No	2	125	No
3	220	Yes	3	150	No
4	220	Yes	4	220	No
5	150	Yes	5	200	No
6	175	No	6	250	Yes
7	190	Yes			

mera analysis of the irradiated CBA recipients of "tolerant" tissue, however, failed in all but one of them to reveal the presence of C_3H cells. Even though the assay sensitivity of C_3H cells in CBA mice is lower than in the reversed direction (in the order of 5 million cells; table 6) these results are nevertheless surprising since the irradiated CBA mice were injected with lymphoid cells from tolerant C_3H mice. Whether there is a preferential proliferation of CBA cells in these irradiated CBA mice is an open question for the moment.

Immunologically Induced Aspermatogenesis

D. W. Bishop's previous studies of the immunologically induced aspermatogenic response in guinea pigs (see *Year Book 60*, pp. 412–416) focused attention on two problems relating to mechanisms of the reaction that are being further explored by Bishop in collaboration with Maurice Lessof, Guy's Hospital, London. The first concerns the significance and possible causal relation between the presence of circulating nonprecipitable antibodies and the appearance of lesions in the germinal epithelium after injection of testicular antigen combined with adjuvant. Although such a causal relation has generally been regarded as relatively heretical by classical immunologists concerned with responses of the delayed hypersensitivity type, the findings of Bishop and co-workers have repeatedly shown a significant correlation and suggest that humoral factors may play an important role. Recent support for this view has been suggested by certain studies of immune induced thyroid disease and skin homograft reactions. Lessof is attempting to determine antibody titer at all stages following sensitization, with particular interest in events when testicular injury first becomes apparent. For antibody assay he is utilizing extracted testicular antigen and serum standards prepared by Bishop with the aid of G. L. Carlson. No results have yet been reported by Lessof, who initiated this phase of the investigation only recently.

The unusual susceptibility of the guinea pig germinal epithelium to autoimmune lesions has been under study by Lessof in an effort to determine what factors or physiological peculiarities render this system responsive, and whether physiological alterations in the testis might so change the reactivity as to shed light on mechanisms of the cellular response. Although many difficulties in technique and interpretation beset such an approach, Lessof has evolved methods involving unilateral treatment of the testis with low temperature or with X

irradiation, the effects of which can be distinguished from autosensitization, in an attempt to modify the onset of immune induced aspermatogenesis. Thus far, cold shock has proved ineffective, but irradiation offers some promise. The effect of pharmacodynamic substances, like hyaluronidase, histamine, and other agents that increase permeability, is being scrutinized.

In last year's report it was noted that studies on the immune capacity of fetal guinea pigs were contemplated with the aim of testing the effect of injection, in utero, of soluble antigen and of the aspermatogenic factor when it can be obtained in a high degree of purity. The operative procedure for this approach has now been refined so that fetal loss is minimized. Intraperitoneal injection with bovine gamma globulin (BGG) of 45- to 66-day-old fetuses results in positive serological reactivity when determined on sera collected at 3 and 8 weeks postpartum. Circulating factors are demonstrable by the passive cutaneous anaphylactic (PCA) reaction but are negative by the gel-diffusion technique, indicating the presence of nonprecipitable or of low-titer precipitable antibodies in serum of newborn animals. Unfortunately, the precise fetal origin of these antibodies is clouded somewhat by positive PCA reactions in maternal serum collected after delivery. More precision in the procedure is necessary to determine whether the pregnant animal also gives rise to specific antibodies as a result of uterine transfer of BGG or whether she is passively sensitized by fetal antibody. The possibility also exists that the mother is accidentally sensitized at the time of fetal injection and her antibodies are transferred to the fetuses in utero or to the newborn during lactation. According to studies by Uhr, the former possibility is remote, since he was unsuccessful in passively sensitizing guinea pig fetuses by injection of the mother with diphtheria toxoid or ovalbumin. As pregnancies become available, the significance of lactation as an anti-BGG transfer mechanism in the guinea pig is being investigated. Although fetal sensitizations with testicular antigen have lagged behind, Bishop has, thus far, been unable to confirm Chutná and Hâsková's finding that the guinea pig can acquire tolerance or be desensitized to the aspermatogenic reaction. The discrepancy may lie in their use of a different and less responsive (aspermatogenetically) strain of guinea pig or in the employment of massive dosages of testicular antigen to "paralyze" the immune reaction.

Previously reported were experiments by Bishop and Gump demonstrating that newborn guinea pigs injected intramuscularly with BGG and adjuvant give rise to circulating antibodies within 7 to 9 days. Two further simple experiments have demonstrated (1) the immunologic competence of newborn animals injected intraperitoneally with antigen alone and (2) the relative efficacy of intramuscular, intraperitoneal, and intravenous injection of adults. The former data are summarized in table 10; of 17 neonatal animals injected intraperitoneally with 20 mg BGG in saline within 24 hours of

TABLE 10. Immunologic Response of Newborn Guinea Pigs to a Single Intraperitoneal Injection of BGG

No. Animals	Injection			Bleeding		PCA Reactions*			Ouchterlony Reaction†
	Age	Route	Antigen	Age	Route	++	+++	++++	
17	18–24 hr	IP	20 mg BGG	21 days	IC	3	3	11	Negative

* PCA sensitization: 0.1 ml antiserum. PCA challenge: 10 mg BGG. PCA scoring: ++ = 11–15 mm, +++ = 16–20 mm, ++++ = >20 mm.

† Ouchterlony plates read daily to 16 days.

delivery, all showed pronounced PCA reactions in sera collected at 21 days of age. Ouchterlony tests, on the other hand, were consistently negative.

In a comparison of circulating antibody titers according to the route of administration, it was found that antigen plus adjuvant injected intramuscularly into the guinea pig gave a higher and more persistent titer than antigen administered alone either intravenously or intraperitoneally (table 11). Adjuvant potentiates the reaction, and is the method of choice. In the intravenous series, antibody titer increased to a maximum at about 10 days and then receded abruptly. The antibody titer of the intraperitoneal series increased for about 2 weeks and leveled off at twice the intravenous value, whereas the titer of the intramuscular series continued to increase for 6 weeks. In this experiment, done in collaboration with Timothy Glover, each animal received 100 mg of BGG, distributed in 5 injections over a period of 9 days in the intravenous and intraperitoneal series and in 2 injections with adjuvant in the intramuscular series. All animals were bled 5, 9, 14, 30, and 42 days after the last injection. PCA tests were carried out according to the procedure developed by Ovary, and the tabulated results indicate the average reaction diameter for 5 undiluted sera. The results of gel-diffusion tests of these sera, also shown in table 11, roughly follow those obtained by the PCA procedure. Double bands appeared in the Ouchterlony plates in 1 serum sample from the intraperitoneal series and in 4 out of 5 from the intramuscular series. In a comparison of the two test methods, the individual data show clearly that the PCA procedure is more sensitive and is capable of detecting precipitable antibody in titers too low to be demonstrated by the gel-diffusion method. That the apparent sensitivity of the PCA test here lies in its ability to detect nonprecipitable humoral antibody is unlikely.

TABLE 11. Immunologic Response of Adult Guinea Pigs to BGG Administered Intravenously, Intraperitoneally, and Intramuscularly with Adjuvant*

No. Animals	Bleeding after Last Injection, days	PCA Reactions†			Ouchterlony Tests		
		IV	IP	IM	IV	IP	IM
5	5	18.4	20.4	21.2	1/5	3/5	4/5
5	9	22.0	24.6	25.2	1/5	3/5	4/5
5	14	21.4	25.6	30.4	3/5	4/5	5/5
5	30	11.6	25.6	35.8	3/5	2/5	5/5
5	42	12.0	25.0	36.6	0/5	2/5	5/5

* Each animal received 100 mg BGG, in 5 injections in IV and IP series, and in 2 injections in IM series.

† Values represent averages (in mm) for 5 sera.

Nature of the Material Inducing Aspermatogenesis in the Guinea Pig

In collaboration with D. W. Bishop, Gerald L. Carlson has continued to investigate the chemical nature of the fraction purified from guinea pig testes which when injected together with adjuvant produces aspermatogenesis in the guinea pig. As was mentioned in *Year Book 60*, pages 412–414, Freund, Thompson, and Lipton had purified an aspermatogenic fraction designated CPM (chlorofo m-purified material) from guinea pig testes and characterized it in a preliminary way. A failure of chemical analysis to account, with reasonable assumptions, for more than 35 to 40 per

cent of the total mass determined in dry-weight measurements by Freund et al. was possibly due to incomplete drying of CPM samples. Dry-weight determinations on CPM samples used in the present studies (table 12) indicate that carbohydrate, measured as reducing power for cupric ion and expressed as galactose, accounts for about 20 per cent, and polypeptide(s), determined by either the Lowry or Biuret method with bovine serum albumin as a standard, for about 70 per cent, of the total mass. Thus, only about 10 per cent of the CPM remains to be assigned to a general chemical classification.

The amino acids composing the polypeptide part of CPM were examined by methods giving greater resolution than techniques previously used. Samples of CPM were oxidized with performic acid to give stable derivatives of sulfur-containing amino acids, and the oxidized CPM samples were then hydrolyzed with 6 *N* HCl in sealed tubes. The hydrolysates of performic-oxidized CPM were used in two-dimensional ionophoretic separations which followed the procedures of M. A. Naughton (the ionophoresis study was made in the Biophysics Department of Johns Hopkins Medical School with his aid). With these methods the presence of eighteen amino acids has been shown in CPM samples, and a quantitative analysis of the amino acids is in progress. Amino acids in CPM that have been identified so far by position in ionophoresis are histidine, lysine, arginine, glutamic acid, aspartic acid, cystine, or cysteine, phenylalanine, tyrosine, proline, threonine, alanine, and serine. Tryptophan is probably present as judged from absorption by CPM at 278 mμ remaining at *p*H 13. One unidentified ninhydrin-reactive compound, present in quantity in all CPM hydrolysates, is under further study.

TABLE 12. Analysis of CPM Samples

Type of Measurement	Milligrams per Milliliter of CPM	
	Preparation A	Preparation B
Dry weight	3.20 ± 0.01	3.10 ± 0.05
Galactose equivalent*	0.60 ± 0.02	0.625 ± 0.01
Bovine serum albumin equivalent (biuret)	2.22 ± 0.04	1.83 ± 0.13
Bovine serum albumin equivalent (Lowry)	2.25 ± 0.07	1.98 ± 0.06

* Reducing material determined after hydrolysis with 0.37 *N* H_2SO_4 in sealed tube at 95–100° for 16 hours and expressed as galactose.

Antibodies as Tools

An Approach to an Immunochemical Study of Neuronal Differentiation

Having only six months to spend in the Department, Arthur LaVelle elected to center his attention on mastering immunochemical techniques and adapting them for the study of developing nerve cells.

The long-range purpose of these preliminary attempts is to endeavor to produce, as one extension of his current research in neuronal differentiation, a specific antiserum to neuronal tissue (neuroplasm). Therefore LaVelle began to develop techniques for the production of antibrain serum in several different strains of laboratory mice.

Because of the ease of dissecting cellular areas from frozen-dried sections of nervous tissue (an advantage that could be of later value) it was decided first to assess the antibody-producing potential of frozen-dried whole brain. Individuals of several series of C57 and

BDF_1J mice (Jackson Memorial Laboratory), 4 to 6 months old, were injected each week for 6 to 8 weeks. At each injection each animal received 0.8 mg (dry weight) of saline-perfused frozen-dried brain combined with saline and Freund's adjuvant (Difco "complete"). This mixture was injected as a 0.2-ml emulsion into multiple sites either intradermally or subcutaneously, depending on the series. At intervals, and at 1 week after the final injection, blood was drawn from each animal by cardiac puncture, and the separated serum was stored in the deep freezer at −40°C for future testing. No physiological signs of experimental encephalomyelitis (EAE) were observed at any time. Also, thus far, titers of the "antiserum" (serum dilutions) against saline suspensions of frozen-dried brain have been negative. Preliminary tests of frozen-dried brain suspensions against the serum from the experimental animals using micro agar-diffusion techniques failed to show any precipitation lines. As this report was being written, saline suspensions of fresh whole brain and frozen-dried brain were being run against serum from the experimental animals on macro-Ouchterlony plates in an effort to obtain precipitation lines. Further tests with the experimental serum will be run, but they will be undertaken relative to experiments using fresh and frozen-dried brain now being injected into other animals. Full testing of this serum will not be finished for several months, in conjunction with correlative experiments now in progress.

Among several reasons for the consistent negative results so far attained, two, in particular, seem pertinent. One is that frozen-dried tissue, aside from brain itself, is in some way altered so that it is a poor antibody stimulus. LaVelle suggests that a mechanism similar to the "enhancement" effect obtained in homograft experiments may play a role; in this type of reaction frozen-dried spleen is particularly effective.

The other explanation is suggested in a report by Lee and Schneider that appeared while these experiments were in progress, stating that a critical relation between the constituents of the antigen-adjuvant emulsion is necessary to produce EAE in completely susceptible mice. These authors also report that the efforts of some others using the Difco "complete" adjuvant in combination with proteolipides failed to produce EAE in susceptible mice.

We might add that the high content of lipide in whole brain makes it particularly difficult to prepare satisfactory suspensions for precipitin tests. Apparently the lipide also interferes with free diffusion on agar plates. As yet, LaVelle has not tested lipide-extracted residue or the extracted total lipide against the experimental serum. The proteolipide, prepared from hamster brain according to the method of Folch and Lees, has not yet been tested either. Experiments will be run to take into consideration these and other factors that need not be detailed here.

Obviously many factors must be considered before a satisfactory resolution to even this modest beginning of the main problem can be obtained.

A New Antigenic System in the Chick Embryo

The following report of R. F. Ruth's activities during 1960–1961 was submitted too late to be included in *Year Book 60*.

With the assistance of F. J. Kupres, Ruth confirmed and expanded his earlier finding of a new antigenic system in the chick embryo. The salient feature of this system is its association with the periphery of the blastoderm. It is at the periphery that the blastoderm attaches to the vitelline membrane, and the effects of antisera were first observed by the separation of the blastoderm from the vitelline membrane. Closer examination of this phenomenon indicates that the peripheral attachment is immunologically related to the erythrocyte.

The detachment of the blastoderm was discovered by treatment of cultured embryos with antichicken erythrocyte serum. More antierythrocyte sera were prepared in rabbits. All such sera, when used fresh or after one freezing and thawing, detached all embryos with which they were in contact between the twentieth and sixty-eighth hours of development. This was true without exception of more than a dozen sera tested with more than 100 embryos. Some antisera were fractionated, and the globulin-rich and globulin-poor fractions were used to treat embryos. The globulin-rich fractions detached the embryos; the globulin-poor fractions did not. Some antisera were absorbed exhaustively with chicken erythrocytes. Despite difficulties associated with the aging of the sera and lysis of many erythrocytes during the absorptions, these sera were only slightly toxic to embryos. They did not detach embryos.

The detachment of embryos by anti-erythrocyte sera and globulin-rich fractions of these sera, and the inability of absorbed sera and globulin-poor fractions to detach embryos, suggested that detachment is effected by globulins that can attach irreversibly to erythrocytes. There remained the possibility that these globulins might be directed toward plasma contaminants present in the erythrocytes used for injection and absorption, despite repeated washings.

Antisera were prepared in rabbits against cell-free chicken plasma. The sera obtained after one series of injections did not detach embryos. This finding indicated that the ability to detach was not due to contamination with any of the principal constituents of plasma. Rabbit antierythrocyte sera, which did detach, were incubated with equal volumes of chicken plasma without altering their ability to detach as compared with appropriately diluted and incubated controls. Various absorptions and technical controls were employed, and these results also were consistent with the hypothesis that detachment is due to antibodies evoked specifically in rabbits by injection of chicken erythrocytes.

This demonstration of the antigenic relationship between the erythrocyte and the peripheral attachment of the blastoderm to the vitelline membrane poses several questions. When do the antigens appear in ontogeny? Are they genetically controlled? Is the detachment due to the presence of the antigens on the vitelline membrane or on the peripheral cells? Are the antigens involved peculiar to the erythrocyte and to the peripheral attachment?

The erythrocytes used to produce sera were obtained from groups of mature chickens. Detachment of treated embryos occurred during the first 3 days of incubation. The effective antigens thus appear early in development and persist into maturity. This observation suggested that the antigens involved might be of the classical Forssman type. Since guinea pigs cannot produce antibodies to Forssman antigens, antierythrocyte sera prepared in guinea pigs were used to treat embryos. These antisera detached all embryos, indicating that the effective antibodies are not peculiar to the rabbit and that the antigens are not of the classical Forssman type.

Presumably, the antigens involved in detachment are genetically controlled. Genetic control could not have been detected in the experiments described, since they were designed to eliminate intraspecies variations in the production of antibodies and in the response of embryos to treatment. The possibility of genetic control was tested in an independent experiment. Embryos known to be of A^1A^1 genotype were treated with chicken anti-A^1 erythrocyte sera, chicken anti-A^5 erythrocyte sera, and normal sera. Equal sets of A^5A^5 embryos were treated with the same reagents. Since the A antigens are present very early in development the A alleles seem to provide the best possible genetic test. The results of the experiment were negative, but a

consideration of the dilutions of the reagents used suggests that the conditions for the test were not the best possible. The embryos and reagents used in the test were provided by Elwood Briles of the DeKalb Agricultural Research Association.

The possibility that detachment is due to a combination of antibodies with the vitelline membrane was tested in several ways. Finely divided vitelline membrane was incubated with antierythrocyte sera. Agglutination could not be detected macroscopically or microscopically. Antisera incubated with divided vitelline membrane retained their ability to detach embryos. These observations fail to support the theory that the antibodies effect detachment by coating the vitelline membrane. These negative experiments cannot be considered conclusive, however. In fact, the only pertinent positive results are consistent with the idea that specific coating of the vitelline membrane may occur.

Vitelline membranes were prepared in the usual fashion. The embryo was removed from each by fine needles and a pipette; the vitelline membranes were incubated with normal sera or antierythrocyte sera for 2 hours at 37°C, washed repeatedly with saline, reincubated with saline at 37°C overnight, and rewashed; and fresh embryos were placed on the membranes. The embryos placed on membranes treated with normal sera attached, and those placed on membranes treated with antisera did not.

The periphery of a detached blastoderm may retain some of its specialized, flattened characteristics, but the beautiful sinuous extremity of the periphery is never present. Typically, the peripheral cells are condensed into a roll of cells some of which retain normal nuclear morphology. Such "rolls" have lost all resemblance to the normal periphery and are largely made up of vacuoles. The remainder of the embryo is usually more normal in appearance, though it may lack hemoglobin or be grossly distorted on occasion. When fresh sera are used individually it is a simple matter to obtain healthy, vigorous embryos which continue development while floating freely in serum. It is not necessary to dilute the serum in order to demonstrate this, but dilution might raise the frequency of good development among detached embryos.

The contraction of the freely floating blastoderm substantiates New's interpretation of the role of the periphery and the vitelline membrane in maintaining tension across the blastoderm. The frequency with which the embryo itself continued development and growth, even though it had to push the contracted blastoderm out of the way, affirms the basic independence of embryonic development and the spreading of the blastoderm. Aside from the birds only one major group of vertebrates, the teleosts, displays an analogous spread of the blastoderm by means of a peripheral attachment.

Composition of the Cell Surface

There seems good reason to hope that immunological methods may be helpful in advancing knowledge of the composition of the cell surface and of the nature of contact-dependent reactions between cells.

The technique suggested by Michael Abercrombie is to apply antisera, made by injecting rabbits with whole cells or cell fractions, to tissue cultures; and to measure the effects of the treatment on some form of cell behavior likely to depend on the nature of the cell surface. Mutual inhibition of cell movement by contact is such a form of behavior. The overlapping of cells in a culture on a plane surface, which largely depends on this inhibition, is the most promising assay system, since it can be precisely quantitated and should be highly sensitive. By this means it is hoped to establish degrees of cross reaction between cells of different types and in different states and to follow up the interesting beginning

made recently by Kite and Merchant in determining the chemical nature of the surface antigens.

The precise nature of the influence of antibodies on the overlapping of cultured cells cannot be predicted from present knowledge and should itself give useful information on the mechanism of the contact inhibition involved. For instance, diminution of overlapping when the cell surface is masked by antibody would make it difficult to suppose that contact inhibition depends on specific adhesion between cells.

CELL INTERACTION IN DIFFERENTIATION AND MORPHOGENESIS

Myogenesis in Vitro

An investigation of the cytodifferentiation of embryonic skeletal muscle cells in dispersed cell culture was originally undertaken by I. R. Konigsberg in an attempt to define a system that would offer greater opportunity for rigorous control of both the quantitative aspects of the cellular population and the extracellular environment than could be achieved either in vivo or in organ culture. The cumulative experience of numerous investigators over many years suggested that such culturing techniques could be expected to promote the loss of differentiative character rather than the progressive increase of the morphological consequences of cell specialization. No generally satisfactory explanation for this observed incompatibility was or is available. The results Konigsberg has obtained with monolayer cultures of embryonic skeletal muscle cells disagree with the general observation of a loss of morphological indices of the differentiated state.

The monolayer cultures prepared from suspensions of 11- to 12-day chick embryonic leg muscle pass through three recognizable phases. The period immediately following plating of the cells is marked by rapid proliferation with a mean generation time of 24 hours. During this period cultures consist exclusively of mononucleated cells and have the general appearance of cultures of fibroblastlike cells such as might be derived from a great variety of tissues. The transition from phase one to phase two occurs very rapidly (in a matter of hours) and is characterized by the formation of long, multinuclear, ribbonlike cells. Formation of multinuclear cells coincides with the attainment of confluency. The involvement of cell density is further suggested by experiments in which the inoculum size was varied. When inoculum size is varied the time of transition from phase one (fibroblastlike cell) to phase two (multinuclear ribbon) can be shifted. The abrupt appearance of multinucleated myotubes is paralleled by an equally abrupt break in the rate of proliferation, which again can be shifted by varying the inoculum size. Differentiation beyond the stage represented by the mononucleated myoblast occurs in culture after rapid cell multiplication has ceased. This is consistent with findings from several laboratories, using such diverse techniques as microspectrophotometry, inhibition of DNA synthesis, microcinephotography, and radioautography, that the myotube nuclei are postmitotic and the mechanism of their formation is cellular fusion. The third phase of muscle differentiation in culture, whose initiation is difficult to time exactly, is characterized by the progressive development of the cross-striated myofibrillar pattern and the initiation of spontaneous contraction (pl. 2, fig. 16).

All Konigsberg's studies before the past year had been restricted to monolayer cultures established with inocula of 2.5×10^6 to 10^6 cells each. Such cultures reach confluency between the second and fourth day of culture, depending on the size of the inoculum. To probe for the lower limit of inoculum size that would

still permit differentiation to occur, Konigsberg turned to the single-cell plating technique of Puck and his associates. In this procedure small numbers of cells are dispersed over a relatively large area. During appropriate periods of incubation the individual cells give rise to macroscopically visible, discrete colonies. The technique has been applied most successfully to permanently established cell strains. Using freshly isolated embryonic muscle cells Konigsberg observes a plating efficiency (approximately 10 per cent) considerably better than that reported for freshly isolated cells of older animals. In plates cultivated for 10 to 13 days approximately 1 in 10 colonies exhibits the unmistakable indices of differentiation of skeletal muscle cells. The proportion of differentiated cells ranges from colonies containing several elongated myotubes in colonies of predominantly mononucleated cells to colonies in which virtually every nucleus is in syncytial association. Examination of the myotubes under polarized light or under bright-field illumination after staining with phosphotungstic acid hematoxylin reveals the presence of longitudinal fibrils which are frequently observed to exhibit the typical pattern of cross striation of mature skeletal muscle cells.

The sequence of events in plating cultures is entirely comparable, except for the temporal factor, to Konigsberg's previous observations on monolayer cultures. Initially, the widely dispersed cells proliferate without exhibiting any overt indications of differentiation by the criteria established. The colonies in cultures observed at the sixth day of incubation are completely devoid of myotubes which are present in cultures fixed at the tenth or thirteenth day of incubation. It is apparent that some cells, at least, can, through a sequence of rapid multiplications, produce a large number of progeny that retain the capacity for differentiation. The two major questions emerging from these observations, however, are:

1. What is the significance of the finding that only 1 in 10 colonies eventually differentiates, and, as a corollary, what are the origin and state of colonies that remain morphologically indifferent?
2. What is the stimulus initiating myotube formation?

The conclusion that 1 in 10 colony-forming centers is different from the others is difficult to escape. The difference may be related to the inherent uncertainty of the plating technique in absolutely ruling out cellular multiplicity of all colony-forming centers. Alternatively, it may reflect the relative ratio of myoblasts to fibroblasts that survive the steps of the culturing technique. Lastly, the difference may be indicative of the attainment of some property by the cells which may or may not be of the type we generally regard as developmentally significant. We must take cognizance of the fact, also, that the final expression by which we infer a preexisting difference may actually be the product of interaction between such a difference and the culture environment. We must bear in mind the possibility that alteration of the culture conditions might reduce or eliminate apparent differences. To extend this thought it would seem incautious in the extreme to invoke, at this time, some fundamental limitation of the cell itself to explain the observations made on cells in vitro. Despite the fact that many of the operations judged to be "impossible" a short time ago are performed with relative ease today, tissue culture is still far from being a perfect tool for all purposes, particularly when applied to the study of cellular differentiation, where the component processes of the phenomenon are so imperfectly defined.

While various approaches to the first question raised are under investigation Konigsberg has chosen to attack the second problem by examining the relation of cell density to myotube formation. Such a correlation can be made in monolayer cultures, where the attainment of confluency and myotube formation are

coincident. In colony formation, also, the cell density seems to increase as a function of the number of cells per colony, the intercellular distances diminishing. In this situation, however, the relation is even more problematical.

Two general mechanisms by which cell density might affect myotube formation have been considered. Since myotube formation is a result of cell fusion, high cell density might ensure that a sufficient number of effective cell-to-cell collisions occur. The possibility seemed equally likely that a high cell density might be either supplementing the medium with cell products or removing some components. Preliminary tests were run by culturing two coverslips seeded with different numbers of cells in a single petri plate bathed with the same medium. The medium was circulated by incubating the petri plates on a slowly rotating tilted turntable. It was found that myotube formation was initiated on the sparsely seeded coverslip, despite the lack of confluency, at the same time the process was initiated in the denser culture and before it occurred in petri plates carrying two sparsely seeded coverslips. The obvious next step consisted in feeding sparsely seeded cultures with medium withdrawn from cultures that had grown to confluency. For these tests the rotating turntable was also employed, to minimize any possible effects of the test cells themselves during the test period. In cultures grown in the preconditioned medium, myotube formation is initiated as much as 24 hours earlier than in cultures initiated with equal numbers of cells from the same cell suspension but cultured in fresh medium. Furthermore, the cells in conditioned medium attach to the glass more firmly, presenting a strikingly different appearance from the control cultures.

It is apparent that the medium has been altered in some way by the metabolic activity of the cells cultured in it, since merely incubating the medium for an equal period of time is without effect. Whatever changes have occurred still exert their effects after filtration through bacteriological filters (Selas, Millipore) or after storage for at least 2 to 3 weeks. Obviously any number of alterations may have occurred, depletions as well as additions. To discriminate between changes in the macromolecular constituents as opposed to alterations in the small-molecular-weight components the latter were replaced by dialyzing conditioned medium against three changes of 4 volumes each of freshly prepared medium over a period of 3 days. Using monolayer cultures on a rotating turntable conditioned medium dialyzed against fresh medium produced results similar to those observed with untreated conditioned medium (pl. 3, figs. 17, 18).

The limitations of tests conducted on mass cultures, particularly in the present application, became apparent. One drawback, only partly compensated for by continuous rotation of the test cultures, is that the changes for which Konigsberg is testing are continuously produced by the test cells themselves. Another is the difficulty encountered in quantitating the response. To circumvent these objectionable features he turned to a plating system.

Using inocula of 50–400 cells he has found that the conditioned media frequently give plating efficiencies much higher than those obtained with fresh medium. Moreover, colony size increases at a strikingly more rapid rate: colonies grown in conditioned medium for 6 days attain a size normally observed in colonies cultivated for 13 days in fresh medium. This increased growth rate was not apparent by inspection in the monolayer test series. Reexamination of the effectiveness of "dialyzed" conditioned medium using plating cultures reveals that, actually, low-molecular-weight as well as macromolecular factors are involved that could not be readily demonstrated in the monolayer test. Colonies grown in "dialyzed" conditioned medium are noticeably smaller than those grown

in untreated conditioned medium, and they exhibit a reduced plating efficiency. They are, however, still vastly different in both respects from colonies grown in fresh medium.

The effects of conditioned medium on differentiation in plating cultures are difficult to interpret. Precocious formation of myotubes also occurs in plating cultures. On day 6, numbers of small colonies composed almost entirely of syncytial elements are observed; myotube formation has never been observed at this time in colonies cultivated in fresh medium. Their frequency is not strikingly different from the frequency of differentiated colonies in fresh medium. Variable results are observed, however, in plates fixed at 13 days. Muscle colonies similar in size to those observed on day 6 are frequently observed. (Precocious myotube formation in colonies consisting of small numbers of cells, if it involves all or most of the cells in the colony, might not increase in size simply because all the proliferative "stem cells" had been recruited into postmitotic syncytia.) Occasionally, these small colonies are either reduced in number or absent at day 13, but, with some lots of conditioned medium, in plating cultures fixed at 13 days muscle colonies fully as large as or larger than those in cultures grown in fresh medium are observed. The frequency of differentiated colonies in such cultures may actually exceed the usual frequency encountered with fresh medium. Konigsberg is now investigating the source of the variability in supporting differentiation among various lots of conditioned medium, his working hypothesis being that with his present conditioning system he is operating close to some threshold condition.

The examination of the second question raised by the plating experiments, incomplete as it is, leads us back to the first one, namely: what is the significance of the finding that only 1 in 10 colonies eventually differentiates? The studies involving conditioned medium suggest a possibility of reconciling the findings on monolayer culturing with the former view that differentiation is promoted by organ culture in which the organization of the tissue is left undisturbed. The necessary condition may in reality be a cell density high enough to correct for the deficiencies of the tissue culture environment. The 1 in 10 colonies that differentiates may represent a population of variants able to express their developmental potential despite the inadequacies of the environment, just as the plating efficiency itself may represent an overlapping class of variants. If we may consider the possibility that a cell type like the myoblast may actually represent a population homogeneous with respect to certain metabolic properties but heterogeneous with respect to others, the effect of conditioned medium on plating efficiency might be interpreted as providing an environment satisfying the requirements for survival and multiplication of a broader spectrum of the myoblast group. An environment adequate for survival and multiplication, however, may not satisfy all the requirements for differentiation. The differences between organ culture, monolayer culture, and plating culture may represent the different degrees to which conditioning or cross feeding may occur.

Regional Localization of Pre-Pacemaker Cells in the Chick Embryo

Previous year books have recorded progress in Robert L. DeHaan's analysis of the formation of the heart in the early chick embryo. Approaches to two sets of questions have been considered: the spatial organization and morphogenetic movements of the precardiac mesoderm; and the formation and early function of the cardiac pacemaker tissue. In the past year these separate lines of investigation have begun to meet in the question of regional localization of pre-pacemaker cells.

It will be recalled that the regions of precardiac mesoderm can be visualized on time-lapse motion-picture films as dark condensed areas silhouetted through the endoderm. The preheart mesoderm is composed primarily of discrete clusters of cells, which migrate with the folding foregut endoderm, using that layer as a substratum for their own independent movements. The migration of these clusters was tracked on such films from their initial positions in the heart-forming region of the embryo at stage 5 into the primitive tubular heart. For the first few hours after these clusters condense out of the background mesenchyme as discrete structures, they appear to migrate in a random fashion within the heart-forming regions. Gradually, the mesoderm becomes arranged into a spatially organized crescentic pattern. The cells destined to form noncardiac structures, like extraembryonic vascular tissue or head mesenchyme, leave the heart-forming regions while each cluster remaining within them takes up a position bearing a definite and constant relation to the part of the heart to which that cluster will contribute. The group of clusters in the anteriormost part of the lateral heart-forming region, for example, migrates into the rostral part of the heart rudiments that develop first, and ultimately forms conus and conoventricular tissue. The clusters in the middle of each cardiac primordium form the belly of the ventricle of the tubular heart; the most posterior clusters in the heart-forming regions enter the heart rudiments last, to form atrial and sinoatrial tissue. Thus, in addition to the prospective heart cells being differentiated, as such, from other mesoderm, at these early stages, the various parts of the heart are also represented as localized parts of the heart-forming regions.

This localization implies more than just a differential spatial distribution. It suggests that cells in each of the prospective regions show distinct differentiative capacities, since, in the later embryonic and adult heart, cells in the conus are quite different, histologically and biochemically, from, say, those that form the posterior wall of the definitive atria. Moreover, it has long been known that the several regions differ in their intrinsic pulsation rate. Alexander Barry has shown that there is a continuous anteroposterior gradient of inherent rhythmicity in the chick heart, so that any fragment of myocardium beats more slowly than those posterior to it, and more rapidly than those anterior, along the axis of the heart. These differences in rate are a function of individual cells within the various regions: thus, after disaggregation with trypsin, isolated cells of the atria beat faster than those from the ventricle.

As DeHaan has argued, the "spontaneous" beat of cardiac tissue depends on stimulation of myocardial cells by the specialized pacemaker cells of the conductive tissue. Therefore, the question arises whether, in the early heart-forming regions, pre-pacemaker cells are already spatially organized in a localized fashion with the rest of the preheart mesoderm and in accordance with the rate gradient seen in the beating heart. Specifically, are there, in the anterior parts of the heart-forming regions, destined to contribute to the distal branches of the Purkinje system in the conoventricular region, pre-pacemaker cells which exhibit a low level of rhythmicity? In contrast, are there, in the posterior parts of the heart primordia, already localized with the prospective atrial and sinoatrial mesoderm, pre-pacemaker cells capable of developing high levels of inherent rhythmicity suitable for the sinoatrial node and atrial conduction tissue? The experiments reported here were designed to answer these questions by separating the heart-forming regions into anterior, middle, and posterior parts and allowing each to develop in isolation.

For these experiments chick embryos were explanted ventral side up, each on its own vitelline membrane. With this technique development progresses nor-

mally for as long as 2½–3 days. For microsurgery, embryos were allowed to develop in culture to the desired stage and were cut into fragments, as diagrammed in figure 19. Fragments 1R and 1L were calculated to include material that would form conus and conoventricular tissue; 2R and 2L included prospective ventricle, and possibly some atrioventricular tissue; 3R and 3L contained the shaped masses of tissue. At the end of 48 hours of incubation, a substantial increase in size is seen, and each culture takes on an appearance characteristic of the original position of the fragment and age of the donor. Figure 20, plate 4, shows three explants after 48 hours of culture, as whole mounts and in cross section. The posterior fragments exhibit distinctly better development than the middle or

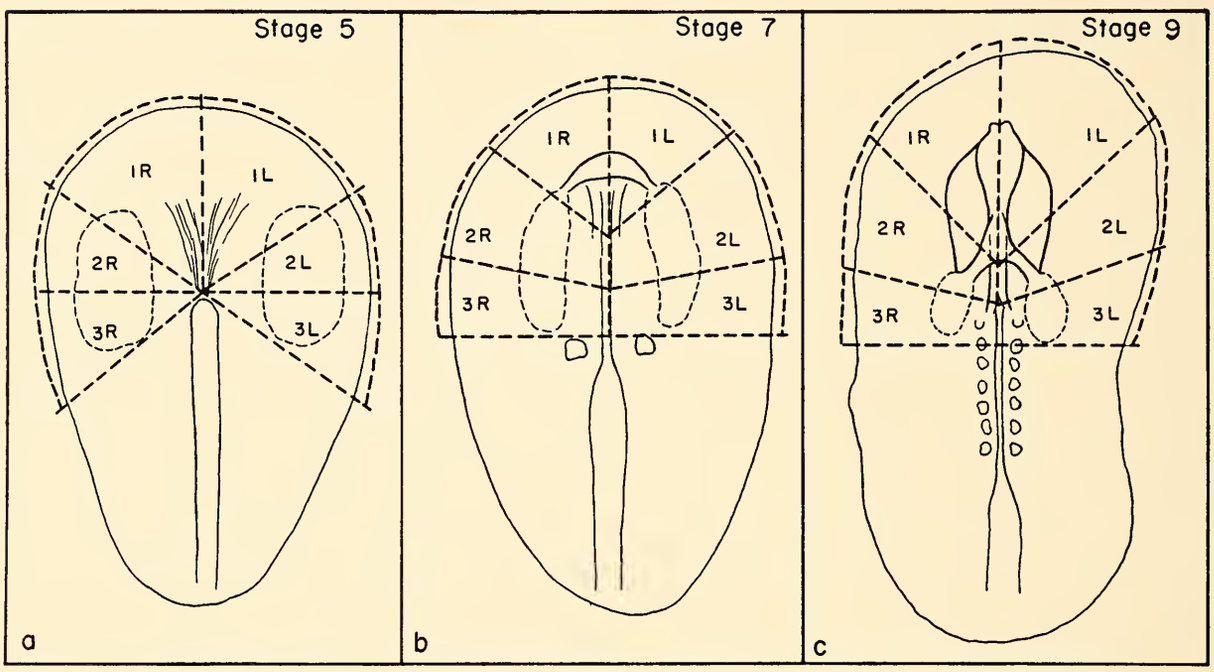

Fig. 19. Diagrams of chick embryos at stages 5, 7, and 9. The heavy broken lines represent cuts made to separate the prospective heart regions into anterior, middle, and posterior fragments. At all stages, fragments 1R and 1L contained prospective conoventricular mesoderm, 2R and 2L included preventricular cells, and 3R and 3L had sinus and atrium. The fragments included all three germ layers.

posterior clusters, destined to form atrial and sinus tissue. Stages 4, 6, and 8 (not shown in fig. 19) were cut in the same fashion. Each fragment was incubated in tissue culture medium for 48 hours, after which it was examined for spontaneous pulsatile activity, and the rate of beating was counted with the aid of a stopwatch. In all, 178 embryos (20–30 at each stage) were operated upon, yielding a total of 1068 cultured fragments, which provided the data for the present study.

After 8–10 hours of incubation in culture medium, explanted fragments tend to round up into solid, irregularly anterior ones, cardiac tissue appearing histologically well differentiated in all.

Figure 21 summarizes the heart-forming potencies of the cultured fragments, in terms of the fraction of the cultures that developed spontaneously beating heart tissue, as a function of the stage of the embryonic donor. The anterior fragments (1R, 1L), destined to form conus and conoventricular tissue, contain very few preheart cells before stage 8, and even at stages 8 and 9 only about half the cultures are capable of forming contractile heart tissue. The middle fragments (2R, 2L) also exhibit very little

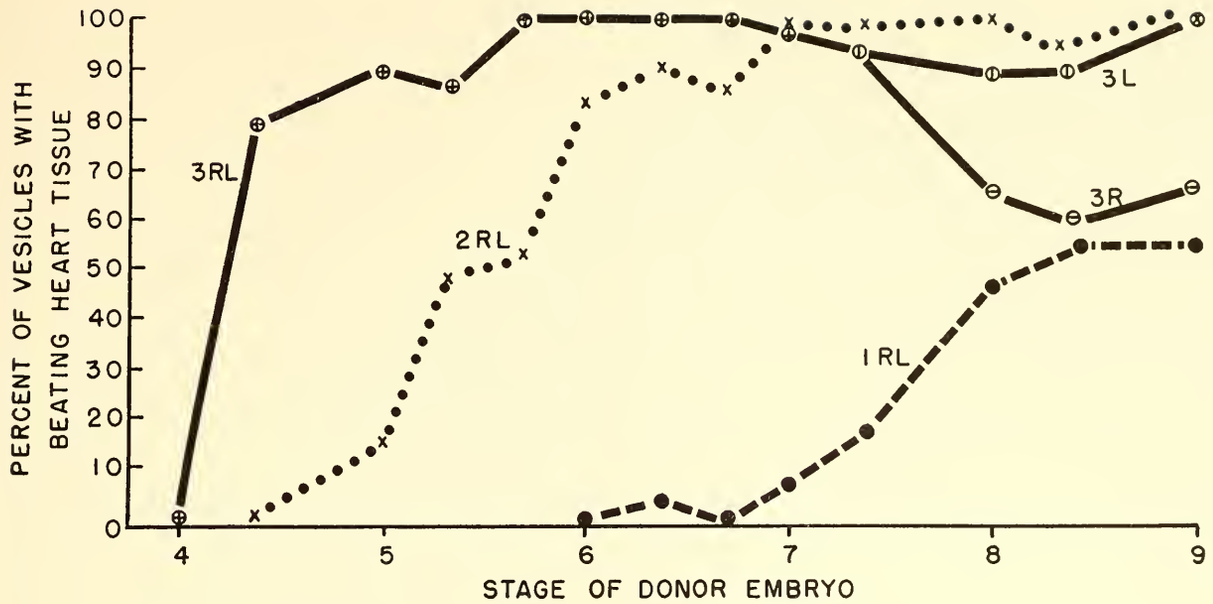

Fig. 21. Proportion of vesicles with beating heart tissue as a function of age of embryonic donor.

pacemaker activity at early stages. By stage 6, however, more than 80 per cent of the cultures of these fragments form beating hearts, and by stage 7 all of them do. The posterior fragments (3R, 3L), containing prospective atrial and sinoatrial cells, are the first to gain the capacity to form pacemaker tissue. At the first sign of notochordal cells pushing out in front of Hensen's node (stage 4+), 80 per cent of these posterior fragments can develop beating heart masses; and this fraction very quickly increases to 90–100 per cent.

Not only are the anterior fragments incapable of forming beating heart tissue until stage 7–8, the vesicles that can beat, as indicated in figure 22 (curve 1 RL), do so, at the low rate of 40–50 beats per minute. The middle fragments (2R, 2L), from the beginning, contain cells with a higher intrinsic rhythmicity, producing vesicles which by stage 7 beat at a rate of 60–85 per minute. The posterior fragments, in accordance with their content of prospective atrial and sinoatrial cells, produce heart vesicles even from very early stages with high rates of spontaneous contraction, which, like those from more anterior fragments, gradually increase in rate with age of the donor, leveling off at stages 7 to 9 at 120–130 beats per minute.

The well defined anteroposterior rate gradient of cells in the heart-forming regions, demonstrated in figure 22, agrees with earlier reports of a similar gradient of inherent rhythmicity in the formed heart after it begins beating. This finding, however, should not be interpreted as indicating, necessarily, a distribution within the heart-forming regions at these early stages of pre-pacemaker cells, already determined to be appropriate for conus or ventricle, atrium or sinus, although this is one possibility. An equally plausible hypothesis is that the heart-forming mesoderm in early stages, like the primordia of brain, limb, or eye, represents an equipotential system in which all parts of the primordial material are competent to form any part of the adult organ. Localized differences in developmental potential would arise as the result of inductive or other influences from the environment. The regional differentiation of a specific group of precardiac mesoderm cells would be a function of the milieu provided by the particular region of endoderm and/or ectoderm with which those cells happened to come in contact. Anterior fragments

1R, 1L would produce slowly beating vesicles, according to this idea, because the endoderm overlying the anterior heart-forming regions induces precardiac cells in contact with it to become pacemaker tissues with low levels of inherent rhythmicity, whereas fragments 3R or 3L develop a sinuslike, rapid beat, in similar fashion, as a result of influences on the mesoderm from the surrounding tissues. Intrinsic activity, therefore, would reside, not in the precardiac mesoderm itself, but in a set of reciprocal interactions between mesoderm and endoderm. Such inductive relations between preheart mesoderm and both endoderm and ectoderm are well documented for other species.

A critical test to distinguish between these two ideas is easily conceived: namely, the recombination of specific areas of endoderm with mesoderm from different parts of the heart-forming regions or from neutral (i.e., nonprecardiac) sites. Such a test awaits the development of practical techniques for recombining tissues from these early stages.

The ability to localize pre-pacemaker cells in specific regions of the early embryo, before they become functional, suggests the possibility of studying the electrophysiology of such cells during their differentiation. Recording with intracellular electrodes during the transition of a cell from a state of quiescence to one of spontaneous rhythmic activity might provide new insight into the mechanism of action of pacemaker tissues in general.

The Cultivation in Fluid Medium of the Labile Chorioallantoic Membrane

In recent years, Ebert and his coworkers, notably DeLanney and Mun, have paid increasing attention to the labile chorioallantoic membrane. In pre-

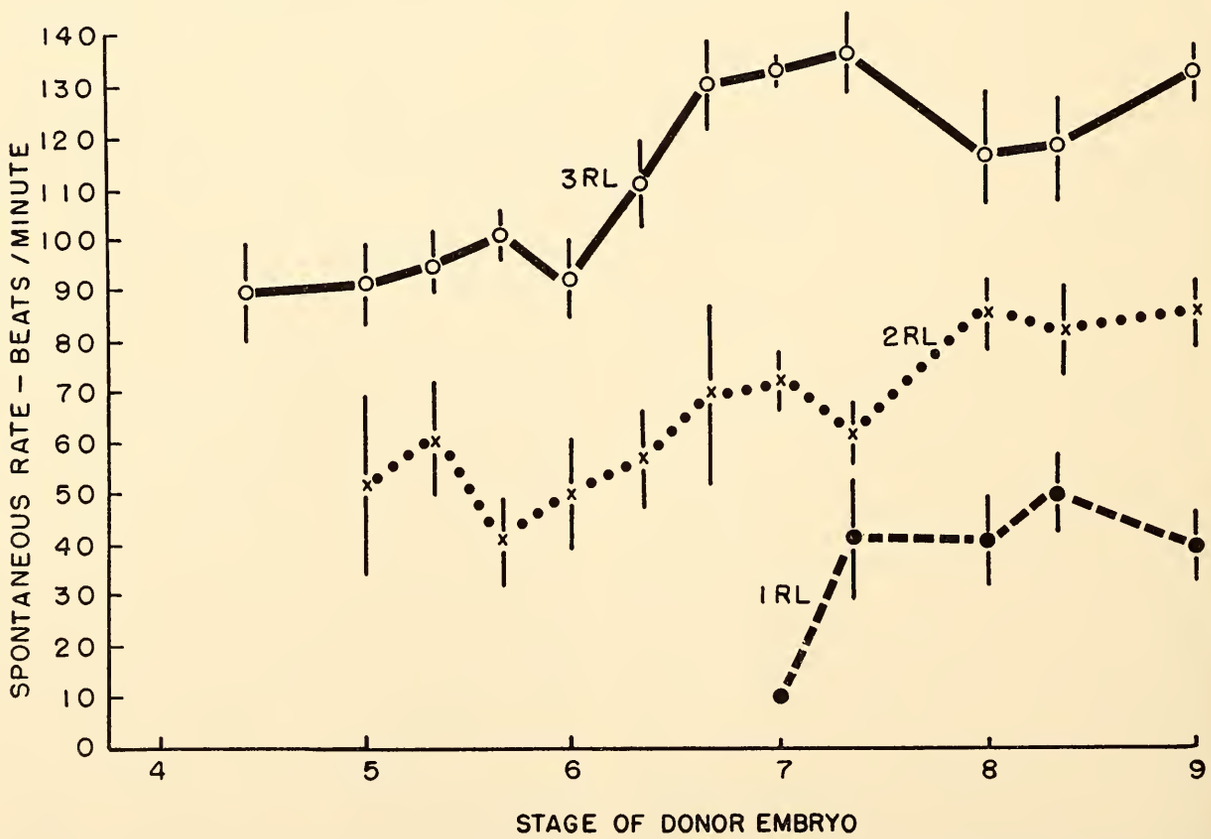

Fig. 22. Spontaneous pulsation rate of the beating heart vesicles as a function of the age of embryonic donor. The vertical bars at each point represent standard error.

vious reports and several other publications evidence has been advanced that the characteristics of the membrane can be altered by a variety of specific and nonspecific stimuli. For example, cardiac muscle is formed in the chorioallantoic mesenchyme after inoculation of a mixture of cardiac microsomes and Rous sarcoma virus. Less convincing but nevertheless suggestive evidence is available for the differentiation of granulocytes in the endodermal layer. And, finally, a graft of spleen and to a lesser extent other tissues, or the inoculation of suspensions of spleen cells, results in the appearance in the membrane of a variety of responding cells, including giant cells, which wall off the homologous cells in cystic masses. Thus, contrary to Burnet's earlier view, such lesions on the chorioallantois are, to a large degree, derived from the host. But whether they migrate in from the embryo, or are mobilized in the membrane in situ, or both, is not yet clear. In an article to appear shortly in *Biological Bulletin*, Mun, Tardent, Errico, Ebert, DeLanney, and T. S. Argyris offer evidence derived from tritium thymidine labeling studies which demonstrates some invasion of splenic grafts by cells of the host.

It became evident that to continue these lines of research effectively it would be necessary to develop a technique for the cultivation of fragments of the chorioallantois in vitro. Such an approach should permit the controlled modification of the membrane on a larger scale than had previously been possible as well as the resolution of the question of the origin of cells reaching homologous grafts.

Moscona has reported that, when small fragments of the chorioallantoic membrane of 8-day chick embryos are cultured on a plasma clot by Fell's watch glass technique, the chorionic epithelium stretched on small squares of rayon acetate net show a keratogenic metaplasia. Earlier, Delson described a method for the cultivation of the membrane supported by filter paper without such metaplasia.

During the past year Chinami Takata and Nancy Sype, in consultation with J. D. Ebert, initiated a study of several techniques for cultivating the membrane. The following preliminary findings may be reported briefly.

In the course of the study to date, several hundred large fragments of the chorioallantoic membrane were taken from 8-day chick embryos. Each fragment, supported on filter paper, was placed on the surface of the medium to be tested in an embryological watch glass and was cultured in a humid chamber for 5 to 7 days at 37°C. The explants were transferred to a fresh medium every second day.

The filter paper (no. 5243–C, Arthur H. Thomas Co.) for supporting the membrane was cut into rectangles about 2.3 cm by 2.0 cm and cut out, leaving a frame about 3 mm wide. The frames were treated with 1 per cent hydrochloric acid for 1 hour, washed in three changes of distilled water, extracted a second time for 1 hour in 95 per cent alcohol, rinsed in several changes of glass-distilled water, dried in an oven (80–90°C), and sterilized by autoclaving. The experimental media were made up from sterile stock solutions in the proportions given in Table 13. Hanks' balanced salt solution was buffered with tris or bicarbonate at *p*H 7.5–7.8.

After cultivation the explants were fixed in Bouin's or Zenker's fluid, stained with Weigert's iron hematoxylin and eosin, and examined as whole mounts. Some explants were sectioned at 5, 6, or 8 microns and stained with Weigert's iron hematoxylin and eosin or Biebrich's scarlet for microscopic observation.

The criteria for determining effectiveness of each medium were degree of maintenance of mesenchyme, ectodermal and endodermal layers, blood cells, and proliferation of cells.

Experimental group A. Series 1, 2, and 3. The fragments of membrane were cultured in media containing horse or calf serum, Hanks' balanced salt solution, and

TABLE 13. Maintenance of Mesenchyme Found in Fragments of Chorioallantois Cultured in Experimental Media

Experimental Group	Experimental Series	Medium	No. Explants Studied	No. in Which Mesenchyme Was Maintained
A	1	10% HS + 75% H + 15% EE	11	8
	2	20% HS + 65% H + 15% EE	46	38
	3	20% CS + 65% H + 15% EE	21	17
B	4	20% HS + 80% H	73	65
	5	40% HS + 60% H	27	19
C	6	10% HS + 50% H + 40% P	39	32
	7	10% CS + 50% H + 40% P	20	18
	8	20% HS + 65% H − 15% P	12	9
	9	20% HS + 40% H + 40% P	27	19

HS = horse serum.
CS = calf serum.
H = Hanks' balanced salt solution containing 3 grams of glucose per liter.
P = Puck's medium N–16, synthetic medium without protein.
EE = 9-day chick embryo extract.

embryo extract. In series 1, most of the blood cells in the blood vessels showed necrosis. The endodermal layer and mesenchyme in this group were kept in healthy condition during the cultivation (pl. 5, figs. 23 and 24). On the other hand, the ectodermal layer changed into a one-cell layer without showing keratinization.

Experimental group B. Series 4 and 5. When the explants were cultured in media containing only horse serum and Hanks' balanced salt solution, the endodermal layer, mesenchyme, and the ectodermal epithelium which consists of a single layer were maintained in good state (pl. 5, fig. 25). Necrosis of blood cells, however, was observed in series 5 containing 40 per cent horse serum.

Experimental group C. Series 6, 7, 8, and 9. In this group, Puck's medium N–16 was used instead of embryo extract. Maintenance of the explants was favorable (pl. 5, fig. 26), and mitotic figures were often observed. Keratogenic metaplasia was not observed in a flattened cell layer of ectodermal epithelium. In series 7, the blood cells were maintained in rather good condition, although the media of series 8 and 9 entirely failed to maintain the blood cells in the blood vessels.

In summary, although the ectodermal layer of the membrane changed to a flattened cell layer, keratogenic metaplasia was not observed; all media tested were effective for maintenance of mesenchyme and endodermal layer.

INDUCTIVE TISSUE INTERACTIONS

Interactions between Dermis and Epidermis from Prospective Feather and Scale Regions after Recombination on the Chorioallantoic Membrane

In a series of experiments with chorioallantoic grafts reported in *Year Book 60,* pages 424–427, in which exchanges were made between epidermis and dermis from prospective feathered and scaled regions of chick embryos, M. E. Rawles observed that epidermis from the foot (tarsometatarsus) failed to self-differentiate when underlain by dermis from the middorsum. In this combination, epidermal differentiation proceeded in a feather direction rather than in a scale direction. Thus

there appeared to be no specific time before or during the formation of the scale ridges when the capacity to form scales is irrevocably fixed or "determined" in the epidermis of the foot region. Differences in the responsiveness of the epidermis with increase in developmental age could be detected, but only by the degree of abnormality displayed by the feathers.

Inasmuch as the dermis in the above-mentioned combination always came from the prospective dorsal feather tract, a region that normally gives rise to feathers only, the question arises about what course the epidermal reaction would follow if underlain by mesoderm (dermis) isolated from the relatively featherless areas lying between the feather tracts—the *apteria*. Results obtained so far from combining epidermis of the foot (9- to 14-day embryos) with dermis from the apteria (6- to 9-day embryos) have, interestingly enough, shown the same type of epidermal reaction obtained previously when the dermis was of feather-tract origin. Feathers, normal and abnormal, depending on the age of the epidermis involved, appeared on the graft surface. Scales could not be recognized.

The fact that the apteria are not always entirely featherless in the normal chick indicates that the skin of these areas is potentially capable of giving rise to down feathers. In other words, the feather-forming potency is not restricted entirely to the feather tracts.

Of the few completely featherless areas of the body of a chick, the beak region seemed to be most suitable for Rawles's purpose. Experiments are now in progress in which exchanges are being made between epidermis and dermis of foot and beak regions. Results obtained, so far, from combinations of epidermis from the foot of 11- to 13-day embryos with dermis from the prospective beak of 5- to 7-day embryos have shown, without exception, beak formation, normal in form and structure.

Results of the various combination experiments mentioned above have given striking confirmation to the principle, heretofore well established for the chick by other workers, that in the formation of epidermal derivations the specificity resides in the mesoderm. Of further interest, however, is the observed fact that epidermis of the foot region at a relatively late developmental stage (13–14 days) after the formation of scales is still labile enough to change its direction of differentiation either into a feather or even more remarkably into a highly keratinized smooth beak.

Lens Induction

Another classic example of induction is found in the interaction of optic vesicle and ectoderm in the formation of the lens. It has long been thought that the formation of the lens offers a favorable target for the analysis of induction at the biochemical level. It was for that reason, in fact, that the goal of one of the more rewarding programs in the Department in recent years, that conducted by John Papaconstantinou (*Year Books 58*, pp. 379–385, and *59*, pp. 378–380) was to describe the normal sequence of protein synthesis in the lens as a basis for combining techniques of biochemistry and experimental morphology.

During the year P. H. S. Silver has begun such a study, partly in collaboration with Papaconstantinou, now at the University of Connecticut, the long-range goal of which is to determine, by biochemical and immunochemical methods, the nature of the proteins formed in interactions of chick and duck embryonic tissues. The analysis has not advanced sufficiently to warrant extensive discussion, but the following brief remarks are appropriate. None of the methods for homoplastic lens induction, described previously in the literature, has proved satisfactory, as the resulting lenses are too small or misshapen for biochemical or immunological study. For this reason an extensive survey has been carried out to find the most suitable experimental

technique. Several different methods, in vitro and in ovo, have been tried. The combination of the primary vesicle with competent ectoderm in ovo has been abandoned, for Silver's material shows that, despite a recent statement to the contrary by McKeehan, the retina of both chick and duck has the capacity itself to produce well formed lens tissue, and so, if the initial experiment is carried out in ovo, and a lens results, there will always be doubt about whether this lens has originated by induction from the covering ectoderm or by regeneration from the retina.

A definitive technique has now been arrived at in which the prospective lens epithelium of the host embryo is replaced by the corresponding tissue of the donor. To do this, the embryo with its blastoderm is explanted in vitro. The blastoderm is folded so that the embryo lies on its side. The graft is fixed by adjusting the level of the culture fluid so that the surface tension presses the graft in place. Once the graft has stuck (about 1½ hours) the blastoderm is unfolded and cultured in the normal way. The thickening and invagination of the graft epithelium can be observed in the early stages of lens formation, and the origin of the lens from the surface ectoderm can be established. The whole eye is then transplanted to the head of another embryo and left for 16 to 17 days. There is no doubt that the head mesenchyme is the best milieu for the growth of eye tissue.

HUMORAL REGULATORY MECHANISMS

Growth Promotion during Regeneration and the Control of Growth

The mechanisms controlling growth and differentiation in adult mammalian tissues are even less understood than those involved in controlling growth and differentiation in the embryo. All students of growth control in adult organs agree that that is, at least in part, a function of intercellular chemical communication. The nature of the chemical communication is unknown. Regeneration is an obvious method for forcing organs to reveal some of their potential for controlling growth. Therefore, it is not surprising that the mechanisms involved in controlling growth during wound healing and compensatory hypertrophy have been intensively studied.

T. S. Argyris has been studying two situations in which damage leads to the stimulation of growth: the stimulation of growth of resting hair follicles surrounding a wound, and the stimulation of cell division in the kidney cortex following damage by insertion of a needle into the contralateral kidney. It is presumed that the stimulation of growth in each situation is due to a diffusible growth-promoting agent from the damaged tissue. Identification of the growth-promoting agents involved is a necessary prerequisite to understanding the mechanisms by which growth is controlled. The aim of Argyris's research program during the past 10 months has been primarily to become acquainted with, and develop, a number of biochemical techniques with which he may try to identify these growth-promoting agents. Specifically, two biochemical tools have been explored: the isolation of cell particulates, and autoradiography. The rationale for the development of these two techniques seems obvious and will not be presented fully. By means of cell particulate isolation he will attempt to determine what cell fraction or fractions are responsible for the observed growth-promoting effects. Once this is accomplished efforts will be made to isolate and identify the specific growth-promoting agent(s) in the active fraction, thus achieving localization as well as identification of the growth-promoting agent(s). By labeling the damaged tissues with an appropriate

isotope and making autoradiograms one may hope to follow the movement of the growth-promoting substances from the damaged tissues to the target tissues.

Argyris further plans to explore the use of these techniques in studying a third growth-promoting interaction, the stimulation of the overlying epidermis by subcutaneous transplants of tumors. Argyris and Argyris have found previously that the subcutaneous inoculation of Ehrlich ascites tumor, sarcoma 180, or adenocarcinoma 755 results in invasion of the skin and the stimulation, at a distance of at least 100 microns, of the overlying epidermis. Contrarily, the resting hair follicles closer to the tumor are never stimulated.

Organ Homogenates and Growth Promotion

Preliminary experiments homogenizing liver, kidney, skin, skin wounds, and the solid form of the Ehrlich ascites tumor (EAT) in a Servall omnimixer or in a number of different kinds of glass homogenizers driven by motor or by hand led to the conclusion that the use of the Ten Broek glass hand homogenizer was the best method, especially for the small amount of material available for homogenization. In all the studies to be described the degree of homogenization was checked by smearing an aliquot of the homogenate on a glass slide, fixing it in 100 per cent methanol for 1 to 2 minutes, and staining with hematoxylin for 2 to 5 minutes. All tissues were homogenized in 0.25 *M* buffered sucrose, *p*H 7.3. Buffer contained 10^{-2} tris and 10^{-3} magnesium chloride. Homogenization was done in an ice bucket, and sucrose homogenizers and other utensils were always precooled before use.

Since the fraction of the homogenate that may contain the active growth-promoting principle cannot be predicted it was necessary initially to inject the whole sucrose homogenate and to begin injecting isolated cell fractions only when the whole homogenate showed activity—an approach containing an element of uncertainty inasmuch as whole homogenates may contain substances that inhibit or inactivate the growth-promoting substance(s).

Effect of subcutaneously injected homogenates of Ehrlich ascites tumor (EAT) on the skin of mice. The EAT was secured from a stock carried by Argyris and Argyris at Syracuse University for the last 3 years. The EAT grows as a fluid tumor in the peritoneal cavity of mice. It is transplanted every 8 to 10 days by removing the tumor with a no. 23 needle mounted on a tuberculin syringe and injecting 0.1 ml of the white fluid material intraperitoneally into 2 female mice. An additional sample of the tumor is diluted with a saline solution of 0.2 per cent Nigrosin, and cell counts are made in a hemacytometer. All cells staining black because of Nigrosin uptake are excluded from the count, presumably being damaged or dead.

Since it is not uncommon to find the loss of stimulating activity of one line of tumor in a newly established subline, 0.1 ml of the EAT was injected subcutaneously into 5 mice with skin in the resting phase to see whether it would invade the skin and stimulate the overlying epidermis. Mice were killed 21 days after tumor inoculation—ample time for the tumor to invade the skin and stimulate the epidermis. Six hours before being killed the mice were injected subcutaneously with 0.01 ml of colchicine (10 mg/25 ml saline) per gram body weight. Biopsy specimens of the skin and tumor were fixed in cold 10 per cent formalin, dehydrated in dioxane, embedded in paraffin, and sectioned at 5 microns. Slides were routinely stained with hematoxylin and eosin. All the sections showed good tumor growth, invasion of the skin, and stimulation of the overlying epidermis, the last being evidenced by thickening of the epidermis due not only to an increase in cell number but also to cell enlargement.

Effect of subcutaneously injected homog-

enates of EAT on the skin of mice. Tumor material for homogenization was provided by injecting 8 to 10 mice subcutaneously with 0.1 ml of EAT. After 7 days the animals were killed, and the solid nodules of tumor were removed, quickly weighed, and minced with scissors. Instead of adding the 9 ml of sucrose per gram of tissue usually recommended, which was found to be effective for normal organs, about 3 ml per gram of tumor material was added and homogenized in an ice bucket with a Ten Broek glass homogenizer. The use of less than expected amounts of sucrose is due to the fact that normal amounts produce homogenates that are too dilute and make breaking-up of tumor cells too difficult. This difficulty might have been anticipated, since the dilutions have been calculated per gram of living tissue. In a 7-day solid growing EAT only the exterior is living, and it is hardly more than a quarter to a half of the entire tumor mass. A drop or two of the homogenate was smeared on a glass slide and stained as described above to make certain that disruption of the tumor cells had occurred.

To ensure that the mice had skin in the resting phase, the entire dorsum of each mouse was plucked 21 days before inoculation. The plucking initiated hair growth in the plucked area only, and the hair follicles completed their growth and by 21 days came to rest again. They usually remained at rest for 4 to 8 weeks. It is necessary to have skin in the resting phase because the epidermis changes in thickness at different stages of the hair-growth cycle, making evaluation of stimulation by experimental factors difficult.

Eighteen female C57 mice were anesthetized with ether, and 0.1 ml of homogenate of EAT was injected subcutaneously. The injected area was marked with a drop of eosin. Nine mice were killed each at 2 and 9 days after injection of homogenate. Six hours previously the mice received colchicine as described above. Biopsy specimens of skin were fixed in cold 10 per cent formalin, routine histological procedures being followed.

Stimulation of the epidermis due either to cell enlargement or to cell division has not been observed. The nuclei of the homogenate persist and are embedded in a blue-staining homogeneous mass. The homogenate often results in the stimulation of the loose areolar connective tissue underneath the skin, a reaction also elicited by the living tumor cells.

Effect of intraperitoneal injections of kidney homogenates on the kidney. Material for kidney homogenate was obtained by killing a female C57 mouse and removing both kidneys. After being weighed, kidneys were quickly minced and placed in a Ten Broek homogenizer and homogenized in 0.25 *M* buffered sucrose as described above. Various amounts of homogenate or buffered sucrose were injected intraperitoneally into female mice; 48 hours later the mice were killed, and their left kidneys were removed and prepared for histological study as previously described. Six hours before being killed mice received 0.1 ml of colchicine (10 mg/25 ml saline) per gram body weight, subcutaneously. The choice of 48 hours as the interval between injection of homogenate and sacrifice was arbitrary, based on the suggestion of Swann that a mitotic "inductive" stimulus of the kind that might be produced by the injection of homogenate requires about 48 hours for its expression because of the time necessary for shifts in intracellular synthesis to occur leading to the building of mitotic protein. Mitotic counts were done on 2 nonadjacent sections per kidney, counting 10 fields per section, using a 44× objective and a 10× ocular. At this magnification there were about 1000 cells per field. Thus the mitotic counts represented the number of mitoses per 20,000 cells. The choice of this counting procedure was based on considerable previous experience indicating that such counts are sufficient to establish obvious differences. As a further

check, a number of kidneys were recounted, counting 10 nonadjacent sections, 10 fields per section, or a total of about 100,000 cells. Mitotic counts were proportionately the same.

Table 14 presents the mitotic counts and shows that the intraperitoneal injection of 0.2 ml, 0.1 ml, or 0.05 ml of kidney homogenate does not increase or decrease the mitotic counts of the kidney cortex as compared with the injection of the same amount of buffered sucrose. Moreover, these counts are the same as he

fore, homogenates were prepared from kidneys at 5 minutes and at 5.5, 24, or 48 hours after damage. Kidney damage was produced in anesthetized female mice by exteriorizing both kidneys and puncturing each kidney 4 times with a sterile no. 23 needle mounted on a tuberculin syringe. Two-tenths milliliter of the homogenate was injected intraperitoneally into each mouse, and the mice were killed 48 hours later, after the subcutaneous injection of colchicine as already described.

TABLE 14. Mitotic Activity of Mouse Kidney after Injection of Homogenates of Normal Mouse Kidney

Material Injected into Each of 6 Mice	No. Mitoses per 20,000 Kidney Cortical Cells	
	Average $\pm SE_m$	Range
0.2 ml kidney homogenate	0.67 ± 0.36	0–2
0.2 ml buffered sucrose	2.0 ± 0.94	0–5
0.1 ml kidney homogenate	0.33 ± 0.36	0–2
0.1 ml buffered sucrose	0	0
0.05 ml kidney homogenate	1.1 ± 0.59	0–3
0.05 ml buffered sucrose	1.0 ± 1.0	0–3

has previously obtained after the injection of saline or from kidneys of noninjected female mice.

The question arises whether the negative results may not be due to the fact that the homogenates are prepared from normal kidneys. But it should be recalled that the original observation in vivo is that damaged kidney results in the mitotic stimulation of the undamaged contralateral kidney. Perhaps if the homogenates were prepared from damaged kidneys kidney cell division might be stimulated.

Since in the original in vivo experiments the increase in mitotic activity of the contralateral kidney is observed 48 hours after kidney damage, it is not known whether the stimulating activity from the damaged kidney is released at once or some time after damage. There-

Table 15 shows that the intraperitoneal injection into a female mouse of 0.2 ml of kidney homogenate prepared from kidneys 5 minutes, 5.5 hours, 24 hours, or 48 hours after damage does not result in any significant increase or decrease of mitotic activity in the kidney.

The effect of homogenates of skin wounds on resting hair follicles. Here the basic premise is that homogenates of skin wounds should be able to induce the growth of resting hair follicles when injected subcutaneously, since experiments in vivo have suggested that the stimulation of hair growth is due at least in part to a diffusible substance from the wounds. Two kinds of stimulation of hair growth can be expected after subcutaneous injection of homogenates: (1) Local stimulation of growth of resting hair follicles directly over the area of

TABLE 15. Effect of 0.2 Milliliter of Damaged Kidney Homogenate on Mitotic Activity of Mouse Kidney

No. Mice	Time between Damage and Killing of Mice	No. Mitoses per 20,000 Kidney Cortical Cells	
		Average ± SE_m	Range
4	5 minutes	1.7 ± 0.97	0–3
8	5.5 hours	0.5 ± 0.28	0–2
6	24 hours	1.5 ± 0.24	1–2
6	48 hours	0.5 ± 0.24	0–1

homogenate injection. This would be due, presumably, to diffusion of the requisite growth-promoting substances into the skin from the underlying injected homogenate. (2) General stimulation of hair growth over the entire back of the mice, this stimulation presumably being due, according to Chase, to an effect of the homogenate on a systemically circulating inhibitor which normally helps to control hair growth. That a substance exists which circulates systemically and can affect hair growth is suggested by recent experiments of Ebling and Johnson. In these experiments, therefore, evidence of both kinds of stimulation was sought.

Wound homogenate was obtained by making three or four wounds in female C57/Black mice immediately after plucking their backs. Four days later the wounds and the surrounding skin were excised, placed in a precooled beaker, and thoroughly minced with scissors.

Minced wounds were then transferred to a homogenizer immersed in an ice bucket; proper amounts of sucrose were added; and the material was homogenized as described above. Since skin wounds were difficult to homogenize, care was exercised that enough of the smear was examined to be sure that the cells were broken up.

To be certain that the mice receiving the wound homogenate had resting hair follicles, their backs were plucked 21 days before injection. Large female mice were used because males tended to fight, and bites induce hair growth, but a few males were included in the final experiment for the sake of completeness.

For injection the mice were anesthetized with ether and the homogenate or buffered sucrose was injected subcutaneously by means of a no. 23 needle mounted in a tuberculin syringe. Before injection mice were clipped. Clipping does not stimulate hair growth, and it makes the detection of new hair growth, observed as a blue area on the skin surface, easier. Mice so treated were examined every day for a month or more for either a specific bluing over the injected site, indicating local hair growth stimulation, or bluing of the entire back, indicating a general stimulation of hair growth.

Of 49 female and 12 male mice so treated none showed any local stimulation over the injected site, with either 0.1, 0.2, or 0.5 ml of wound homogenate or sucrose (table 16).

Similarly, these animals showed no enhanced general stimulation over their entire backs (table 17).

Since it is known that hair follicles that have been resting for some time and are about to grow can be prematurely brought into the growth phase by a variety of stimuli (Chase), it was decided to repeat the experiments using mice whose hair follicles had been at rest for 18 days. Perhaps the ability of the homogenate to induce hair growth would then be enough. Sixteen female mice were injected as described above with 0.2 ml of homogenate 18 days after their hair follicles had come to rest. Again, neither

TABLE 16. Local Effect of Wound Homogenate on the Stimulation of Growth of Resting Hair Follicles in Mouse Skin

No. and Sex of Mice	Material Injected	Presence of Local Hair Growth Stimulation
7 F	0.1 ml wound homogenate	–
16 F	0.2 ml wound homogenate	–
6 F	0.3 ml wound homogenate	–
6 F	0.3 ml wound homogenate	–
8 F	0.2 ml buffered sucrose	–
8 F	0.5 ml buffered sucrose	–
6 M	0.2 ml wound homogenate	–
6 M	0.2 ml buffered sucrose	–

TABLE 17. General Effect of Wound Homogenate on the Stimulation of Growth of Resting Hair Follicles

No. and Sex of Mice	Material Injected	Time, in Days, of Appearance of General Hair Growth on Backs of Mice	
		Average ± SE_m	Range
7 F	0.1 ml wound homogenate	42.9	39–58
16 M	0.2 ml wound homogenate	30.7	16–45
6 F	0.3 ml wound homogenate	33.3	24–58
6 F	0.5 ml wound homogenate	43.3	37–52
6 F	0.2 ml buffered sucrose	22.5	13–30
6 F	0.5 ml buffered sucrose	41.1	28–52
6 M	0.2 ml wound homogenate	32.3	13–36
6 M	0.2 ml buffered sucrose	27.5	5–50

specific nor nonspecific stimulation of hair growth was seen.

The possibility exists that, although the amount of homogenate is sufficient to induce hair growth, not enough can diffuse through the skin muscle (panniculus carnosus) to stimulate hair growth locally. That skin muscle may act as a barrier to growth-promoting substances has been suggested by previous work in which the growth-promoting effect of tumors on the mammary gland and skin of mice has been studied. In these experiments it had been noticed that the presence of muscle between the target tissue and the tumor is always associated with an absence of stimulation of the target tissue. Therefore, the experiments were repeated using 22 female and 14 male C57/Black mice which had their skin muscle removed in a small area of skin by a special procedure developed by Argyris at Syracuse University. As before, these mice were plucked and their hair follicles were allowed to grow and reenter the resting phase. They were then subcutaneously injected with 0.2 ml of wound homogenate or buffered sucrose near the area of skin that lacked its muscle.

No stimulation of hair growth was ever observed with the dosage of homogenate used (table 18).

Since there is a measure of thoroughness in this study, it might appear that the negative results strongly suggest that wounds do not contain growth-promoting substances. This can hardly be true. We must keep in mind that the wound tissue used is that present 4 days after injury.

Perhaps the use of wounds from earlier or later stages would reveal growth-promoting substances. Moreover, the wound homogenates were prepared from whole wounds, which have a variety of tissue components, such as epithelium and granulation tissue, and the growth-promoting activity may be inhibited. It may well be that homogenates of each kind of tissue will have to be prepared and tested separately. To this end Argyris, sodium acetate-1-C^{14} and tritiated water being used.

Sodium acetate-1-C^{14} had a specific activity of 5.90 mc/mM. It was diluted to the desired concentration with sterile saline. Eight female C57/Black mice were injected intraperitoneally with 50 μc of the labeled acetate. Mice were killed at 1, 8, and 24 hours after the injection. Biopsy specimens were fixed in 10 per cent formalin, dehydrated in dioxane,

TABLE 18. Local Effect of Wound Homogenate on the Stimulation of Growth of Resting Hair Follicles in Mouse Skin in Which the Skin Muscle (panniculus carnosus) Had Been Removed

No. and Sex of Mice	Material Injected	Presence of Local Hair Growth Stimulation
11 F	0.2 ml wound homogenate	–
11 F	0.2 ml buffered sucrose	–
9 M	0.2 ml wound homogenate	–
9 M	0.2 ml buffered sucrose	–

under the guidance of Mary E. Rawles, has developed trypsinization procedures that permit him to separate the wound epithelium from the rest of the wound components.

Autoradiography of skin, wounds, and Ehrlich ascites tumor. As was stated above, Argyris hoped to develop techniques for labeling the damaged tissues and following the released growth-promoting substances to the target tissues. To accomplish this, some prerequisites have to be met in addition to learning the technique of autoradiography: (1) an isotope must be found which enters into as many metabolic pools as possible, thus labeling as many classes of substances as possible; (2) the distribution of the labeled material in the normal and damaged skin and kidney tissue, and in the EAT, must be determined.

These prerequisite studies have been carried out in a preliminary fashion for resting and growing skin, for wounds, and for subcutaneously inoculated Ehrlich ascites tumor, the labeled compounds embedded in paraffin, and sectioned at 4 or 5 microns. Sections were then rehydrated and dipped in Kodak NTB–2 emulsion according to Quastler. After drying, the coated slides were stored for 10 days, 60 days, and 6 months before being developed in DK–19, and fixed according to the technique of Quastler. The developed slides were then counterstained lightly with hematoxylin before being mounted.

Twelve C57/Black females were injected intraperitoneally with tritiated water of low specific activity. The isotope was diluted with saline, and mice were injected with 50, 100, 200, 1000, 2000, or 5000 μc each. The mice were killed 1, 5, and 8 hours after injection of the isotope. Biopsy specimens were taken, and autoradiograph sections were prepared from them as described above for sodium acetate-1-C^{14}.

Skin with resting hair follicles shows much radioactivity in the epidermis, hair follicles, and sebaceous glands within 1 hour after injection of the labeled acetate.

Seven days after initiation of hair growth the growing hair follicles show intense activity, as does the hyperplastic epidermis. The radioactivity in the dermis and subcutis is high in skin with either resting or growing hair follicles.

Four days after wounding, the epidermis is markedly enlarged as the result of both cellular hypertrophy and hyperplasia. The hair follicles lose their sebaceous glands and are converted into hyperplastic cords of cells. Concomitantly, the wound is filled with the hyperplastic epidermis, hair follicle cords, and granulation tissue.

The EAT, 7 days after subcutaneous inoculation, is composed of an outer rim of rapidly proliferating cells and a necrotic center. Surrounding the tumor is a tumor-induced hyperplastic connective tissue sheath. Except for the necrotic tumor mass, which exhibits little activity, all these areas show high radioactivity within 1 hour after injection of the labeled acetate.

In all the materials studied—normal skin, wounds, or tumor—the pattern and intensity of radioactivity are the same at 8 and 24 hours as at 1 hour.

The distribution of the labeled water at 1, 5, and 8 hours after injection is similar to that of the acetate. The activity, however, is not as intense, irrespective of dosage. Also, in contrast to the labeled acetate, which shows high activity within 1 hour after injection, the activity of tritiated water is not high until 8 hours. This gradual increase in activity from 1 to 8 hours after injection is evident in mice injected at all doses tested, ranging from 200 to 5000 microcuries.

Reproduction and Sex Differentiation in the Opossum

R. K. Burns's studies on young opossums in Florida consisted entirely in a continuation of the experiments of recent years on the effects of administration of steroid sex hormones on the early differentiation of the gonads, and in the study of the reproductive life of the opossum in a state of nature, a study essentially completed with accumulation of sufficient data on most points to permit general conclusions, which can be briefly summarized as follows:

1. In northern Florida breeding is sharply limited to a period of 2 to 3 weeks in late January and the first half of February, more than 80 per cent of all births occurring then. The first appearance of young is very constant from year to year and apparently has no relation to local climatological conditions, suggesting that in all probability length of daylight is the determining factor in the onset of breeding.

2. Young females may produce their first litters at an age of 6 to 7 months, and at a weight of less than 1 kilogram, some 2 years before they attain their final size. Nevertheless, the average litter closely approximates the average for mature females.

3. In spite of the mild climate and relatively favorable conditions in Florida, the average number of young opossums per litter is definitely lower than in any other part of the United States for which data are available. This number is about 6.2 based on more than 200 litters.

4. The opossum in Florida does not seem bound in any way to a definite breeding territory or a home range. Year after year trapping data reveal a completely new population of adults in the same area. It is extremely rare to recapture an adult of either sex after the lapse of a year. This does not preclude the possibility that, over shorter periods of time, an animal may remain in a neighborhood for a while because of plentiful food or other favorable conditions.

The study of histogenesis in the embryonic gonads continues to follow the patterns of previous years. The testis is readily transformed under the influence of estradiol into an ovotestis or an almost typical ovary. In these experiments attention has been chiefly concentrated

on the role of the secondary sex cords (cortical cords) in the survival and multiplication of the germ cells. Large doses of estradiol typically result in delayed appearance of the cortical cords; germ cells do not long survive, and the developing cortex is sterile. Administration of androgens, in an attempt to transform the embryonic ovary, has continued to yield negative results.

BIOCHEMICAL CHANGES DURING METAMORPHOSIS

Acid Deoxyribonuclease in Amphibian Metamorphosis

In *Year Book 60*, pages 401–402, John R. Coleman's preliminary findings, indicating that *Rana pipiens* tadpole tails exhibit an increase in acid deoxyribonuclease (DNase II) activity concomitant with resorption of the tail at metamorphosis, were described. Since this enzyme is one of the hydrolytic group of enzymes found in rat liver lysosomes by de Duve and co-workers and is generally considered to serve more of a catabolic than an anabolic role in the cell, a more rigorous examination of its changes during tail resorption was considered worth while. For convenience, and because this project was undertaken in the autumn when *R. pipiens* tadpoles are difficult to obtain, the investigation was conducted on *R. catesbeiana* tadpoles, which are commercially available (Carolina Biological Supply Co.). The techniques for enzyme assay were essentially as described in last year's report.

Tadpoles about to undergo spontaneous metamorphosis were not available. Assays of DNase II activity in all the available stages showed considerable variability from tadpole to tadpole with no significant trend of increase or decrease with advancing development (fig. 27, open symbols). To obtain tadpoles in the process of tail resorption, thyroxine (Na salt, California Corporation for Biochemical Research) was added to the medium in a final concentration of 0.1 μg/ml. Under these conditions, measurable growth in both hind limb and tail occurred within the first 4 to 6 days. No gross signs of tail resorption were evident until 10 to 11 days, at which time the extent of resorption varied from disappearance of the tail fin with no shortening of the tail to 25 per cent reduction in tail length. The only tadpole that survived 14 days of thyroxine treatment had undergone 43 per cent tail resorption.

DNase II activities in the tails of the thyroxine-treated tadpoles were not significantly different from those of untreated tadpoles except in tails that had begun to be resorbed (fig. 27). But when the DNase II activity was plotted as a function of the time in thyroxine it appeared to have reached a maximal rate of increase by 8 days (fig. 28), before any extensive resorption had occurred. Tails in the process of resorption (11–14 days) showed a 2- to 3-fold increase in enzyme activity over the average value obtained from tails not in the process of resorption. These results are in agreement with the earlier findings of Weber that the specific activity of cathepsins begins to rise in the *Xenopus* tadpole tail shortly before metamorphosis. However, Weber found a logarithmic increase in cathepsin activity throughout resorption, which is not true for DNase II activity. The changes in DNase II activity more closely parallel the changes in acid phosphatase activity in *Xenopus* shown by Weber and Niehus than changes in cathepsin activity.

No evidence is available about the possible lysosomal compartmentalization of these enzymes in tadpole tail tissues, but their patterns of activity are consistent with the theory that they play primarily a catabolic role.

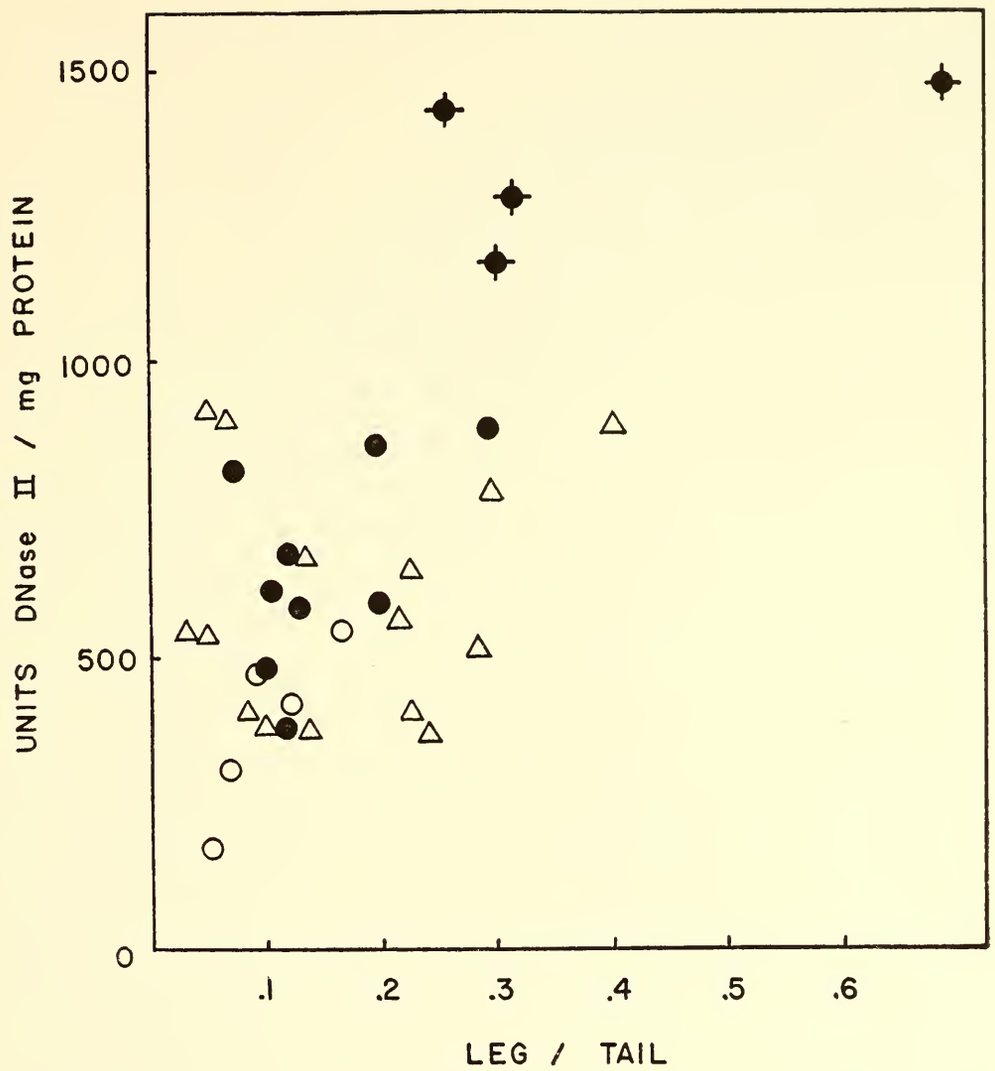

Fig. 27. DNase II activity per milligram of protein in isolated tadpole tails at various stages of development as indicated by the ratio of hind-limb length to tail length. Shaded symbols represent tadpoles kept in the presence of 0.1 μg of thyroxine per milliliter for various periods; open symbols refer to nonthyroxine-treated tadpoles. Each point represents duplicate assays on a single tail. Triangles indicate determinations made before thyroxine experiment; open circles, control determinations carried out in parallel with thyroxine experiment; closed circles, determinations on tails of thyroxine-treated tadpoles before resorption had begun; closed circles with crosses, same, but undergoing resorption.

An Amylase Activity in *Rana pipiens* Serum

Frieden and co-workers have described the appearance of an albumin in serum of amphibians during metamorphosis and have suggested that the albumin appearing during metamorphosis is a preparation for the terrestrial environment of the adult inasmuch as an increased amount of osmotically active material in the blood would aid in the maintenance of blood volume. These earlier studies of blood proteins had been made by means of paper electrophoresis, and G. L. Carlson thought it useful to reexamine the findings taking advantage of the greater resolution of the starch gel electrophoretic technique.

In electrophoresis of adult *Rana pipiens* sera on starch gel, an unusual degradation of the starch near the point of application of sera demonstrated the existence of glucan hydrolase (amylase) activity in the sera. Of the proteins resolved on starch gel electrophoresis, amylase has been studied most thoroughly to date.

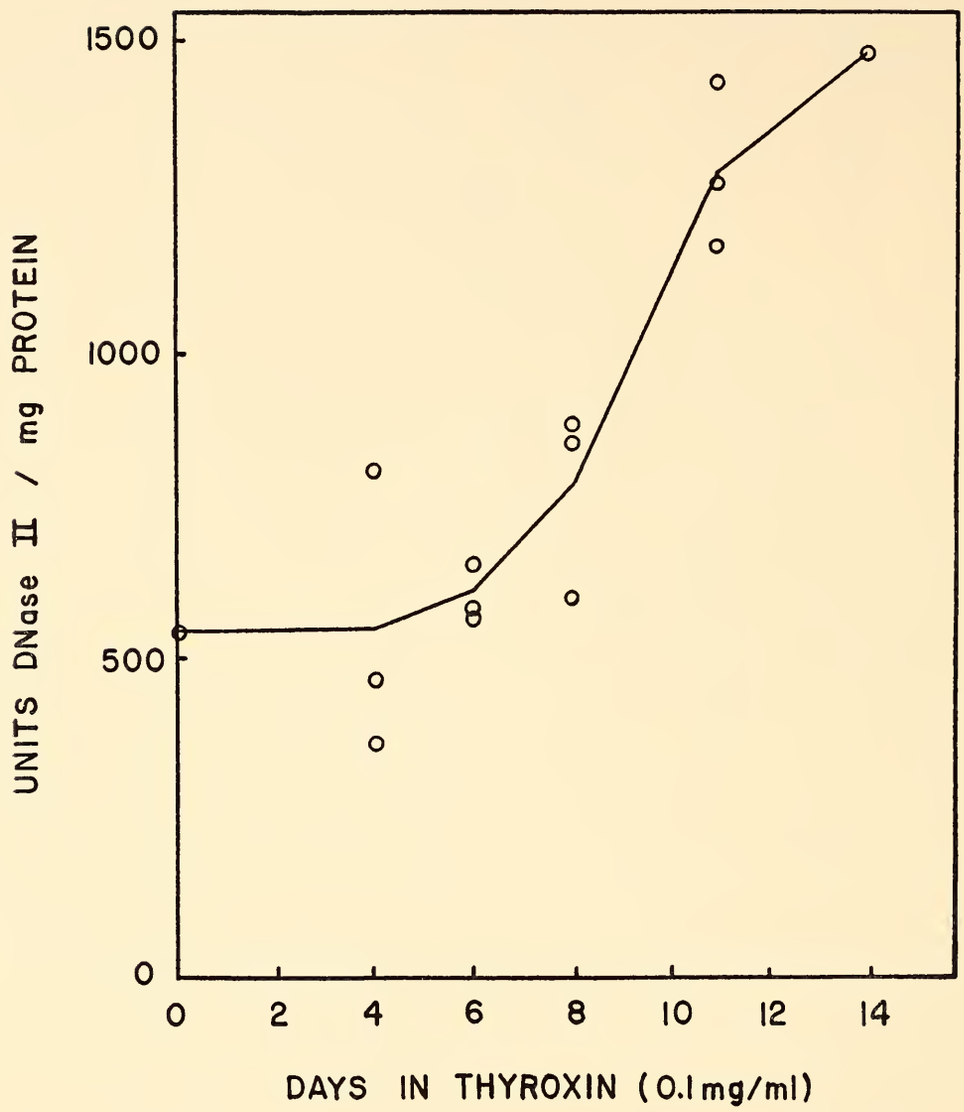

Fig. 28. DNase II activity per milligram of protein in isolated tadpole tails after exposure of tadpoles to thyroxine for various periods. Thyroxine was added to the medium in a final concentration of 0.1 μg/ml. The medium was changed every 2 to 3 days. Each point represents duplicate assays on single isolated tails except at 0 days, where the point represents an average of all control data shown in figure 27. No tail resorption was apparent before 10 days in thyroxine, but by 11 days all tails exhibited some degree of resorption.

A quantitative assay for the amylase was devised taking as a measure of enzyme activity the appearance of compounds reducing cupric ion after incubation of aliquots of serum with potato starch in *p*H 7.5 tris buffer at 30°C for 25 minutes. Under these conditions, a linear proportionality between the amount of serum added and the amount of reducing material liberated could be shown. With color developed from the reduced copper by means of Nelson's arsenomolybdate reagent, it is easily possible to assay the amount of amylase activity in as little as 1 microliter of adult serum. The assay conditions were chosen after determining that the enzyme activity of adult serum had a *p*H optimum between 7.5 and 8.0. On a volume basis it is interesting that the amylase activity in adult *R. pipiens* serum obtained by heart puncture is about one-tenth that of human saliva.

Further definition of the nature of the amylase activity was obtained by paper chromatographic examination of the products of the action of adult *R. pipiens*

serum amylase on glycogen at various times during hydrolysis. When aliquots of reaction mixtures were chromatographed with an isopropyl alcohol–water (4:1) solvent system and reducing compounds were detected with alkaline silver nitrate reagent, a complex mixture of reaction products was observed as expected of endoamylase (α-amylase) activity. The chromatographic pattern of reducing products compared well with that obtained by digestion of glycogen to approximately the same fraction of free reducing groups with a human salivary α-amylase preparation, suggesting strongly that the frog serum amylase consists mainly of α-amylase(s). Reducing material with an R_f of maltose in this solvent system is formed in quantity later than the slower-moving dextrins, and it therefore seems highly unlikely that a β-amylase (exoamylase) is present. This conclusion is supported by the observation that chromatograms of a reaction mixture of maltose incubated with *R. pipiens* serum show that only glucose is produced as a reaction product, and this reaction proceeds at a very slow rate.

A quantitative analysis of the levels of serum α-amylase of *R. pipiens* during metamorphosis is now being made in order to relate the serum amylase levels to changes in β-amylase activity previously noted by E. Urbani in studies on homogenates made from amphibian tadpoles during metamorphosis.

THE EMBRYO IN RELATION TO ITS ENVIRONMENT

Mechanisms of Implantation of the Ovum

In the rabbit. As has been reported in *Year Books 58* (pp. 368–370), *59* (pp. 359–362), and *60* (pp. 431–432), Bent G. Böving's proximate objective is to analyze the mechanisms of implantation of the rabbit blastocyst. He has elected to study a single species rather than adopt the traditional comparative approach. Fragments of knowledge of the implantation process suggest that there is considerable species variation; it appears likely, therefore, that a better view of the implantation process as a whole may be obtained by following it in one species. Ultimately, we should like to know to what extent the major conclusions from studies of the rabbit apply to other species, particularly the macaque and the human.

As Böving has shown, implantation embraces a series of mechanisms grouped by him into three main categories: muscular, adhesive, and invasive. The last two (adhesion of trophoblast and uterine epithelium, and penetration of epithelium by trophoblast) have been shown to occur selectively where there is a capillary at the base of the epithelium. Both phenomena have been attributed to a local alkalinity elicited when bicarbonate from the blastocyst passes through the epithelium and there dissociates into (alkaline) carbonate and carbonic acid, which with enzymatic assistance dissociates into water and carbon dioxide, the carbon dioxide being removed by the maternal circulation.

During the year covered by this report, Böving has centered much of his attention on the uterine lumen and the uterine epithelium.

Little is said of the uterine lumen in most treatises, and what is said is largely incorrect, for there are several popular, but mistaken, impressions about it. In particular, it is supposed to be occupied by "free" blastocysts and uterine fluid. The "free" blastocysts not only are confined within the uterus but also depend on it for their motion and presumably for some aspects of their metabolic maintenance. That is "freedom." When the uterus of the rabbit is opened at the time of implantation, its lining is covered with a thin layer of mucus that is barely moist. That is "uterine fluid." Still, what there is of it

is possibly interesting, if the usual assumption is correct that all chemical exchange between mother and unattached blastocyst occurs through the fluid rather than directly in regions of contact. Böving believes that, except for the rather viscous uterine secretion and debris remaining adherent to the blastocyst as the gloiolemma, any fluid interposed between epithelium and blastocyst would be squeezed out to unoccupied segments of the uterus as the blastocyst expands and pushes out the uterine wall around it to form its dome.

The amount of uterine fluid in a uterine horn was estimated by Kulangara (*Year Book 59*, p. 361) to be about 0.1 to 0.5 ml. Assuming a length of 200 mm, the lumen accommodating that amount of fluid would have an average cross-section area of 0.5 to 2.5 mm. The open spaces seen in histological sections are generally greater, the difference being almost certainly evidence of tissue shrinkage during preparation. Böving has developed a new way of visualizing the distortion; it is derived from a test of the distribution of a small amount of fluid injected into the uterine lumen. About 0.5 ml of 0.5 per cent $AgNO_3$ solution was injected into a segment of uterus about 20 mm long. That is to say, the segment had added to it about 10 times as much fluid as it would normally contain. Even with that excess, the fluid stained, and presumably touched, only the tips of endometrial folds nearest the center of the uterus (pl. 6, figs. 29, 30). Since the now open "glands" were not entered by the fluid, we may reason that they were closed in the living state. That relation may be reconstructed if we envision the black-stained tips of the endometrial folds pulled together to compensate for the shrinkage that pulled them apart. The remaining space may then be considered to be 10 parts artifact produced by injecting the $AgNO_3$ solution and 1 part uterine fluid, representing the extent of the uterine lumen in vivo. The uterine lumen, then, is small in life.

Böving's estimate of the distribution of fluid injected into the uterine lumen provides the background for a study of the effects on cohesion of uterine epithelium of various solutions in the carbonate family. His working hypothesis (that bicarbonate from the blastocyst yields carbonate in epivascular epithelial cells, where it raises the *p*H and so causes epithelial dissociation) suggested to him that a bicarbonate solution of appropriate concentration (0.15 *N*) in the lumen might produce the same effect. Slight dissociation was noted, but the evidence was not convincing. Earlier, Böving had observed that general epithelial disruption was produced by 0.05 *N* NaOH. It seemed possible, therefore, that the bicarbonate solution was being handled in a way significantly different (mixed with uterine fluid and buffered?) from bicarbonate coming from a blastocyst held in apposition to uterine epithelium. Hence it became necessary to provide a direct test of the action of 0.15 *N* Na_2CO_3 instead of depending on it to be produced in the epithelium after injecting $NaHCO_3$ solution into the uterine lumen.

As has been reported earlier, there was little effect of bicarbonate solution, even when charged with CO_2, and little effect of Na_2CO_3 charged with CO_2; but there was a whole range in severity of effects produced by alkaline Na_2CO_3 (pls. 7, 8, figs. 31, 32, 33). Except for differences in degree, the kinds of effects observed were similar to those seen in the invasion by normal trophoblast at 7 days after mating. Not only does carbonate dissociate the epithelium (and occasionally stroma) but it generally spares the underlying blood vessels (pl. 8, fig. 33). (It is a day or two later that the blood channels normally lose the endothelium, after they have been enveloped by trophoblast.) Thus, not only does the experiment lend support to Böving's hypothesis; it also points out that maternal tissues have different degrees of susceptibility to dissociation.

Previously, the idea of differential

dissociability had been applied to the invasion of trophoblast only in the all-or-none sense: penetration was explained as the persistence of trophoblast by reason of the syncytial structure of its invading knobs, which resist dissociation in an alkaline microenvironment where the epithelial cells suffer loss of cohesion. It remains to be explained why the cellular trophoblast between knobs does not dissociate, although it may adhere to epithelium. Conceivably, it may have a high degree of resistance to dissociation at high *p*H, as does the maternal capillary endothelium. That idea is being tested, along with the idea that trophoblast spread is arrested by the uterine epithelium's becoming converted by progesterone into a syncytium and thereby becoming insusceptible to dissociation. The experiment of putting carbonate solution into rabbit uteri at 9 days after mating has been done, but the manipulation was suspected of being improper, and repetition is planned.

The foregoing questions of dissociability will be approached by technical means that may be expected to provide data on the amounts of force necessary to separate the tissues and to determine whether the binding strength varies with *p*H. A Duryee-Bush-Hastings electric micromanipulator is being adapted as a microtension measuring device. The associated equipment for cinematographic recording is essentially complete and has performed adequately in a single preliminary test.

The question whether bicarbonate forms carbonate in the epithelium is being approached not just by the substitution methods described but also analytically. Nickel chloride has been found to precipitate sodium carbonate but not bicarbonate. A perfusion with it has been done, but the histology, which is to depend on development with sodium sulfide, is just beginning.

Finally, it should be noted that the peeling of epithelium (pl. 7, figs. 31 and 32) induced by carbonate resembled that observed previously, both histologically and in an experiment with a plastic chamber in vivo, mentioned in *Year Book 59* (p. 362) as a possible basis for the rise in the concentration of protein in the uterine fluid that Kulangara found to begin about 4 days after mating. Could it be that the blastocyst "turns on" the increase of uterine fluid protein by a *p*H mechanism? The idea is difficult to test, for comparisons of protein concentration of fluids in empty vs. gravid horns must get around the possibility that higher values in the gravid horn may derive from blastocysts, living or dead, rather than from the uterus on which they are presumed to act. Further caution is suggested by evidence of epithelial shedding in cysts (pl. 6, figs. 29 and 30), where it may be assumed no blastocyst reached. Such cysts, surprisingly common once one is alert to them, may also be contemplated as a source of protein of epithelial cell origin, but that idea should not be taken too seriously until timed histological studies confirm that they are formed and rupture at an appropriate time.

In the mouse. The factors effecting the orientation and spacing of blastocysts in the mouse uterus have never been clearly delineated. It is known that the normal orientation of the blastocyst and the sites of implantation are not affected by reversal of the gravitational axis of the uterus. Evidence for and against implantation's being invariably antimesometrial because of the influence of a chemotactic stimulus (from epithelial lipides) has been presented in the literature, and the balance seems to be against such a stimulus. Spacing of blastocysts along the length of the uterus (whether even or random) is thought to be caused by peristaltic movements of the uterine smooth muscle.

In earlier experiments involving the transplantation of melanotic tumor tissue to the pseudopregnant mouse uterus, Ian Wilson found that such grafts "implant" in the endometrium invariably antimeso-

metrially, as do blastocysts. Grafts of muscle tissue (autografts of body wall) also "implant" at the same site, and it has been reported that even wax or glass beads, when inserted into the uterus, become antimesometrially located.

The above observations, considered together, suggest that the antimesometrial siting of implantation is brought about by a simple, nonspecific mechanism. It is possible that the uterine contents, whether blastocysts or wax beads, are forced into the antimesometrial area simply by compression of the uterine lumen caused by contraction of the circular muscles.

Melanoma cells in suspension, injected into the uterus, segregate into clumps, often spaced out along the uterus exactly as blastocysts are. This finding supports the idea that spacing, in the mouse, is a result of random scattering of the uterine contents by peristaltic muscular movements and not a result of specific interactions between the blastocyst and the uterus.

It was thought that a direct test of the effect uterine muscular movements exert on the spacing and localization of implantation sites might be made by injecting mice, in early pregnancy, with a drug that would inhibit the activity of uterine smooth muscle. Such a drug, isoxsuprine [2-(phenoxypropylamino)-1-('*p*'-hydroxyphenyl)-1-propanol-HCl], has become available recently. It is reported to have a relatively specific inhibitory effect upon the smooth muscles of the uterus (as opposed to the gut), although it is also a vasodilator.

It was supposed that the muscle activity that causes spacing of the blastocysts preceded, and was relatively independent of, the activity that causes their antimesometrial propulsion. To separate these two stages for experimental work it was necessary to find out, from a series of normal pregnancies, the exact location of the blastocysts (and, incidentally, their orientation) at any given time before their implantation.

Wilson's preliminary observations show that, in the period up to about 90 hours postcoitum, blastocysts become randomly located throughout the uterine lumen; they may be found in the mesometrial or antimesometrial region, and their embryonic-abembryonic axis is haphazardly oriented (pl. 9, fig. 34). From 90 hours postcoitum onward the blastocysts are invariably found in antimesometrial "pockets" properly oriented with their embryonic mass toward the mesometrium (i.e., orientation does not occur until the blastocysts occupy their prospective implantation sites). It is clear that, at this time, the uterine lumen is completely occluded except for the small antimesometrial pockets containing the blastocysts. Although the walls of the lumen are pulled apart during fixation and mounting of the sections, the silhouettes of the lateral walls are complementary; on the free surface of the epithelial cells (except those in the "pocket") there are present, during this period, small conical protrusions that correspond exactly with small depressions on the opposite side of the lumen (pl. 9, fig. 35).

The dose of isoxsuprine needed to inhibit uterine contractions is being measured by observations in vivo upon the rabbit and the rat, the mouse being too small. It is hoped to test the effect of the drug during the two established periods of early pregnancy: (1) the period during which the blastocysts become randomly scattered in the uterus, and (2) that in which they become antimesometrially located.

Trial injections of isoxsuprine into 5 mice (over days 3 and 4 of pregnancy) inhibited, or interrupted, pregnancy in 4. In all 5 animals the blastocysts had reached their prospective implantation sites. In 2 animals the blastocysts were judged to be healthy but retarded in development by about 36 hours (no egg cylinder formed, no sign of any reaction in the uterine mucosa). In 2 others it appeared that the blastocysts had actually started to implant when the vaso-

dilator action of the isoxsuprine precipitated rupture of subepithelial capillaries and extravasation of blood into the uterine lumen. The exudate dislodged the blastocysts from their implantation sites. In 1 animal pregnancy was apparently unaffected by the drug. At the time of these trials, however, neither the inhibitory effect of the dosage used nor the stage of pregnancy at which injections were started had been determined critically.

Anatomy and Physiology of the Placenta

The team of E. M. Ramsey, G. W. Corner, Jr., and M. W. Donner sustained a severe loss in the fall of 1961 in the untimely death of their technical associate Herbert M. Stran. Mr. Stran's understanding of biology and of the point of view of scientific investigation, as well as his training and skill in mechanics and electronics, made him uniquely valuable. His death constitutes a scientific loss as well as a source of personal regret.

Problems associated with the move to the new laboratory were agreeably few and easy of solution, thanks to the valued assistance of Arthur G. Rever and William I. Cleary and his staff.

Adjustment to new conditions has been the rule on all fronts throughout the year. During the winter and spring the Department of Radiology at the Johns Hopkins Hospital installed new and improved equipment for cineradioangiography. Ramsey and her associates assisted by providing monkeys for tests and calibration studies involving higher radiation dosage than is permissible for use in human patients.

In the experimental procedures themselves unanticipated areas of ignorance became manifest requiring reorientation of the schedule of operations. It was found necessary, before all else, to acquire background information about labor and parturition in the rhesus monkey.

As was noted in *Year Book 60* it had been planned that the first studies of placental circulation to be undertaken in the season just ended would deal with the final stage of pregnancy. Inasmuch as symptomatic diagnosis of impending parturition in the monkey cannot often be made long enough in advance of birth to permit adequate study of the process, it was proposed to induce labor at full term (as judged by the duration of the pregnancy) by administration of hormones. Unexpectedly, it was found that this cannot be done in the monkey with the same ease as in human patients. High dosage and prolonged administration of the standard oxytocic drug Syntocin failed to effect adequate cervical dilation and effacement. Furthermore, in some of the extended studies the drug seemed to be incapable of maintaining such progress as was achieved. As for the activity of the uterus, recordings of intraamniotic pressure during administration of Syntocin showed progression from the late-pregnancy type of infrequent, uncoordinated, myometrial contraction waves of medium strength to characteristic rapid, coordinated, high-amplitude labor waves, but the latter type of activity was intermittent and unsustained.

These observations afford renewed evidence that the technique of intrauterine pressure recording permits accurate evaluation of clinical status and prediction of clinical performance; it is gratifying to find them in harmony with previous observations on the early and middle stages of pregnancy, yet they do present a serious dilemma as far as the study of placental circulation at term is concerned. First, why is Syntocin less effective in inducing productive labor in monkeys than in humans? And second, how can labor be diagnosed and studied? The probable explanation appears to be that the attempts to initiate labor were made before physiological "term" had been reached, no matter what the chronological age of the pregnancy. Almost nothing is known about prelabor developments in the monkey. The observations

and motion pictures of parturition made by Carl Hartman more than 30 years ago remain the only recorded observations, and they are entirely gross and external and deal with the final stage of birth alone. The current pressure studies have at least made it possible to state when the myometrium is *not* ready to respond promptly to oxytocics and to expel the fetus. Although negative, this information should make it possible in the future to study the response to hormones of the "ready" myometrium on a basis of better understanding of the animal's physiology at the end of pregnancy. Since it is impractical to diagnose impending labor by the fairly elaborate process of intrauterine pressure recording, intensive clinical studies have been made in pregnant monkeys approaching term for the purpose of developing criteria that can be established by vaginal palpation of the cervix in the unanesthetized animal.

The urgency of the above problem has made it necessary to defer radioangiographic study of venous drainage of the placenta, which was the second series of studies planned for the current season, as noted in *Year Book 60*.

In reviewing for publication the data assembled in the 1960 season on the effect of administration of progestins at various stages of pregnancy it was found possible to amplify the preliminary statements made immediately after the conclusion of the experiments and, setting the observations in the framework of recent studies by other workers, to propose the following generalizations.

1. In terms of amplitude and rate of myometrial contraction, activity at the beginning and end of pregnancy is more intense and rapid than in midpregnancy. In midpregnancy, rates and intensities similar to those prevailing at the beginning and end foreshadow labor or abortion.

2. The contour of the contraction wave is as important as its amplitude and frequency. Despite the strength and frequency of the myometrial contractions in *early pregnancy*, the contour of the contraction wave indicates a high degree of myometrial incoordination. Under such conditions the uterus is incapable of emptying itself. On the other hand, the contraction wave of *midpregnancy* indicates relative myometrial coordination, but the contractions are of insufficient amplitude and frequency to expel the uterus. The mounting myometrial activity associated with *preabortion* or *prelabor* is indicated by the return of a complex wave which gradually breaks up into true labor units. The simplicity, the high amplitude, and the rapidity of the *labor* wave show that both myometrial coordination and force have attained the degree necessary to effect delivery.

3. In speculating on the possible role of progesterone in the observed phenomena, Csapo's suggestion that the local action of progesterone may enhance muscle incoordination is to be considered. The monkey's bidiscoid placenta, supplying two foci of progesterone formation, certainly contributes to uterine asymmetry. In addition, the marked reduction in uterine circulation just before conversion, found by Reynolds, tends to enhance this action.

The observation, previously reported (*Year Book 59*), of the slight quieting effect of progesterone administered in early pregnancy can then be explained on the basis that, because of poor circulation, endogenous progesterone is reaching the muscle in so reduced a quantity that the additional dosage counteracts the asymmetry. In midpregnancy, when uterine conversion has reestablished circulation, and the myometrium already has a full supply of progesterone, added quantities merely increase the surplus of progesterone and can have no additional effect. It may be postulated that the reduced circulation of the late-pregnant uterus permits the two foci of placental production of progesterone to reestablish their dominance. Thus there is a return of the complex wave pattern. Then, as the uterus gradually escapes from proges-

terone domination, coordinated myometrial activity supervenes. Until a clearer overall picture is available, however, such speculations upon the role of progesterone in the control of myometrial activity cannot be considered more than provocative conjectures.

Study of Human Placental Vasculature

During his year in the Department, J. W. S. Harris made good progress in working up the pregnant human uteri in the Carnegie Collection. This material has been accumulated over a period of years with the ultimate objective of preparing a study of the vasculature of the human placenta along lines parallel to the study of the placenta of the rhesus monkey previously carried out by Ramsey.

It was envisaged that the human study would fall into three phases:

1. *Analysis of available material.* The material consists of specimens submitted to the Department either as unsolicited contributions or by obstetrical colleagues throughout the country who have cooperated in preparing and injecting uteri specifically for the project. Upon receipt, the specimens from both sources were screened and if considered suitable were placed in carefully adapted storage containers. Only a few sample blocks from some half dozen of the specimens were sectioned.

Harris's first concern was to cull and evaluate this special material and to survey the collection as a whole to determine what older specimens might also be included in the study. Not only has he completed this part of the program, but he has also prepared for more general use of all those handling the collection a definitive list of all suitable placental material on hand through 1961. Specimens are seriated by age; the state of preservation and type of preparation have been noted; and gaps in the series that should be closed in future collecting have been marked.

2. *Study of the placental series and modeling of representative examples.* Preparation of the material is, of course, preliminary to study. It has been a basic premise that the preparation should be carried out in the same way as the preparation of the monkey material, so that results will be closely comparable. Refinements and additional lines of investigation are by no means excluded and are particularly to be welcomed in so far as they may yield data on points remaining obscure in the monkey series. The fundamental pattern of serial sectioning through the placenta and uterine wall will be maintained, and transparent sheet reconstructions of key stages will be prepared.

Somewhat more than half of the technical work was done before Harris returned to London, and arrangements have been made for completion of the remainder.

Study of specimens was commenced as soon as selections were ready, and remodeling proceeded both as an element in the study serving to elucidate relationships and as a first step in readying the material for eventual publication. Numerous sketch models have been made, and several plastic sheet reconstructions have been brought close to finished form —one, entirely so.

3. *Preparation of manuscript for publication.* This step will be a joint project of Harris and Ramsey. Its completion is tentatively scheduled to be in time for volume 38 of the *Contributions to Embryology.*

The services of Mrs. Ranice Davis, Director of the Department of Art as Applied to Medicine of the Johns Hopkins University, have been enlisted for the artistic rendering of the models as illustrations for the publication. Mrs. Davis, a former student of the late James F. Didusch, who drew the illustrations for the monkey papers, and herself both skilled and experienced, is well fitted to prepare drawings that will be comparable to Mr. Didusch's.

A Composite Drawing of the Placenta to Show Its Structure and Circulation

Diagrammatic representations of the placenta and its circulation, as shown in scientific papers and textbooks, have not kept pace with advances in knowledge and understanding of this organ. Revisions of diagrams based upon long-outmoded data and theories—and snowballing revisions of revisions—have produced confusion and inaccuracy. A fresh start and a rethinking of the whole problem of how to represent this complicated organ are long overdue. In an attempt to meet this need, figure 36 (pl. 10) has been drawn by Mrs. Davis, in consultation with Ramsey. The goal has been to embody current knowledge of placental structure and current concepts of fetal and maternal circulation in a drawing that reconciles realism and interpretation as far as possible. The usual diagram, which frequently resembles a blueprint of hydraulic channels, has been replaced with a drawing in which students can immediately recognize the placenta as they see it in the delivery room and under the microscope. At the same time it has been considered desirable to express clearly the course pursued by maternal blood in traversing the intervillous space and to represent the forces propelling it. To achieve these diverse purposes the drawing has been made in five separate panels, each telling a single facet of the total story, as indicated in the legends. Taken together they illustrate the "physiological concept" of circulation in the maternal placenta, which has been deduced from anatomical, physiological, and radiological studies carried out by Ramsey and her associates over a period of years (see *Year Books 48* to date). This concept may be expressed as follows:

Maternal blood enters the intervillous space of the placenta from the endometrial arteries in fountainlike jets or "spurts" produced by the higher pressure in the maternal vessels as compared with the low pressure prevailing in the amorphous intervillous pool. The head of maternal arterial pressure drives the blood well along toward the chorionic plate before lateral dispersion occurs, thus preventing short-cutting from arteries into adjacent venous orifices before the blood has circulated through the intervillous space. Gradually, however, the head of pressure is spent, and general spreading throughout the space occurs. The villi, acting as baffles, aid this dispersion and promote slowing and mixing. Their own pulsation also effects a mild stirring. Finally, the blood drains from the intervillous space into the maternal uterine veins, where pressure is even lower than in the placenta.

Dr. John W. Crawford of Glasgow, Scotland, has kindly checked the representation of the fetal circulation as it appears in this drawing. Dr. Crawford's extensive investigations over the past decade have largely illuminated this aspect of placental circulation. His helpful suggestions are gratefully acknowledged. The fetal circulation is less fully portrayed here than the maternal circuit, in part because problems of magnification preclude representation of the villous capillary bed, which is the fundamental area of metabolic exchange on the fetal side.

DIFFERENTIATION AND MORPHOGENESIS IN THE HUMAN EMBRYO

The Collection of Human Embryos

In the year covered by this report, Elizabeth M. Ramsey examined 30 specimens sent by physicians and laboratories from three states and one foreign country. Of these specimens, 22 were discarded as of no research value, at the

end of three months after reporting to the donor and in the absence of instructions to the contrary. Eight specimens had sufficient research value to justify preservation.

In the six months before moving to the new laboratory all the gross specimens in the Collection of Human Embryos were reviewed carefully. All tissues of important early stages were retained, whether any part of the specimen had been sectioned previously or not. All specimens exhibiting anomalies were retained unless the state of preservation precluded any possible future study. This was rarely true even among specimens collected as much as 45 years ago. Normal material in grade 2 condition (adequate for gross study but not for histological) was reduced sharply. Material in grade 1 condition was reduced to an inventory of about 10 specimens for each 10-mm increment in crown-rump length through 150 mm. Older specimens up to term were kept to an average of 5 per 10-mm increment.

Discarded specimens in good condition were presented to the Departments of Anatomy of the Johns Hopkins Medical School and the University of West Virginia, School of Medicine.

Development of the Eye

Ronan O'Rahilly's main objective has been to expand a program of work on the development of the eye by (*a*) undertaking an experimental embryological study of the chick eye, and (*b*) making a detailed analysis of the development of the human eye in staged embryos.

Some experience in several experimental techniques on the chick embryo, such as intracoelomic and chorioallantoic grafting, has been attained, and it is proposed to make use of these methods subsequently.

A detailed analysis of the development of the human eye, from its initial appearance at horizon x (3 postovulatory weeks) until the beginning of the fetal period at horizon xxiii (7 postovulatory weeks), is under way. Specimens acquired during recent years, particularly those stained by the "azan" method, have been especially valuable in this investigation. Considerable attention has been paid to the basement and other membranes of the eye, and an abstract has already been published in the *Anatomical Record.*

During the closure of the retinal, or so-called "choroid," fissure of the eye, the generally accepted view that "fusion of course must occur before the internal limiting membrane is differentiated along the margins of the cleft" (Mann, 1949) has been found to be incorrect. The optic cup is covered by, and lined with, a continuous basement membrane from its initial formation from the neural tube, and, at the site of obliteration of the retinal fissure, this membrane disappears during closure. A similar occurrence has been seen during the complicated morphogenesis of the membranous labyrinth, and preliminary observations have also been made on the early development of the otocyst in the collection of human embryos.

Early Development of the Brain

In the large and closely spaced series of well preserved embryos in the Carnegie Collection the stage at which structural differentiation appears in the various centers of the brain has been determined by G. W. Bartelmez and A. S. Dekaban and an account has been published in *Contributions to Embryology,* volume 37. Description of the progressive development of the human brain from early neural folds is of value from the practical as well as from the embryological viewpoint. Analysis of various complex congenital malformations of the nervous system can be understood only in the light of normal morphogenesis. It is well known that the nervous system is highly susceptible to various noxious factors during prenatal life.

To follow the centers of the brain from later to earlier stages it was necessary to

establish a series of landmarks. The most obvious are the boundaries between the major subdivisions that characterize the brain of all vertebrates: namely, the forebrain, midbrain, and hindbrain. These have been traced from embryos of the eighth to ninth weeks, when all the fundamental centers can be recognized, to the neural-fold stages of the early fourth week.

The Carnegie Collection has photomicrographs of the serial sections of many embryos, each section accurately oriented by guide lines according to the Born-Lewis procedure. It is accordingly possible to reconstruct any internal organ with the control of the external form of the embryo, controlled in turn by photographs of the intact specimen. Such controls are especially important for reconstruction of the brain with its flexures.

By means of graphic reconstructions and models the exact location of histological differentiation could be determined in each embryo of our series. In the earliest stages the primordium of the optic vesicle from which the retina develops, and the characteristically shaped subdivisions of the hindbrain associated with the fifth and seventh cranial nerves, mark the segment of the neural folds between them as the midbrain, which is at this period, as well as throughout development, associated with an abrupt shift in the axis of the central nervous system, namely the "cranial flexure."

In the early stages of human development the hindbrain is the largest subdivision, as it is in all other vertebrates. The earliest differentiations appear in this region and rapidly proceed forward to the basal (efferent) region of the midbrain and thence to the hypothalamus and the basal centers of the olfactory system. Neuroblasts then appear in other phylogenetically old centers of the forebrain, the corpus striatum, and the epithalamus. As was first reported by Hines, the first region of the cerebral cortex to differentiate is the hippocampus, which is the dominant center of the forebrain in many lower vertebrates. With the appearance of differentiation in the neopallium the associated thalamic centers can be recognized. Retinal fibers do not reach the brain until the end of the second month. The cerebellar cortex differentiates later than all others, and its cells are still undergoing rearrangement and maturation after birth. It is perhaps significant that it is the most frequently observed site of neoplasms in the central nervous system.

Bartelmez and Dekaban are currently attempting to follow the primary projection centers of the cerebral cortex, motor, visual, and auditory, to stages of the third fetal month.

STAFF ACTIVITIES

One of the most perplexing problems with which all scientists have to deal is the ever-mounting tide of publications. There can be little doubt but that the next decade will see far-reaching changes in the nature and scope of scientific journals and in other means of dissemination of data. Already specialized journals are being established to speed the flow of announcements of discoveries in brief form, and the number of review journals is being increased. It is not hard to imagine that soon each biologist will receive his daily or weekly newspaper of biology; extensive documentation will be available in centers established at several strategic locations, and copies of data will be made available to persons working on a given problem. Journals, in the usual sense, might then be devoted almost entirely to synthesis and evaluation.

Similarly there may have to be a change in the direction of conferences and symposia, which have multiplied rapidly

in recent years. We are beset by vast numbers of conferences with attendance in the range 150 to 300 persons, too large a group for effective discussion, too small to make full use of a speaker's talents. Often the intermediate-size meeting is justified by the statement that the proceedings will be published, but such volumes are all too often collections of ill-assorted papers.

The activities of our own staff members and visiting investigators reveal that they have found no simple solution to these problems. Of all the ventures in which the staff was engaged one stands out as an unusually promising approach. Elizabeth M. Ramsey organized and conducted a meeting of an informal group of 16 physicians engaged in laboratory and clinical studies of uterine motility and the physiology of the placenta. This conference was a three days' session of reports and consultations, November 3–5, 1961, constituting the first conference to be held in the new laboratory. The writer, who was privileged to attend several of the sessions, found the frank, uninhibited discussions highly rewarding.

An effective training program in which two members of the group took part was the Summer Institute in Developmental Biology for College Teachers held at Brevard, North Carolina, during August 1961, under the auspices of the National Institutes of Health. Other activities directed principally toward teaching included the following: One member of the staff served as a consultant in developmental biology in the Baltimore Public Schools, under the BSCS Teacher Training Program developed by the American Institute of Biological Sciences; another lectured at the Summer Institute for Secondary School Teachers, held at the University of Maryland; and still another presented a lecture-demonstration in the Basic Science Program of the Washington Hospital Center. As in the past, several members of the group took limited part in formal courses in several departments of the Johns Hopkins University and School of Medicine, including Anatomy, Biology, and Pathobiology. Other teaching activities included lectures in experimental embryology given by one staff member at the University of Miami, and the participation of two others in the Embryology Training Program of the Marine Biological Laboratory, Woods Hole, Massachusetts.

More difficult, but equally rewarding, are lectures directed toward lay audiences; several members of the staff lectured before such groups, which included the Maryland Academy of Sciences Public Lecture Series and the Baltimore City-County High School Science Seminar. Attention was focused on congenital defects for general audiences in Providence and Washington, D. C. One member of the group took part in the Columbia Broadcasting System's College of the Air television series *The New Biology*.

Among the principal lectures presented by members of the staff, the following should be mentioned: a series of lectures delivered under the auspices of the Cleveland Foundation at Western Reserve University, the Hoffman-La Roche Lectures at Rutgers University, a Macy Lecture at Harvard Medical School, and a Sloane Lecture in the Department of Obstetrics and Gynecology of Columbia University. Other lectures were offered at the following research centers and universities: University of Aberdeen, Scotland; University of Alabama School of Medicine; University of Florida; Harvard Medical School; Johns Hopkins University; University of Miami; National Institutes of Health; State University of New York; University of Washington.

Staff members and fellows took part in several international meetings, including the Fifth International Embryological Conference, held in London; the First Inter-American Conference on Congenital Defects, held in Los Angeles; the Conference on Specificity of Cell Differentiation and Interaction, Gatlinburg, Tennessee; the Thirteenth Conference on Problems of Nephrosis, Princeton, New

Jersey; and the First International Conference on the Biology of Skin Cancer, Philadelphia.

Other meetings of learned societies in which members of the group participated were the American Association for the Advancement of Science, American Association of Anatomists, American Institute of Biological Sciences, American Society of Biological Chemists, American Society of Zoologists, Biophysical Society, Federation of American Societies for Experimental Biology, Johns Hopkins Medical Society, Society of American Bacteriologists, Society for the Study of Development and Growth, Society of General Physiologists, and the Tissue Culture Association.

Advisory and consultative services included membership on the editorial boards of the *American Zoologist*, the *Biological Bulletin*, the *Journal of Embryology and Experimental Morphology*, the section on Human Developmental Biology of *Excerpta Medica*, and the board of consulting editors of *Developmental Biology*. One member of the staff was appointed to the Divisional Committee for Biology and Medicine in the National Science Foundation, served on the Cell Biology Study Section of the National Institutes of Health, the Subcommittee on Congenital Malformations of the U. S. National Committee on Vital and Health Statistics, and the Visiting Committee, Department of Biology, Massachusetts Institute of Technology.

Among the services rendered by several members of the staff were the following: Vice-President, AIBS; Vice-Chairman, Scientific Council, Maryland Academy of Sciences; President, Maryland Section, Society of Experimental Biology and Medicine; Chairman, Division of Developmental Biology, American Society of Zoologists; Chairman, American Organizing Committee for the forthcoming International Conference, International Institute of Embryology; Secretary, Society of General Physiologists; Secretary, Section F, AAAS; Chairman, Publications Committee, American Society of Zoologists.

A distinction won by one member of the group must be singled out for special mention: Virginia LaFleur, a senior at College of Notre Dame of Maryland, who has for the past year been carrying on research in the Department on the graft-versus-host reaction in consultation with J. D. Ebert, was awarded first prize for her paper presented at the Northeastern Regional Convention of the undergraduate biological society, Beta Beta Beta, held at American University, Washington, D. C.

Seminars. The roster of speakers at the Seminar organized by the Department to serve all those working in developmental biology in the area included Robert Auerbach, University of Wisconsin; J. D. Biggers, University of Pennsylvania; J. Chutná, Prague; A. L. Colwin, Queens College; A. J. Coulombre, National Institutes of Health; I. Finger, Haverford College; C. E. Ford, Harwell; V. Hâsková, Prague; T. J. King, Institute for Cancer Research; H. Kroeger, Zurich; J. W. Lash, University of Pennsylvania; A. B. Pardee, Princeton University; P. Perlmann, Stockholm; R. B. Roberts, Department of Terrestrial Magnetism; J. Runnström, Stockholm; L. Saxen, Helsinki; F. Seidel, Marburg, Germany; P. Sengel, Paris; A. K. Tarkowski, Warsaw; T. Vainio, Helsinki.

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PERSONNEL

Year Ended June 30, 1962

(including those whose services began or ended during the year)

Research Staff

David W. Bishop, General Physiology
Bent G. Böving, Physiology
Robert K. Burns, Experimental Embryology
Robert L. DeHaan, Experimental Embryology
James D. Ebert, Director
Irwin R. Konigsberg, Experimental Embryology
Elizabeth M. Ramsey, Placentology; Pathology
Mary E. Rawles, Experimental Embryology

Assistant Investigators

Alton M. Mun, Experimental Embryology
Chinami Takata, Experimental Embryology

Research Associates (Extramural)

Louis B. Flexner, Philadelphia
Arthur T. Hertig, Boston
Chester H. Heuser, Augusta, Georgia
Samuel R. M. Reynolds, Chicago

Fellows

Michael Abercrombie, Fellow of Carnegie Institution of Washington
Bertie F. Argyris, Fellow of the U. S. Public Health Service
Thomas S. Argyris, Fellow of the National Science Foundation
Donald D. Brown, Fellow of Carnegie Institution of Washington
Gerald L. Carlson, Fellow of the Given Foundation–National Research Council
J. Douglas Caston, Fellow of Carnegie Institution of Washington
Timothy Glover, Fellow of the Population Council
John W. S. Harris, Fellow of the Rockefeller Foundation
Tom Mori, Fellow of the Rockefeller Foundation
Ronan O'Rahilly, Special Fellow of the U. S. Public Health Service
Peter H. S. Silver, National Institutes of Health Postdoctoral Traveling Fellow
Ian B. Wilson, Fellow of Carnegie Institution of Washington

Visiting Investigators

Frank D. Allan, Washington, D. C.
George W. Bartelmez, Missoula, Montana
George W. Corner, Jr., Baltimore
Anatole S. Dekaban, Bethesda
Arentje Dekker, Leiden, the Netherlands
Louis E. DeLanney, Crawfordsville, Indiana
Martin W. Donner, Baltimore
W. Richard Ferguson, Baltimore
Arthur LaVelle, Chicago
Ali Mehrizi, Baltimore
Sheila J. Moody, London and New York
E. Carl Sensenig, Birmingham, Alabama

Students (in cooperation with the Johns Hopkins University and College of Notre Dame of Maryland)

Timothy Bishop (student assistant)
John R. Coleman (graduate, biology)
James Errico (medicine)
Charles B. Kimmel (student assistant)
Virginia LaFleur (undergraduate, biology)
John Rowse (medicine)
Gretchen Schabtach (graduate, biology)
R. Owen Sear (medicine)

Clerical and Technical Staff

Leon Allen, Custodian
Mary N. Barton, Librarian
Franklin R. Baytops, Custodian
George Boettinger, Porter
William Bouchat, Assistant Recorder
Barbara Brown, Dishwasher
William I. Cleary, Recorder
Lloyd Crane, Technician
Lawrence A. Dorsey, Custodian
William H. Duncan, Technician
Ernest W. Edwards, Custodian

Linda Fuson, Technician
Wilma Gabbay, Technician
Wilbur F. Garde, Assistant Recorder
Thomas F. Garnett, Technician
Richard D. Grill, Photographer
Ernest Harper, Chief Custodian
Elaine Kerby, Stenographer
Francis J. Kupres, Technician
Edna G. Lichtenstein, Secretary
Ellen P. Monaghan, Technician
John Pazdernik, Building Engineer
Margaret J. Proctor, Secretary
Arthur G. Rever, Office Manager
James Roland, Custodian
Nancy J. Sype, Technician
John L. Wiser, Machinist

Special Technical Assistant pro tempore

Joseph P. Drane

PLATES

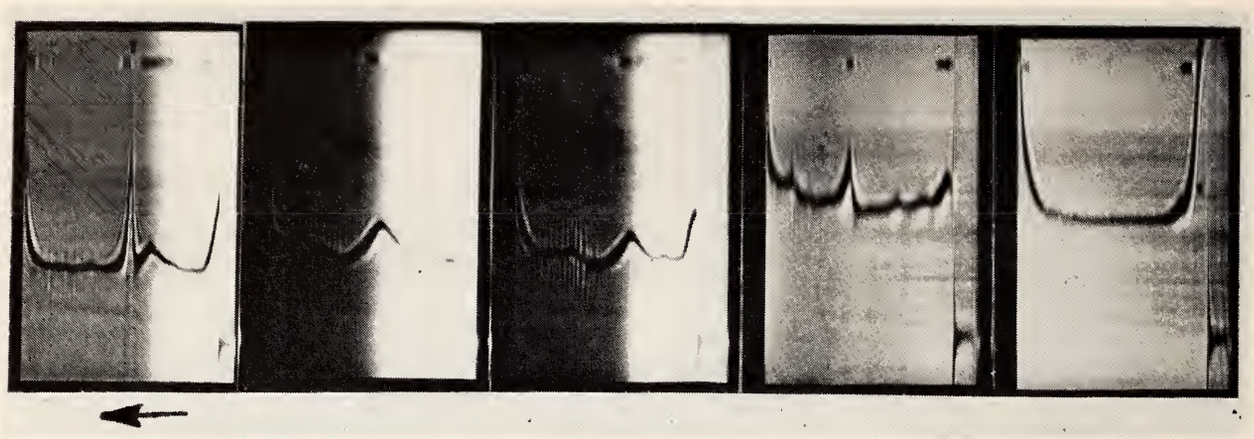

Fig. 1. Schlieren patterns of ribosome fractions. Centrifugations were done at 50,740 rpm, and pictures were taken 320 seconds after reaching speed. Preparations are, from left to right, adult frog liver; ovarian eggs; ovarian eggs treated in versene; stage 25 embryos; stage 25 embryos in versene. Arrow shows direction of sedimentation.

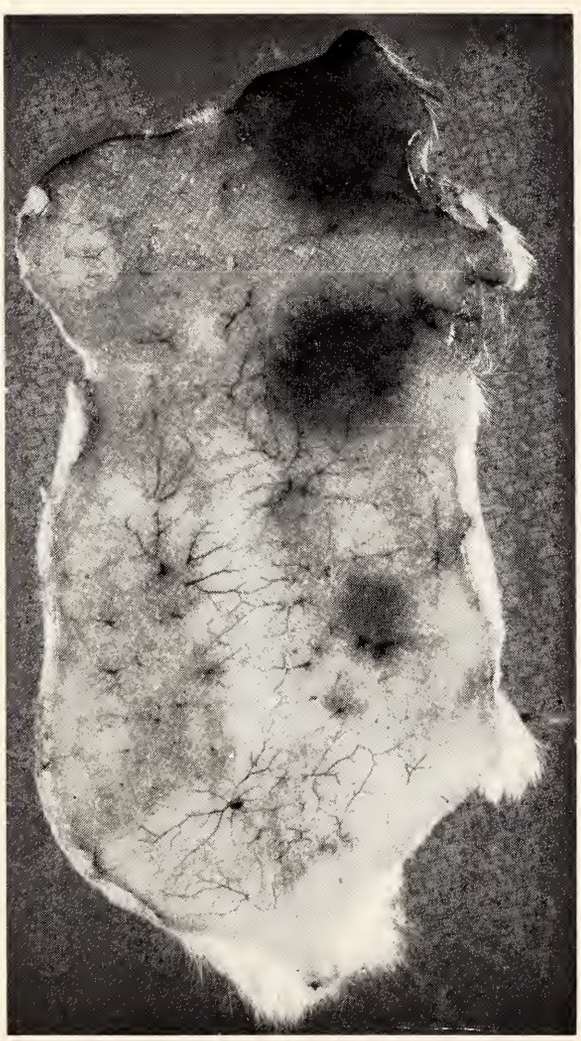

Fig. 3. Passive cutaneous anaphylaxis reaction elicited by intracutaneous antiferritin serum in decreasing concentrations from top down on right. Compare with injection of control serum at top left.

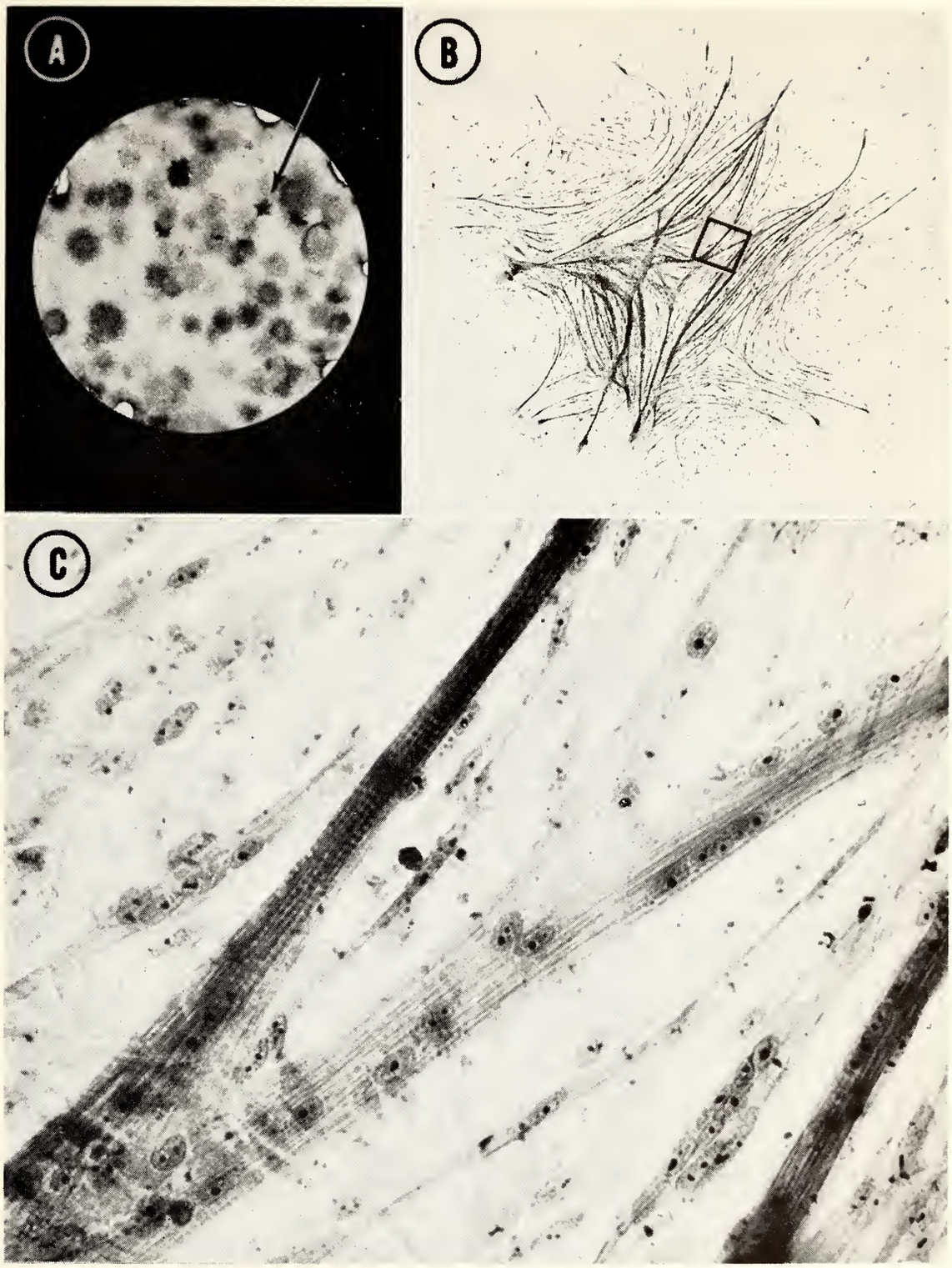

Fig. 16

A: Culture initiated with 400 cells and fixed on the tenth day of incubation. Fixation: Bouin's. Stained in phosphotungstic acid hematoxylin. Arrow indicates colony enlarged in *B* and *C*. Actual size.

B: Colony indicated in *A* above. Area encompassed by the square outline is enlarged in *C*. Magnification 20×.

C: Area of *B*. Magnification 370×. Note the prominently cross-striated segment of the left branch of central myotube. In the same field, smaller, less well differentiated myotubes can be seen as well as mononucleated cells.

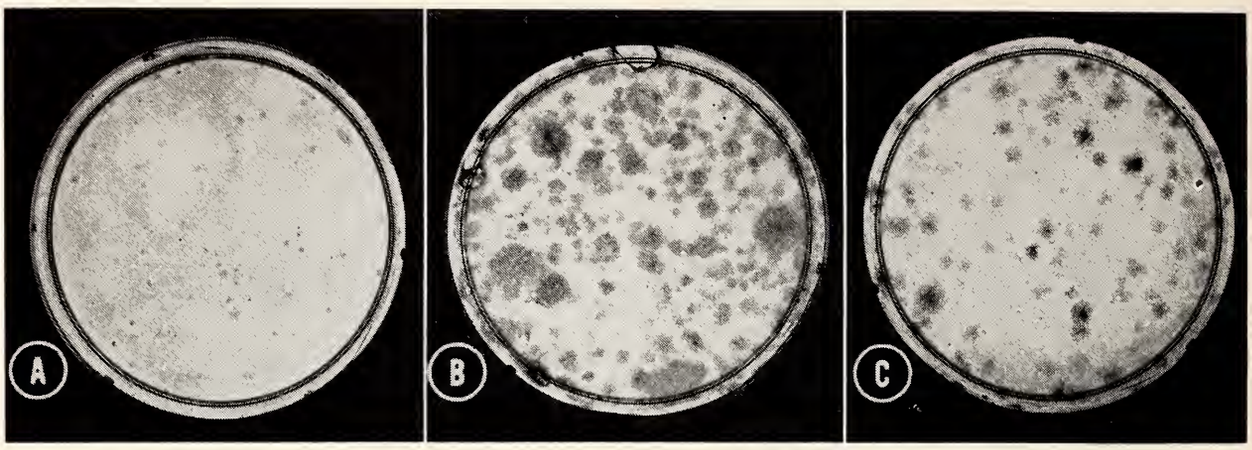

Fig. 17. Cultures initiated with 400 cells each. Fixed in Bouin's on the sixth day of culture. Stained in Ehrlich's hematoxylin. 0.75 actual size. *A*, colonies in fresh medium; *B*, colonies in conditioned medium; *C*, colonies in conditioned medium which had been dialyzed against fresh medium as described in text.

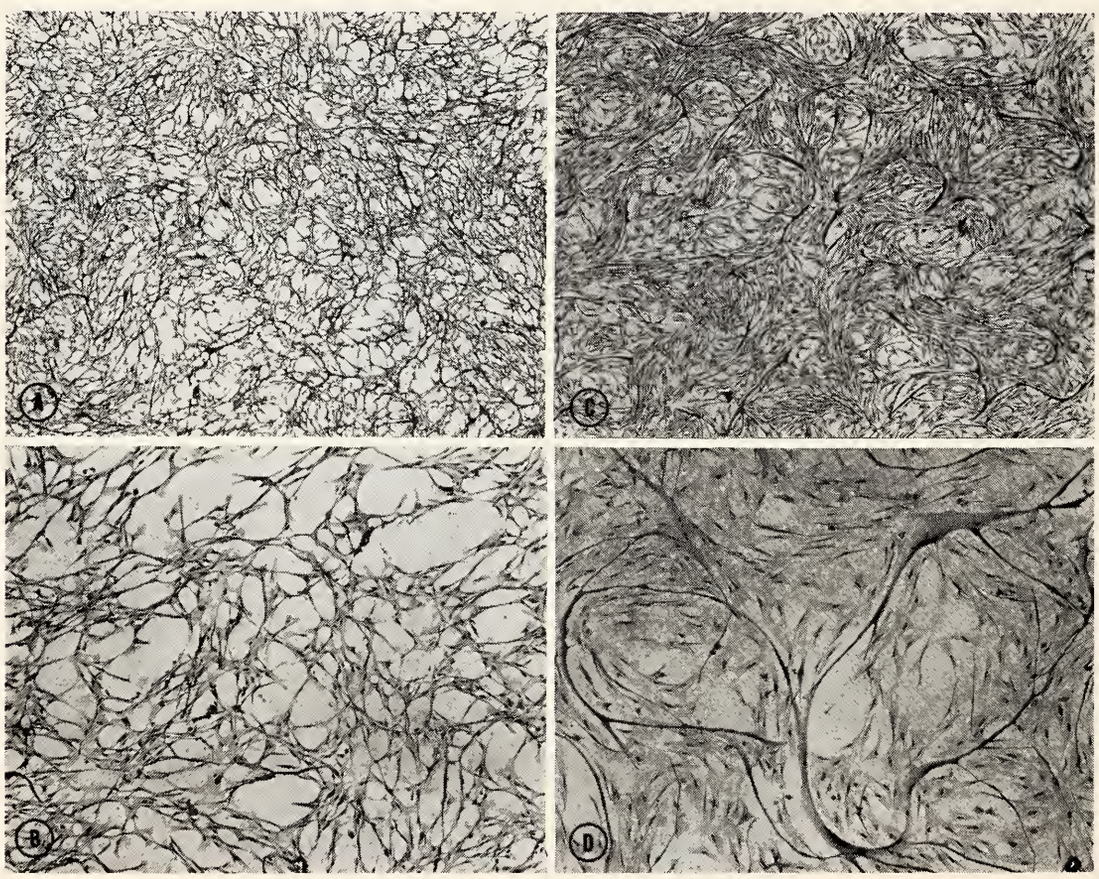

Fig. 18. Monolayer cultures initiated with 7.5×10^5 cells in 5-cm petri plate. Cultures fixed and stained after 2 days of cultivation on slowly rotating turntable. *A*, culture in fresh medium, ×17.5; *B*, as above, ×50; *C*, culture in conditioned medium, ×17.5; *D*, as above, ×50.

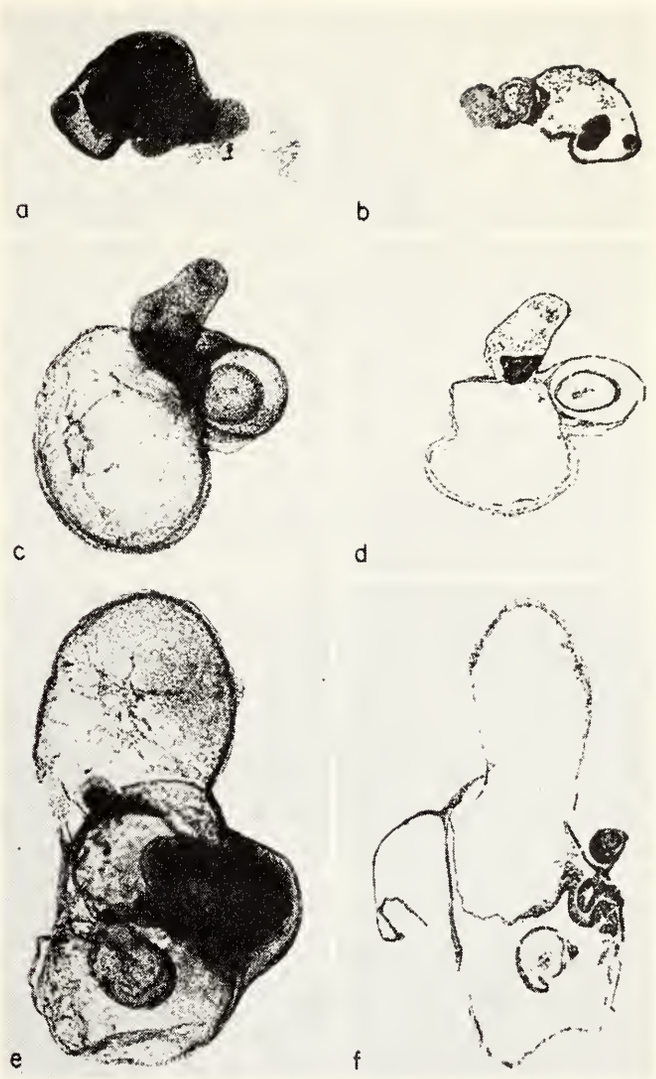

Fig. 20. Vesicles formed from fragments of heart-forming tissue, after 48 hours of incubation. Whole mounts and cross sections, ×50.

a, b vesicle 2315–10–1L
c, d vesicle 2215–8–2R
e, f vesicle 2222–15–3R

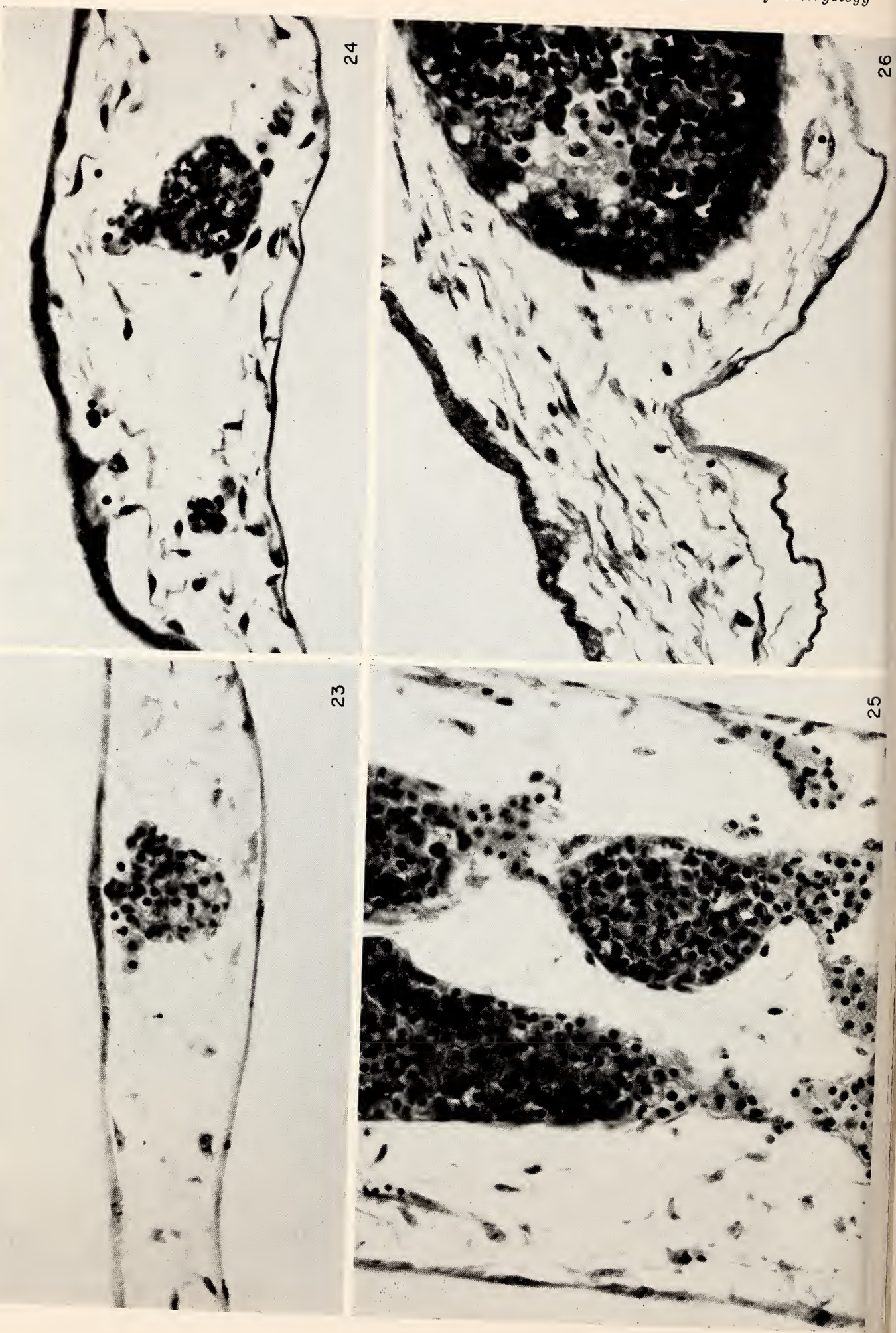
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Fig. 23. Cross section of a CAM fragment cultured for 6 days. Medium: series 1 (10% HS + 75% H + 15% HEE). ×400.
Fig. 24. Cross section of a CAM fragment cultured for 6 days. Medium: series 3 (20% CS + 65% H + 15% EE). ×400.
Fig. 25. Cross section of a CAM fragment cultured for 5 days. Medium: series 4 (20% HS + 80% H). ×400.
Fig. 26. Cross section of a CAM fragment cultured for 6 days. Medium: series 7 (10% CS + 50% H + 40% P). ×400.

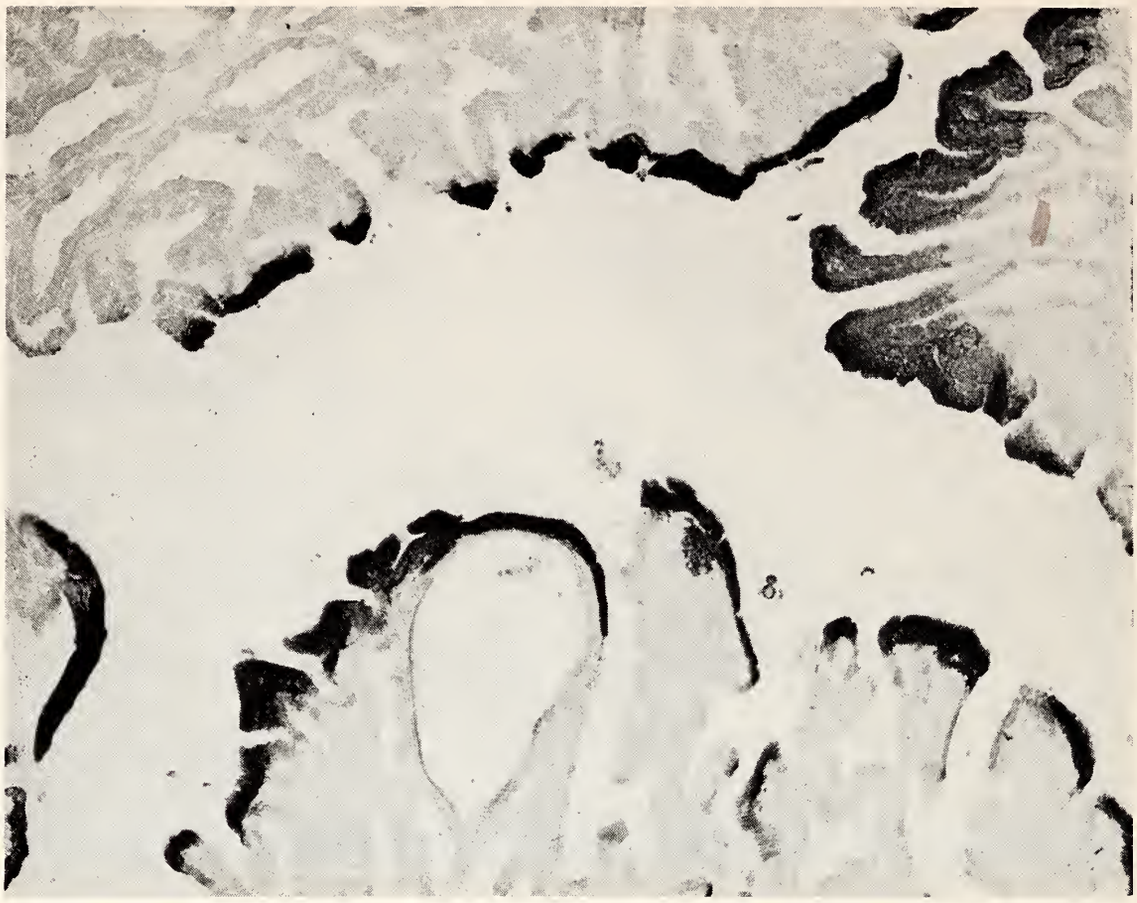


Fig. 29. Black staining by 0.5 per cent silver nitrate solution shows the distribution of a small amount of fluid injected into the uterine lumen. It did not enter glands, which presumably are closed in the living state. From the relationships shown, the original extent of the uterine lumen may be estimated. Dislodged cells are present in the uterine lumen, and a cyst near the center of the figure also contains some. L–441/3–4. ×150.

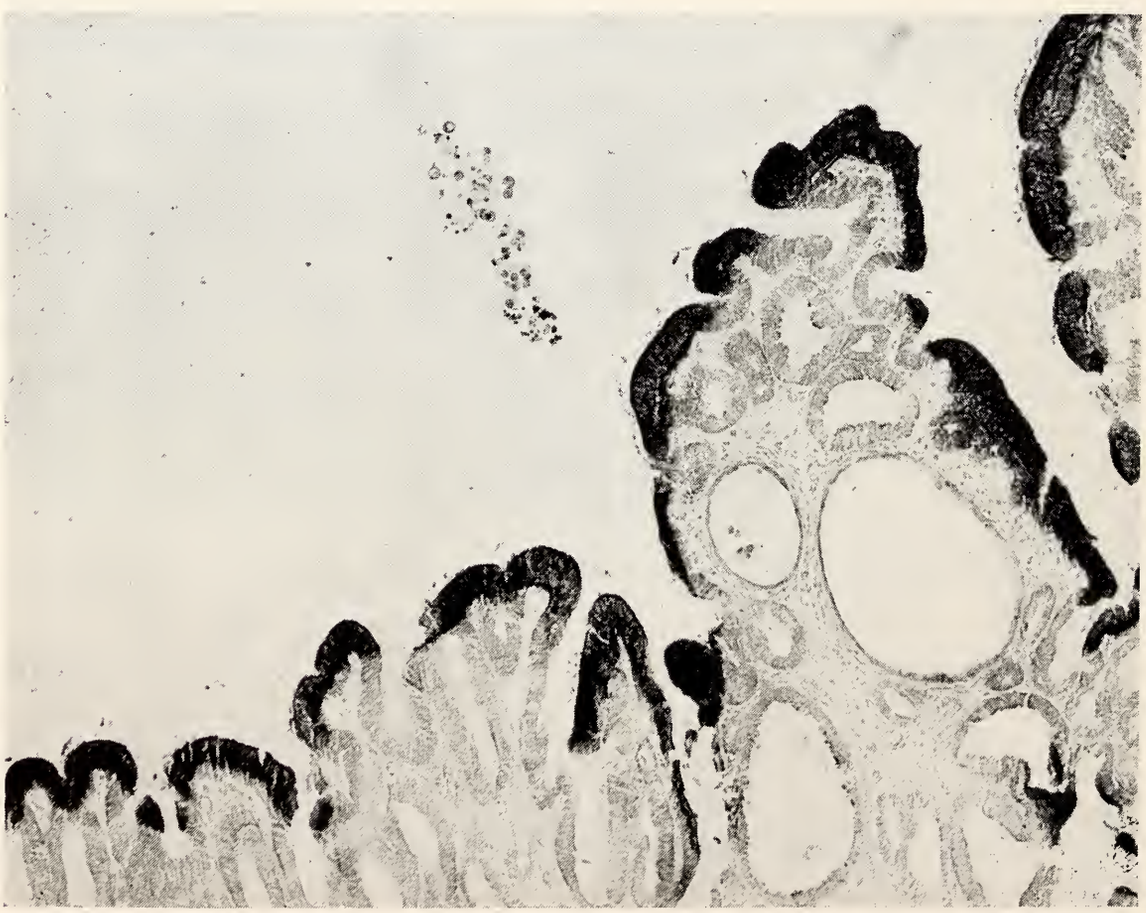


Fig. 30. A second view of the specimen of figure 29 shows shed cells in lumen and in cysts to better advantage. L–441/4–7. ×150.

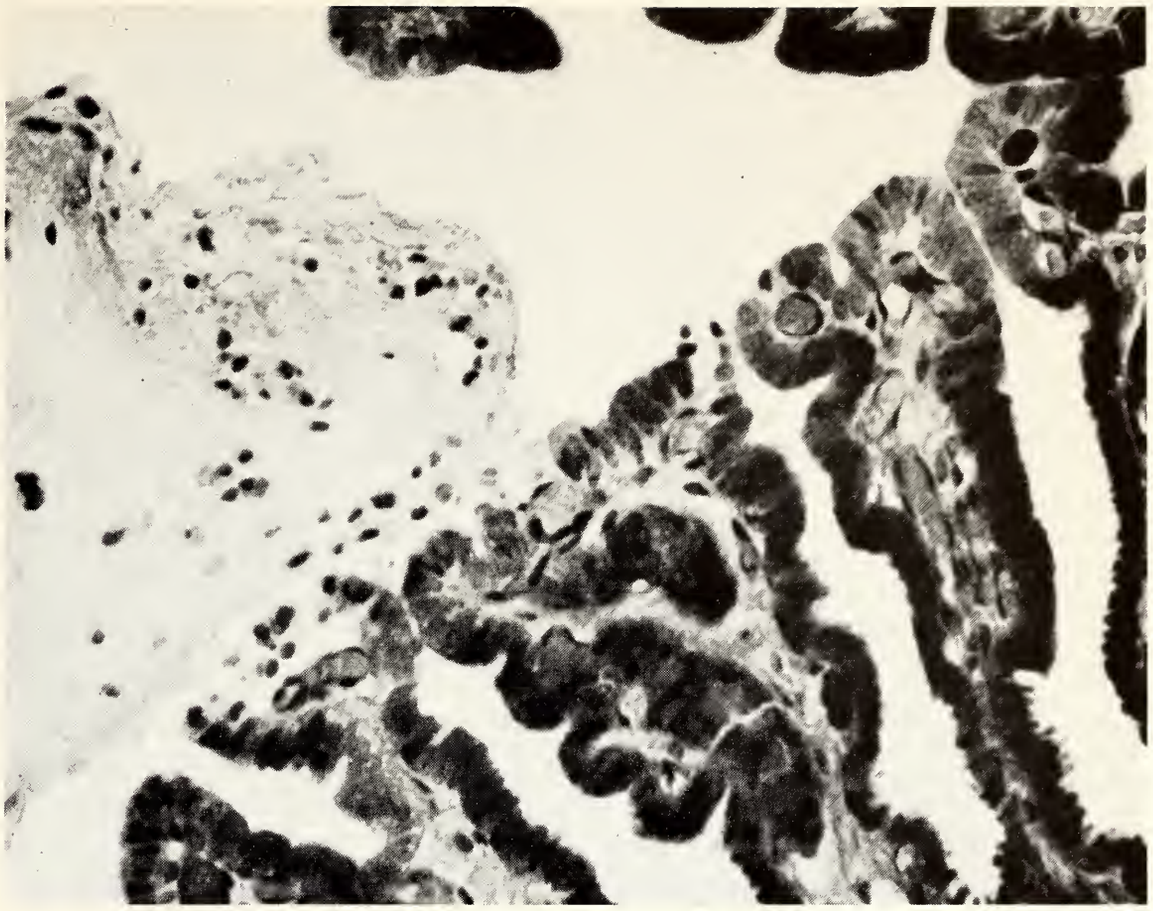


Fig. 31. Sodium carbonate–induced epivascular dissociation of uterine epithelium at the tips of endometrial folds exhibits several degrees of increasing severity progressing diagonally downward from right to left. A mass of cells, debris, and perhaps uterine secretion is in the uterine lumen. L–443–A/3–3. ×400.

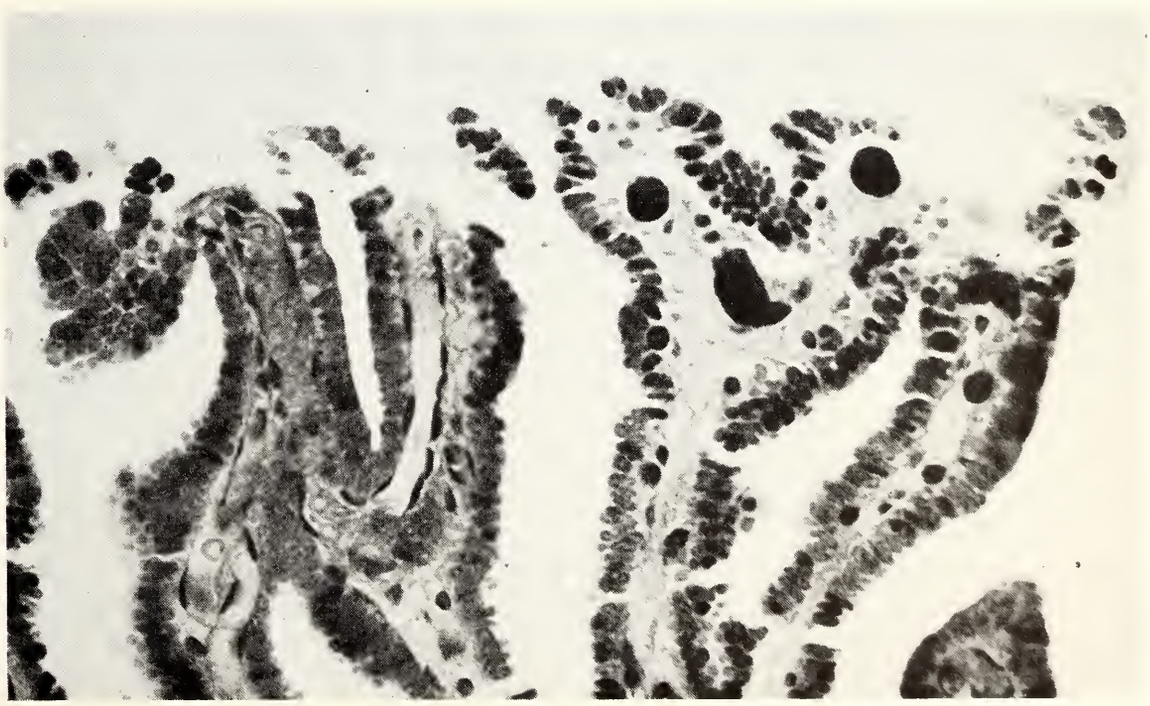


Fig. 32. Another region of the same specimen as figure 31 shows more severe alkali-induced epithelium loss. L–443–A/4–3. ×400.

Fig. 33. A third region of the same specimen shows extreme and even complete loss of epithelium, some loss of stroma, and (in the upper right corner) complete stripping down to capillary endothelium. The absence of hemorrhage is surprising. L–443–A/test slide-2. ×400.

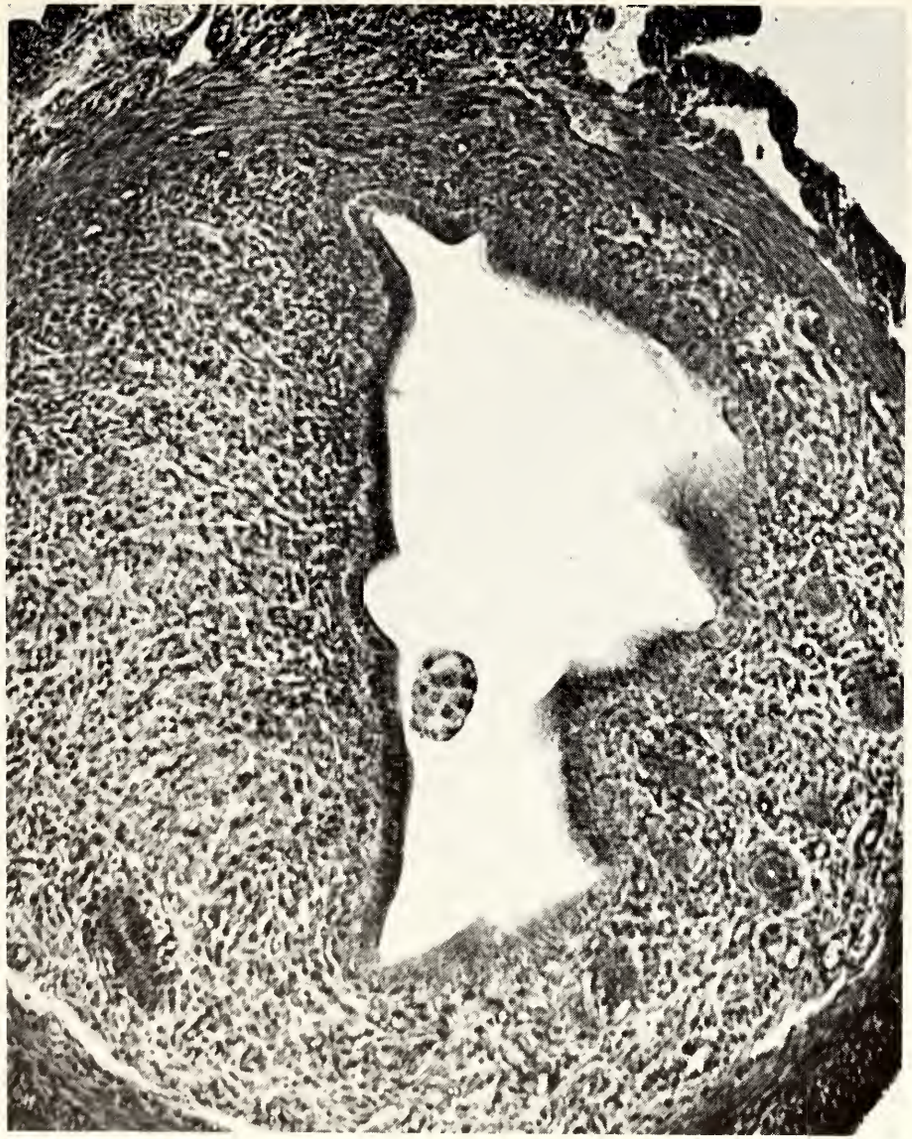


Fig. 34. Blastocyst with abembryonic pole toward mesometrium, lying in the middle of the uterine lumen. 80 hours postcoitum. ×150.

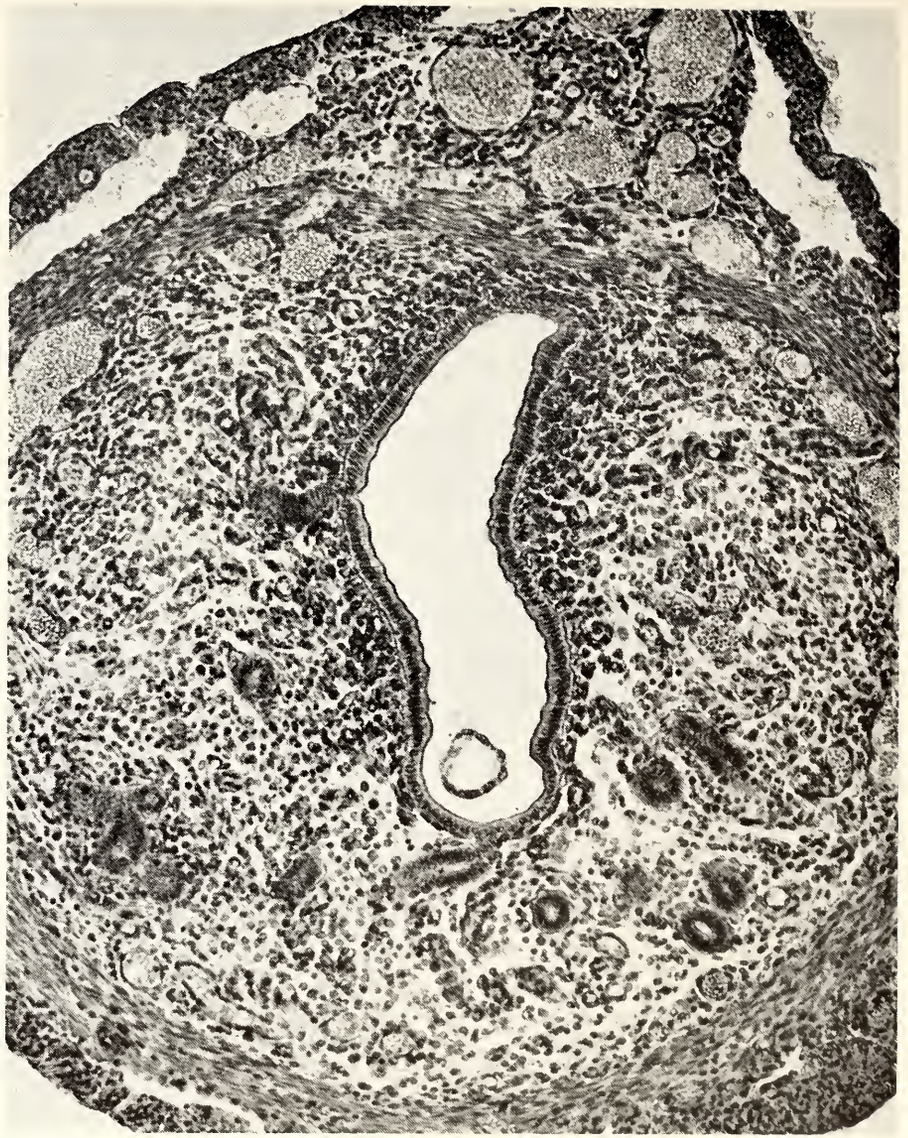


Fig. 35. Blastocyst properly oriented in antimesometrial "pocket." Walls of lumen pulled apart in preparing section. Blastocyst not yet attached to epithelium. 96 hours postcoitum. ×150.

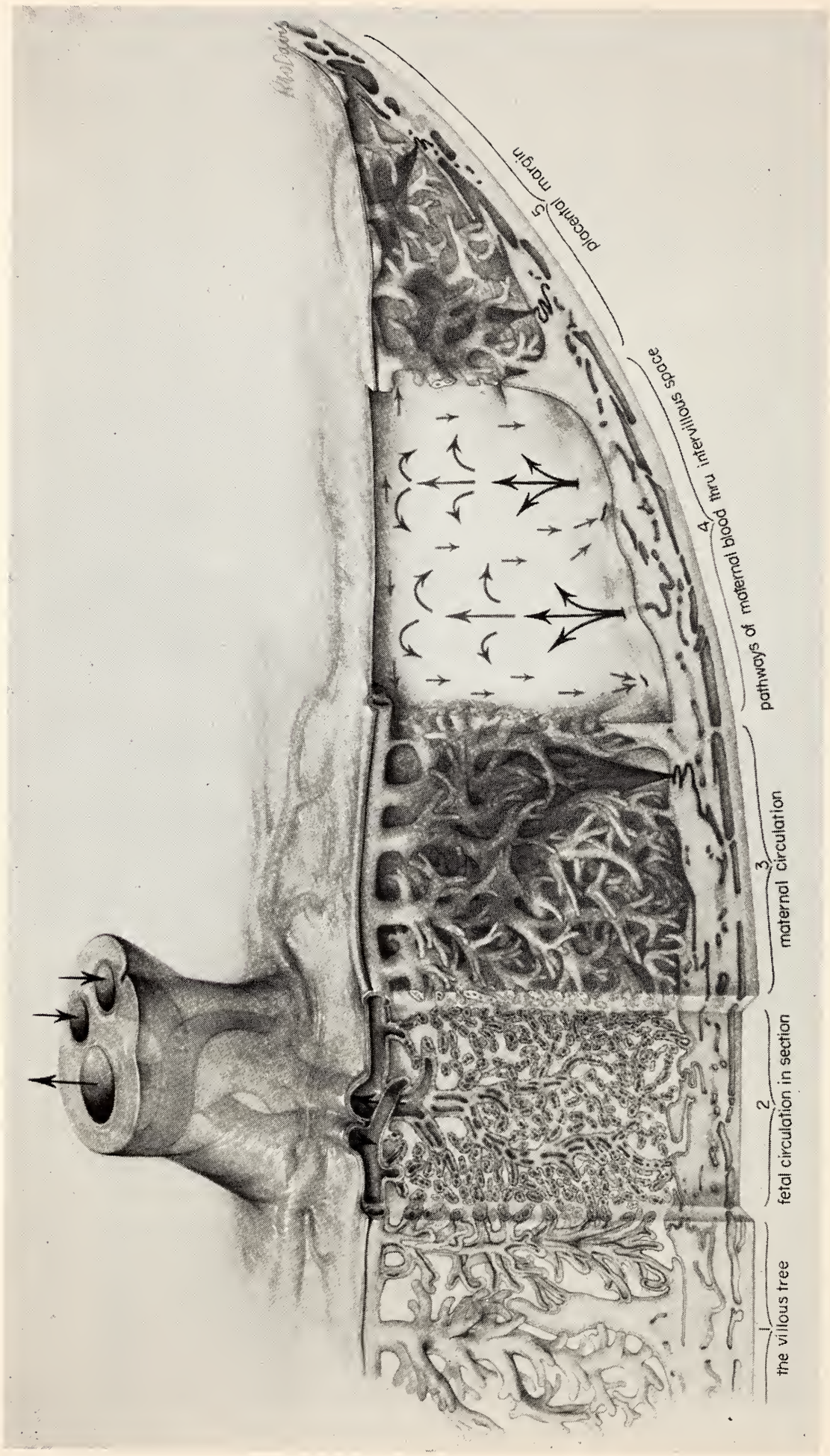


Fig. 36. Composite drawing of the placenta.

Department of Genetics

Cold Spring Harbor, New York

Berwind P. Kaufmann
Director

Contents

INTRODUCTION

"A BRIGHT prospect opens before us," said Hugo de Vries in his address at the dedication of the Station for Experimental Evolution in Cold Spring Harbor on June 11, 1904. "The matter of the evolution of organic life on this earth . . . is to be investigated to its very core. . . . We want to have a share in the work of evolution, since we partake of the fruit. We want even to shape the work, in order to get still better fruits."

Speaking as the chief apostle of the emerging science of genetics, de Vries could envision the future with a prescience that stemmed from his own pioneering efforts at the turn of the century in the rediscovery of Mendel's fundamental work and the formulation of the mutation theory. Within the framework created by this active participation, de Vries found himself in a position to serve as godfather of the new laboratory and offer advice to its staff about the experimental procedures requisite to its success and perpetuation. "Increase of knowledge of all the peculiarities which accompany the phenomenon of mutability is the most immediate requirement," he told them. "A broad foundation knowledge of phenomena is the most assured way to success. . . . During a long series of years I have fostered my conception of sudden mutability and cultivated my primroses for myself and for myself only. . . . [Now] I have to yield my much beloved child. But I do it gladly and without regret. It is the interest of the child itself which commands me. It will be better in your hands. . . . Pray have good care of it and educate it assiduously, that it may become one of the most brilliant parts of your work, a glory to this laboratory and to the institution that founded it, a pride to your country, and a bliss for humanity." (*Year Book 3*, pp. 39, 48, 49, 1905.)

Fifty-eight years later, having attained full stature among the biological sciences, genetics is providing man with the essential tools and techniques that will enable him to shape the work of evolution as envisioned by Hugo de Vries. In its progress from a nascent to a mature science, genetics has benefited immeasurably from the work carried on in the Station for Experimental Evolution—later to be known as the Department of Experimental Evolution and finally the Department of Genetics. The entreaty voiced by de Vries in 1904 has not been forgotten, and his expectations have been realized.

A survey of the accomplishments of the departmental staff up to the year 1942 was made by Dr. M. Demerec when he assumed the office of Director (*Year Book 41*, pp. 169–172). The scientific productivity of the two previous directors, C. B. Davenport and A. F. Blakeslee, and of their associates (including G. H. Shull, C. W. Metz, John Belling, A. J. Harris, Oscar Riddle, C. C. Little, and E. C. MacDowell) had indeed been "a glory to the laboratory and to the institution that founded it." Among biologists, at least, the name of Cold Spring Harbor had gradually acquired the connotation of pioneering effort and major discovery.

The pattern set in the early years has been maintained during the past two decades, which lie fresh in the memory of the writer. Investigations for the most part have fallen within the purview of cytogenetics in its broadest implications —an elucidation of the mechanisms of heredity in terms of the structure and behavior of chromosomes and other cellular constituents—utilizing the facilities that have become available in recent years for exploring the details of genetic mechanisms down to the level of molecular organization. Microorganisms have played a significant part in facilitating the meticulous analysis of genic fine structure by M. Demerec and his associates, and the skillful diagnosis of mechanisms of bacteriophage reproduction by Alfred

Hershey and his colleagues. Chromosomes of higher forms have served as material for Barbara McClintock's penetrating investigation of the elements that control gene action, and for the detailed studies, made by Helen Gay, Margaret McDonald, and the writer, of linear and lateral patterns of organization in euchromatic and heterochromatic materials and their roles in facilitating nucleocytoplasmic exchanges.

This, then, is a brief measure of past accomplishment and continuing endeavor. As this report goes to press, the Department is being terminated as a separate administrative unit of the Institution. Those of its staff who remain at Cold Spring Harbor in the Genetics Research Unit will cooperate with the new Laboratory of Quantitative Biology now being organized. It is anticipated that the accomplishments of this group will continue to sustain the international image of Cold Spring Harbor as a great scientific center.

The termination of the Department of Genetics as an administrative division of the Institution has been a matter of concern to many geneticists. But it is obvious that in a sense the Department as such has fulfilled its mission. Genetics now stands as "the core science of biology." It serves in this capacity to integrate the efforts of physicists, chemists, and biologists in resolution of the basic problems of living systems concerned with heredity, growth, and development. Its practitioners are now firmly established in numerous elaborately equipped laboratories in many lands. Nonetheless, much work remains to be done by all of us, under one patron or another, including the continuing Genetics Research Unit of the Institution. In the words of Thomas Jefferson, "Truth advances, and error recedes step by step only; and to do to our fellow men the most good in our power, we must lead where we can, follow where we cannot, and still go with them, watching always the favorable moment for helping them to another step."

Educational Programs

The scope of these activities was detailed in *Year Book 60*. More up-to-date information with respect to certain categories is given below.

Drosophila project. Distribution of mutant stocks of *Drosophila melanogaster* and copies of the *Drosophila Guide* has been continued throughout the year. A further impressive increase in the number of distributions is reported by the Curator of Stocks, Mrs. Buchanan. As compared with the figures recorded last year for the eight and a half months from October 15, 1960, to June 30, 1961—namely, 2704 Drosophila cultures and about 505 copies of the *Guide*—this year's tally shows that between July 1, 1961, and June 30, 1962, 4484 cultures were sent out in addition to 876 copies of the book. On a prorated basis, this represents an average increase in the neighborhood of 20 per cent.

As usual, the shipments were made to almost every state in the country, and a few went as far afield as Europe, India, and Australia. In this connection it is interesting to note that the Comisión Nacional de Energía Nuclear, of Mexico, completed during the year the publication of a Spanish translation of the *Drosophila Guide*, to be distributed to students of biology in Mexican schools.

It appears that this year by far the largest proportion of the requests, perhaps more than 70 per cent, came directly from students; about 20 per cent were made by high school teachers, and about 9 per cent by college teachers. The correspondence files record eight science fair awards to students who enlisted the aid of the service during the year, and in addition one honorable mention in the Westinghouse Science Talent Search.

Because of the widespread demand for this service, and the steadily increasing costs, a decision was made to institute on April 1 a small charge, sufficient to cover the expense for materials and postage involved in the distribution.

Other educational services. As reported

in *Year Book 60*, the Department has continued to respond, when possible, to requests for information, illustrative material, and bibliographic references received from teachers, students, and other members of the public. The resources of the library have remained available to students and biologists in the vicinity.

Drs. Gay and Kaufmann again participated in graduate courses in biology at Adelphi College. One graduate student of the College, Miss Myrna Thomas, sought and gained permission to utilize our facilities in undertaking the research required for the M.S. degree. Carried out under Dr. Gay's supervision, her project has been completed and the degree granted. Dr. Kaufmann has been serving as Director of Graduate Program Development in the Department of Biology at Adelphi, in an effort to establish a Ph.D. program. During March 1962, Dr. McClintock delivered several lectures in a course in advanced genetics at New York University. In February she took part in a conference at Raleigh, North Carolina, for the purpose of organizing a program of research in maize cytogenetics for four Latin American fellows who will be in residence at North Carolina State College during the coming academic year. Sponsored by the Inter-American Maize Improvement Program of the Rockefeller Foundation, this project will be under the direction of Dr. McClintock and Dr. William L. Brown.

Cooperative Activities

In the course of the year Dr. Gay presented seminar talks at the University of Michigan and Cornell University; Dr. Kaufmann was a symposium speaker at the A.I.B.S. meetings at Purdue University; Dr. McDonald attended the meetings of the Federation of American Societies for Experimental Biology in Atlantic City and a symposium on proteins and nucleic acids at Columbia University; Dr. Hershey lectured at the University of Oregon; and Dr. McClintock delivered lectures at Columbia University, New York University, North Carolina State College, and Yale University.

Under the joint chairmanship of Dr. McDonald and Dr. H. E. Umbarger of the Biological Laboratory, an interesting program of seminar lectures included the following invited speakers: Dr. Edward A. Adelberg of Yale University; Dr. V. G. Allfrey, Rockefeller Institute; Dr. Elias Balbinder, Scripps Institution of Oceanography; Dr. Maurice Bessman, Johns Hopkins University; Dr. Liebe F. Cavalieri, Sloan-Kettering Institute; Dr. Ludwig E. Feinendegen, Brookhaven National Laboratory; Dr. Allen Fox, Michigan State University; Dr. Kathryn Fuscaldo, St. John's University; Dr. A. F. Graham, Wistar Institute; Dr. Samson R. Gross, Duke University School of Medicine; Dr. Berwind N. Kaufmann, Johns Hopkins Hospital; Dr. Victor R. Larsen, Adelphi College; Dr. Arthur B. Pardee, Princeton University; Dr. J. L. Sirlin, University of Edinburgh; Dr. Stephen Taub, Harvard University; Dr. H. Ursprung, University of Zurich and Johns Hopkins University; and Dr. Geoffrey Zubay, Brookhaven National Laboratory. Other meetings were devoted to discussion of work within the Department or at the Biological Laboratory, presented by Drs. Arthur Chovnick, Helen Gay, Edward Goldberg, Christoph Jungwirth, B. P. Kaufmann, Margaret McDonald, Paul Margolin, and H. E. Umbarger.

As was reported briefly last year, the twenty-sixth annual Cold Spring Harbor Symposium on Quantitative Biology, sponsored by the Biological Laboratory, was held in the Department's Lecture Hall from June 4 to June 12, 1961. This symposium on regulatory mechanisms, with its brilliant and timely contributions to an understanding of gene action and interaction, served to underline the central role played by modern genetics in effecting a rapprochement among scientists of divers backgrounds and thus

facilitating the "breakthroughs" that are the hallmark of present-day genetics.

Although the laboratories at Cold Spring Harbor have always provided in many ways an ideal setting for such meetings as the Symposia on Quantitative Biology, an element of physical comfort has sometimes been lacking in assembly rooms during hot and humid weather. Therefore it is a pleasure to report that an air-conditioning system, installed in the Lecture Hall during the winter and spring of 1962, was completed in time for the twenty-seventh Symposium, which was held June 7 to 13, 1962. Fifty-four speakers took part in this program, "Basic Mechanisms in Animal Virus Biology," which was attended by approximately 200 participants.

The Lecture Hall was also utilized, in the summers of 1961 and 1962, for lectures and seminars of the series of advanced courses, offered by the Biological Laboratory to research workers, entitled Bacterial Genetics, Bacterial Viruses, and Microbiology of Vertebrate Cells and Quantitative Animal Virology. From August 29 to September 1, 1961, the annual Phage Meeting, organized by Dr. Hershey and Dr. Burgi, was attended by about 100 workers engaged in bacterial virus research. Abstracts of the talks presented at that meeting were mimeographed at the Department and issued in the form of a *Phage Information Service* bulletin. The 1962 Phage Meeting was scheduled to be held in Cold Spring Harbor from August 21 to 24.

The departmental program was augmented during the year by the presence of several fellows and guest investigators. Dr. Fred R. Frankel, Postdoctoral Fellow of the U. S. Public Health Service, continued to work in Dr. Hershey's group, which was joined in October 1961 by Dr. Edward Goldberg, Fellow of The National Foundation. Dr. C. C. Das, on leave from Allahabad University in India, held an appointment from the Biological Laboratory as an exchange visitor to work in Dr. Kaufmann's laboratory. During a visit to Cold Spring Harbor, Dr. Berwind N. Kaufmann extended his studies of the action of deoxyribonucleases and base analogues on chromosomes of human blood cells maintained in culture.

During the past year Dr. McClintock has continued as a research associate of Columbia University, and Drs. Gay, Kaufmann, and McClintock as guest investigators of Brookhaven National Laboratory; Dr. McClintock grew her maize crop at Brookhaven during the summer of 1962. Gay has served as an associate editor of *Biological Abstracts*; Kaufmann as an editor of *Biological Abstracts* and associate editor of *The Nucleus* and the *International Journal of Radiation Biology*; and Hershey on the editorial or advisory boards of *Genetics*, *Virology*, and the *Journal of Molecular Biology*. Kaufmann's term as a member of the Executive Committee of the Division of Biology and Agriculture, National Research Council, terminated on June 30, 1962, after many years of active association. As past-president of the Genetics Society of America, he has continued to serve on the Executive Committee of the Society.

Terminal Activities

As staff members in the Genetics Research Unit of the Institution, Dr. McClintock and Dr. Hershey are continuing to carry on their work in the laboratories at Cold Spring Harbor.

Dr. Gay, while remaining on the Genetics Research staff of the Institution, transferred her program in cytogenetics and electron microscopy to the University of Michigan, where she has been accorded the title Professor of Zoology. During the summer of 1962 she attended the Second International Congress of Radiation Research, at Harrogate, England, and participated in the International Symposium on Repair from Radiation Damage and Differential Radiosensitivity in Germ Cells, in Leiden, the Netherlands.

After his retirement Dr. Kaufmann traveled to Europe to participate in the international congresses on radiation biology. When he returned to the United States he took up research activities at the University of Michigan, where he had been appointed to the posts of Professor in the Department of Zoology in the College and Senior Research Scientist in the Institute of Science and Technology.

The Library

Mrs. G. C. Smith, Librarian, reports that between July 1, 1961, and June 30, 1962, 256 books were added to the Department library. Of these, 53 were purchased, 22 were received by gift or exchange, and 181 were volumes of newly bound periodicals. The number of books now catalogued, exclusive of unbound publications, exceeds 20,000.

The recent acquisition of volumes I (1857) and III (1860) of *Contributions to the Natural History of the United States*, by Louis Agassiz, which were presented to the library by Mr. Walter K. Earle of Oyster Bay, completes a set of four volumes of this historic work. Volumes II and IV represent an earlier gift from Mr. Earle.

The library served investigators and visitors at the Biological Laboratory as well as those at the Department of Genetics. In addition, interlibrary loan facilities were extended to Adelphi College, the Canadian Department of Agriculture in Ottawa, Dartmouth College, Glen Cove Community Hospital, Haskins Laboratories, Inc., New York Botanical Garden, Republic Aviation Corporation, State University College on Long Island, *Time* and *Life* magazines, Tufts University, University of Vermont, and Wilbur Cross Library in Storrs, Connecticut.

In the summer months of 1961 and 1962, supplementary library services were provided by the Biological Laboratory on evenings and weekends, for the benefit of students in the Laboratory's summer courses. Miss Ann Carroll served as assistant librarian during the extra hours of coverage.

Research Programs

Kaufmann and Gay and their associates have continued with cytogenetic and cytochemical studies of the changes occurring in the organization of chromosomes and cytoplasmic organelles during growth and differentiation of cells in higher organisms. Several new experimental approaches have shown that chromosomes behave at times as integrated fabrics, although their essential coding material is DNA. Agents reacting specifically with DNA—the enzymes DNase I and II, and the base analogue 5-bromodeoxyuridine—have been found to induce mutations and chromosomal aberrations; but a refined analysis of the results suggests that the whole DNA-RNA-protein chromosomal complex responds as a unit, not the DNA alone.

The finding that far-red radiation increases the frequency of double-crossover types in *Drosophila* has been interpreted by this group as evidence of the essential role of RNA in crossing over. The marked changes in basicity of the DNA-associated protein during late cleavage mitoses and during spermateleosis in *Drosophila* appear to be significant indices to functional states. Other changes of function have been detected in studies of cytoplasmic fine structure in the vegetative cell during microsporogenesis in *Tradescantia*. The highly metabolic vegetative cell increases greatly in volume during this process, and the cytoplasmic organelles—endoplasmic reticulum, mitochondria, and Golgi bodies—increase greatly in numbers. The changes observed in the Golgi body show that this organelle has different forms, depending on its functional or developmental state.

In continuation of her studies of DNase II, McDonald has noted that there is more than one enzyme of this type in calf spleen but apparently only one in salmon testes. The salmon-testis enzyme has a

molecular weight of about 52,000. It degrades heat-denatured DNA at only one-tenth the rate of its degradation of native DNA; nevertheless, its action on heat-denatured DNA appears to be due to an intrinsic property of the enzyme and not to an impurity.

Many samples of crystalline RNase contain a nonenzymic component which, on the basis of its ultraviolet absorption spectra and dialytic properties, is believed to be polynucleotide in nature. McDonald has isolated this component from various RNase samples and is now characterizing it. She has found that the sugar component is primarily deoxyribose.

McDonald and Gay have shown that the ability of crystalline RNase to reduce methyl green stainability of fixed biological sections without impairment of Feulgen colorability is due not to RNase per se, nor to the polynucleotide impurity, but to a heretofore unrecognized contaminating protein. This material has been separated from the bulk of the RNase by ion-exchange chromatography, but its chemical action has not yet been identified. Until that has been established, the previous hypothesis of these workers, that chromosomes contain a type of structural RNA connecting chains of DNA, should be held in abeyance. On the other hand, McDonald and Kaufmann have noted that even "chromatographically pure" cytochrome *c* is capable of reducing pyronin stainability, a phenomenon for which they still have no reasonable explanation.

McClintock reports that some mechanisms associated with control of gene action appear to be similar in maize and in bacteria. Certain gene-control systems, in both these organisms, are composed basically of two elements, an operator and a regulator. In bacteria, both elements may be located near the gene controlled by the system (structural gene); or only the operator may be located there, and the regulator elsewhere in the genome. In maize, a number of examples of the second type have been detected among both the *Spm* (Suppressor-mutator) and *Ac* (Activator) systems. Sometimes, however, in each system, it is evident that the regulator element initially occupied a position close to the structural gene. If the circumstances are similar to those in bacteria, the operator element likewise should have been present near the gene locus. Experiments have been conducted to test this inference, and all the results support the supposition that in such instances both the operator and regulator elements of a system are originally located close to the structural gene.

Hershey has found that measurement of the shearing forces required to break DNA molecules, combined with chromatographic measurement of the breakage, apparently offers a general method for determining molecular weights of DNA. This method reveals for T5 DNA a molecular weight of 84 million, indicating that there is one DNA molecule per phage particle.

The DNA of phage lambda is characterized by an unusual intramolecular heterogeneity of interbase bonding strengths; it also exhibits readily demonstrable intermolecular interactions. Hershey and his colleagues think these properties may be related, and may offer a clue to the forces that operate in synapsis of chromosomes.

Shearing forces that break DNA molecules can also produce local defects in structure. The two phenomena occur independently, however, and stirring at low temperatures and high salt concentrations permits breakage without denaturation, as required in biological experiments with molecular fragments.

GROWTH AND INHERITANCE IN BACTERIOPHAGE

A. D. Hershey, Elizabeth Burgi, Fred Frankel, Edward Goldberg, and Laura Ingraham

Several methods applicable to the characterization of DNA molecules have been developed in recent years. Examples are the optical analysis of thermal denaturation, chromatographic analysis, measurement of fragility, measurement of buoyant density, and specific enzymatic tests. Such methods make it possible to distinguish DNA's from different sources and to detect alterations produced experimentally. They do not, however, yield direct information about molecular structure. Structure is a more or less plausible inference that serves to unify diverse measurements, as did, for example, the notably successful model of Watson and Crick.

These remarks sufficiently explain why our work, though directed toward rather specific biological goals, is for the moment devoted to the exploration of physical techniques.

The Molecular Weight of T5 DNA

The above generalities are illustrated in experiments of the following type, by which we arrive at an estimate of the molecular weight of the DNA of T5 and, more important, evaluate a novel technique.

As a preliminary, a sample of DNA isolated from phage T2 is stirred under conditions that produce single, clean, transverse breaks near the centers of molecular length. The DNA is labeled with radiophosphorus, because the tracer permits analysis of extremely dilute solutions (less than 0.1 μg of DNA per ml) in which molecular interactions can be neglected. The stirred solution now consists of fragments of DNA molecules ranging in length from about $\frac{1}{3}$ to $\frac{2}{3}$ of the length (50 μ) of unbroken T2 DNA molecules. The fragments are next sorted out into length classes by chromatography on a column of methylated bovine serum albumin. The separation is possible because the basic protein in the column acts as an ion exchanger from which the acidic DNA fragments are removed at different salt concentrations, depending on their length. The resulting fractions are listed in table 1, where they are characterized by their sedimentation coefficients and corresponding molecular weights (*Year Book 60*).

The DNA of phage T5 exhibits a sedimentation coefficient of 48.5 S. From table 1 we see that fragments of T2 DNA sedimenting at the same rate have a

TABLE 1. Equal Fragility under Hydrodynamic Shear of T5 DNA and Fragments of T2 DNA of Similar Sedimentation Coefficient

Sample	Chromatographic Interval, %	Sedimentation Coefficient, S	Molecular Weight, $\times 10^{-6}$	Breakage at 630 rpm, %
T2 DNA fragments				
tube 30	10.6	31.8	37	--
tube 31	23.4	36.2	47	--
tube 32	36.6	38.6	55	--
tube 33	49.4	42.4	64	0
tube 34	60.7	44.1	71	15
tube 35	69.8	44.9	73	27
tube 36	77.9	47.2	79	51
tube 37	83.8	48.5	82	60
tube 38	88.5	49.5	86	73
tube 39	92.3	51.0	89	95
T5 DNA	----	48.5	?	65

molecular weight of 82 million. It does not immediately follow that this is the molecular weight of T5 DNA, however, because sedimentation constants depend on molecular shape as well as molecular weight, and are also influenced by molecular interactions that may differ from one DNA to another.

To circumvent the difficulties last mentioned, we apply a very different criterion to the same materials. The last column in table 1 gives the results of fragility tests, in which we measure the fraction of DNA broken when very dilute solutions are stirred for 30 minutes at 630 rpm. From the results with the T2 fragments, it is clear that molecular fragility is a sensitive index of molecular weight. T5 DNA exhibits a fragility corresponding to that of T2 fragments of molecular weight lying between 82 and 86 million. In other words, T5 DNA matches practically the same fragments of T2 DNA either in terms of sedimentation coefficient or in terms of fragility. The agreement permits the following conclusions.

1. A single relation between molecular weight and sedimentation velocity applies to both T2 DNA and T5 DNA.
2. A single relation between molecular weight and fragility under hydrodynamic shear applies to both DNA's.
3. The bonds broken by stirring must be of equal strength in both DNA's.
4. The molecular weight of T5 DNA is about 84 million, as compared with 130 million for the DNA of T2.
5. Since T2 DNA shows about the proper mass per unit length for a double helical structure, the DNA of T5 must also have this structure.
6. Since particles of phage T2 contain a single molecule of DNA, the same must be true of T5 particles, which are somewhat smaller.

These conclusions are interrelated in such a way that all must be correct or all or most of them incorrect. That they are correct follows as a plausible inference from the data presented, although no single measurement forces this conclusion. The alternative can be rejected on the basis that it would require an improbable set of coincidences.

Finally, independent checks of any of the stated conclusions reinforce them all. Such checks will be reported in another publication.

The DNA of Phage Lambda

Comparisons of the sort illustrated in table 1 can also bring to light DNA's having unusual properties. The DNA of phage lambda is an example. It is unusual in the following respects.

1. It does not emerge from our fractionating column in a single band, but trails over a wide range of salt concentrations and fails to elute completely.
2. It shows a broad range of denaturation temperatures, similar to that of the bacterial DNA's and in contrast to the exceedingly narrow range characteristic of other phage DNA's. A comparison with the DNA of phage T1, chosen for its similar composition and molecular size, is made in figure 1. The flatter curve for lambda DNA probably reflects the character of the individual molecules, though the possibility that the DNA is composed of a mixture of species with different

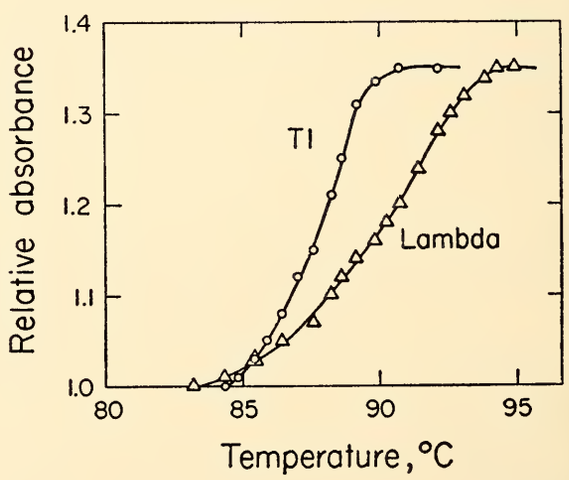


Fig. 1. Thermal denaturation of DNA from phages T1 and lambda.

melting temperatures has not been excluded.

3. Lambda DNA forms a broad or double boundary in the optical ultracentrifuge. Zone centrifugation according to methods developed by Britten and Roberts at the Department of Terrestrial Magnetism confirms that even in very dilute solutions the DNA exists in two or more differently sedimenting forms.

4. On stirring at low speeds, too low to break DNA molecules of the maximum molecular weight (50 million) represented by the DNA content of the phage particle, lambda DNA is converted into a form homogeneous by ultracentrifugal criteria and exhibiting the sedimentation coefficient (32 S) corresponding to the slowest form present in unstirred DNA. The behavior on heating and chromatography is not affected by this treatment.

These properties suggest a diversity of molecular shapes or aggregation products, together with and perhaps related to other structural peculiarities responsible for the unusual response to heating. It is evident that different DNA's do exhibit idiosyncrasies not yet accounted for in structural terms, and it is interesting that an example should be found in phage lambda, itself a biological curiosity in several respects.

A cursory examination of DNA from phages T1 and P22, as well as more thorough studies of T2 and T5, has not revealed comparable unexpected properties. Since lambda is a phage of well known and complex genetic properties, we propose to continue our physical studies in the hope of reaching conclusions of biological pertinence.

The Nature of Self-Protection

Some years ago we noted that the breakage of DNA by stirring depends strongly on the concentration of the solution. In more recent work we find that, as more and more dilute solutions of T2 DNA are subjected to stirring, susceptibility to breakage increases to a maximum at a concentration lying between 0.2 and 0.1 μg/ml. Further dilution is without effect. At 0.2 μg/ml, the molecules are separated by an average distance of about 28 μ, or half the actual length of the molecules. Self-protection is therefore a remarkably sensitive index of molecular interactions.

Such interactions could involve specific intermolecular forces of biological interest. We have looked for evidence of such forces by testing the ability of T2 DNA to protect that of T5, and vice versa. We find that the protection is not specific and therefore does not involve specific intermolecular bonding.

The protective effect greatly depends on the length of the molecules. We suspect that it results simply from their viscous drag, which may reduce the maximum local shear rate in the stirring vessel without, of course, affecting the average rate of shear in the vessel as a whole.

Local Denaturation by Hydrodynamic Shear

For a number of experimental purposes it is desirable to be able to fragment DNA molecules without producing unwanted side effects. In the course of our work with T5 DNA we found, however, that stirring can produce denaturation as well as breakage. In order to understand, and the better to avoid, such denaturation, we studied it in some detail. The principal facts are the following.

1. When T5 DNA is stirred in salt solution at 25°C at speeds just sufficient to initiate breakage of the molecules, subsequent chromatography reveals that a considerable fraction of the DNA has been altered in such a way that it attaches irreversibly to the basic protein in the column.

2. Such denaturation can be demonstrated also by the action of Lehman's phosphodiesterase, a bacterial enzyme that acts on denatured but not native DNA.

3. The extent of denaturation, measured by either of these methods, increases

as the salt concentration at the time of stirring is lowered or the temperature raised. Salt concentration and temperature do not affect breakage, however. At 5°C and 0.6 *M* NaCl, or at 25°C and 2.6 *M* NaCl, DNA can be broken with very little denaturation.

4. The speed of stirring is also critical, depending, of course, on the other variables mentioned. At 25° in 0.6 *M* NaCl, little denaturation is produced at speeds too slow to cause breakage. Remarkably, rapid stirring also fails to produce denaturation, which requires a critical stirring speed just sufficient to break the molecules slowly. At higher temperatures and lower salt concentrations, denaturation can be produced either without breakage, by stirring at low speeds, or in spite of rapid breakage caused by stirring at high speeds.

5. The existence of a critical stirring speed for denaturation is explained by the fact that DNA fragments produced without denaturation by stirring at a low temperature are relatively resistant to denaturation on restirring at a higher temperature.

6. The critical speed at which denaturation occurs varies with DNA concentration in the same way as the speed required to produce breakage: high concentrations protect against both denaturation and breakage.

7. If a sample of DNA is stirred under conditions that produce both partial denaturation and partial breakage, the denaturation can be demonstrated in both broken and unbroken molecules. Similarly, molecules previously denatured without breakage can be shown to exhibit about the same susceptibility to breakage on restirring as their native counterparts. Breakage and denaturation are independent events.

8. The denaturation produced by stirring does not alter the characteristic melting curve of the DNA.

9. When DNA denatured by stirring is restirred at low temperature to reduce the size of the fragments, the fraction that will subsequently pass a column is greatly increased but the susceptibility to phosphodiesterase is not affected.

10. This type of denaturation cannot be repaired by a regime of gentle heating and slow cooling.

11. Denaturation showing all the above characteristics can also be produced by heating DNA in the absence of shear to temperatures (84° to 88°) near the midpoint of the melting curve.

These results can be interpreted in the following way.

When a DNA solution is heated to a characteristic range of temperatures, the weak bonds responsible for maintaining the two-stranded configuration are loosened and local separation of strands is permitted, even though a sufficient number of bonds persists to hold the two chains in apposition. This effect can be measured by the increased absorption of ultraviolet light as the temperature is raised. A major factor in the process is the purely mechanical effect of Brownian motion. When the solution is cooled at this stage, the original structure is regained as far as can be determined by optical measurements. By chromatographic or enzymatic tests, however, local molecular lesions can be shown to persist. If the molecules are now fragmented without further denaturation, the denatured and undenatured regions of individual molecules are separated and the weight fraction of the DNA that can pass a column is increased.

Subjecting the molecules to shearing forces can produce the same result at low temperatures: in effect such forces merely lower the temperature of thermal denaturation, as does decreasing the salt concentration. The stresses generated by shear are of a special character, however, in that they depend not only on the speed of stirring but also on the length of the molecules. They are localized, moreover, near the centers of molecular length. Thus if a collection of fragments of different lengths is subjected to stirring at a given speed, the shortest ones will

survive because subjected to little stress, the longest will quickly be broken to fragments that are likewise resistant to denaturation, and only those of a critical length will resist breakage and at the same time undergo the repeated mechanical distortions that eventually produce permanent local denaturation. Only if the original population consists of molecules of uniform length can a majority of them be denatured in this way at low temperatures, and then only at the critical speed of stirring appropriate to that length, namely, the maximum speed that just fails to cause rapid breakage. If the temperature is sufficiently low and the salt concentration sufficiently high, the stresses required for denaturation exceed those required for breakage, and denaturation cannot be observed.

As far as we can surmise, permanent denaturation of this type, whether the consequence of gentle heating or of stirring, must result when locally separated strands rejoin out of register, producing unpaired loops or similar structural irregularities. As can be demonstrated with fully denatured DNA, such structures have a strong affinity for the basic protein of our fractionating columns and are the natural substrate for Lehman's phosphodiesterase.

In short, local denaturation of DNA molecules can be produced by either heating or stirring, the effects of which are identical except in one respect. When hydrodynamic shear is a dominant cause of denaturation, the molecular lesions tend to be central with respect to molecular length. Heating in the absence of shear, on the contrary, tends to produce terminal defects. These differences are readily accounted for theoretically and can be demonstrated experimentally.

The Reversibility of Thermal Denaturation

When a solution of T5 DNA is heated to 100°C for 10 minutes and then quickly cooled in ice water, relatively little denaturation is evident by optical criteria and then only if the measurement is made at about 45°C. By chromatography or by tests with phosphodiesterase, however, complete denaturation can be demonstrated. Thus denaturation produced at 100°C is irreversible on rapid cooling, but optical criteria are poor measures of it.

As is well known, measurements of the absorption of ultraviolet light serve admirably in following the progress of denaturation during the actual heating (fig. 1). According to this criterion, T5 DNA is half denatured at 85°C. However, if the solution is heated to 85° and then cooled, either rapidly or slowly, little denaturation is detectable by enzymatic tests. What little there is is due to small imperfections in many of the molecules, which prevent them from passing a fractionating column.

Thus denaturation on heating occurs simultaneously in all the molecules but is largely reversible unless a temperature of about 90° is exceeded. The irreversible event occurring at this temperature is not reflected in the optical density measurements; it undoubtedly consists in the unwinding of the polynucleotide chains of the helix, which have already largely separated at lower temperatures.

The behavior described above is presumably characteristic of a molecularly homogeneous DNA and explains a puzzling feature of experiments performed in the past with inhomogeneous DNA preparations. In the earlier experiments it was found that progressive denaturation involved the irreversible "collapse" of individual molecules at different temperatures, indicating that the denaturation of a given molecule was an "all or none" event.

It is now clear that it is not the denaturation measured by optical means (i.e., the loss of regular structure) that constitutes the all-or-none event, but the subsequent separation of chains. And the failure to observe partly denatured molecules after heating to intermediate temperatures and subsequent cooling (by electron microscopy, for example) is due

to the fact that partial denaturation of individual molecules is reversible on cooling. Our experiments now show that the recovery is often imperfect, but the resulting local imperfections that we see were not detected by the means previously employed.

It may be added that the reversibility of denaturation of a sample of DNA heated to the middle range of its melting curve should provide a new criterion of molecular homogeneity applicable, for instance, to the DNA of phage lambda.

Replicating DNA of Phage T2

DNA undergoing replication during growth of phage T2 can be studied in two ways: first, by infecting bacteria with isotopically labeled phage particles and then examining the labeled DNA subsequently isolated from the infected cells; second, by labeling DNA synthesized after infection, which is exclusively viral, and examining it. In either method chloramphenicol can be added to prolong the period during which DNA synthesis can be observed without complications due to the re-formation of phage particles.

Experiments of this type reveal at least two forms of DNA that differ from the finished molecules finally incorporated into phage particles. These are as yet poorly characterized, and little can be said about them except that they do not include any appreciable fraction of low-molecular-weight DNA, either single or double stranded.

On the other hand, the cells always contain a considerable fraction of their total DNA in a form indistinguishable from that found in finished phage particles. This finding shows that a mechanism for the preservation and determination of molecular length operates continuously during replication, and not only at some terminal stage in the formation of a phage particle—a conclusion pertinent to several hypotheses concerning genetic mechanisms.

Conclusion

The phage DNA's provide favorable materials for investigation, for three reasons. First, they can be isolated in a molecularly homogeneous state, a circumstance that permits for the first time satisfactory correlations between gross structure and biological function. Second, the synthesis of phage DNA can be studied in infected cells that have proved amenable to metabolic experimentation in the past. Third, intensive genetic study of a few phage species is yielding results to which physical and chemical findings can be constantly referred.

In the present state of knowledge concerning both genetic mechanisms and molecular structure, work of a purely exploratory nature is called for as well as efforts directed toward well defined goals.

TOPOGRAPHICAL RELATIONS BETWEEN ELEMENTS OF CONTROL SYSTEMS IN MAIZE

Barbara McClintock

In *Year Book 60*, parallels were drawn between gene-control systems in maize and those in bacteria. In both organisms, the comparable systems are composed, basically, of two genetic elements: an "operator" element, located adjacent to the structural gene(s) and directly controlling genic activity; and a "regulator" element that in turn controls the functioning of the operator element. Each operator responds only to a specific regulator. Therefore, each operator with its corresponding regulator represents a gene-control system. In bacteria, the position of the regulator element on the chromosome is not the same in all examined systems: it may be located either near by or at a distance from the

operator. Probably the elements of a control system in maize express similar topographical relationships; evidence supporting that probability will be presented here.

In maize, the controlling elements of a system are transposable. Therefore, different genes may come under the control of the same system, or the same gene under the control of different systems. Inception of control of gene action by a particular system sometimes occurs when the operator of the system is inserted near the locus of the gene, the regulator element being located elsewhere in the chromosome complement. At other times, inception of control is associated with the appearance of the regulator near the locus of the gene. In each such example that has been examined adequately, however, a clearly expressed two-element system has subsequently arisen, the operator element residing near the gene locus and responding to the regulator element, now located elsewhere in the chromosome complement.

With advancing knowledge of control systems in bacteria and of the topographical relations among individual components of a system, the cases in maize in which the regulator element of a known system initially occupies a position close to the structural gene require close scrutiny. In maize genetics, the devised test methods allow ready detection of the presence of the regulator element of a system, either when it is located close to the gene under the control of the system or when it is located elsewhere. When the regulator is close to the gene locus, the concomitant presence of the operator element is not so readily detected. Nevertheless, as was stated above, a clearly expressed two-element system of control of gene action does subsequently appear. The origin of such a system poses several critical questions: Are both elements of the system initially located close to the structural gene, and does the two-element system arise by removal of the regulator only? Or can a regulator element alone directly modify the action of a gene when located close to it, and does the two-element system that subsequently appears result from an alteration of the regulator element which converts it into an element that thereafter behaves as an operator?

Experiments eliciting direct answers to the above questions may not be conducted readily with maize. Nevertheless, certain relevant facts are known. Although it is possible to consider that insertion of a known regulator element close to a structural gene may initiate control of action of the gene, and also that an operator element may originate by some modification of the regulator, no certain evidence has yet been obtained of the reverse process, that is, conversion of an operator into a regulator. Moreover, examination of the behavior of each of the elements of a two-element system has shown that transposition of both elements to new locations in the chromosome complement may occur coincidently in a single nucleus, so that the operator is removed from the locus of the gene and the regulator is moved to a new location in the chromosome complement. In other cells, on the other hand, only one of the elements is transposed at a given time.

Now, if it is assumed that both an operator and a regulator are located adjacent to the structural gene in those instances where only the presence of the regulator at that location can be determined with certainty, the following possibilities are predictable. The operator and regulator might undergo simultaneous transposition, leaving the structural gene with neither element adjacent to it. Other transpositional events might remove only one of the elements, the complementary element of the system remaining in location. With regard to control of gene action, the effects produced by these kinds of transposition would lead to three distinctly different consequences. Removal of both elements would release the structural gene from control by the system to which the

elements belonged, and genetic tests would reveal the absence of the regulator element from the vicinity of the gene. Removal of the operator element only would likewise release the structural gene from control by the system to which it belonged; but the regulator element would still be located close to the gene locus. Removal of the regulator alone, however, would give rise to a clearly expressed two-element system of control of gene action—the same system that was operating before the transposition. Moreover, the location of the regulator element at some distance from the structural gene, or in another chromosome of the complement, could readily be determined.

The Cold Spring Harbor cultures include five identified instances in which the initial location of the regulator element of a known control system close to a structural gene resulted in control of the gene's action by the system to which the regulator belonged. In the three instances that have been adequately examined so far, all three of the expected consequences of transpositions outlined above have been confirmed. The evidence is reviewed below.

Three of the five examples involve the *Ac* (Activator) system, and two the *Spm* (Suppressor-mutator) system. Insertion of *Ac*, the regulator element of the *Ac* system, close to the locus of the bronze (*Bz*) gene in chromosome 9 initiated control of action of this gene by the *Ac* system. The modified locus was designated bz^{m-2}, and some discussion of it appears in *Year Books 54* and *55*. Two independently occurring insertions of *Ac* close to the locus of the *Wx* (waxy) gene in chromosome 9 resulted in control of action of this gene by the *Ac* system. These modified loci were designated wx^{m-7} and wx^{m-9}. Insertions of *Spm*, the regulator element of the *Spm* system, near the locus of the A_1 (anthocyanin) gene in chromosome 3 gave rise to two modifications designated $a_1{}^{m-2}$ and $a_1{}^{m-5}$. Extensive examinations have been made only of bz^{m-2}, $a_1{}^{m-2}$, and $a_1{}^{m-5}$.

Origin from $a_1{}^{m-5}$ of a Two-Element Control System

The original isolate of $a_1{}^{m-5}$ had an *Spm* element located close to the A_1 gene; the degree of closeness was not made apparent in the initial tests of 28 plants carrying $a_1{}^{m-5}$. In 18 of these plants, more than two *Spm* elements were present; 6 had two *Spm* elements, one obviously linked with $a_1{}^{m-5}$; and 4 had one *Spm* element, linked with $a_1{}^{m-5}$. The location of *Spm* in the immediate vicinity of the A_1 gene was not recognized in the tests conducted with these last 4 plants, because frequent transpositions of *Spm*, occurring in some sporogenous or presporogenous cells, resulted in the production of a number of gametes in which *Spm* occupied a new location in the chromosome complement. The intimate proximity of *Spm* to the A_1 locus was made evident, however, in tests of certain progeny of these plants, in which transpositions of *Spm* occurred so late in development that few or no gametes carried a transposed *Spm* element.

With respect to A_1 gene action, transposition of *Spm* away from the locus of $a_1{}^{m-5}$ leads to one or the other of two quite different results: either release of gene action from the control of the *Spm* system, or continued control by that system. Approximately half of the transpositional events that effect release from control of the *Spm* system result in an A_1 gene capable of a high level of action. The other half of these events bring about a much lower level of A_1 gene action, or occasionally the absence of such action, in both plant and kernel.

That the *Spm* system can continue to control A_1 gene action after some transpositions of *Spm* away from the $a_1{}^{m-5}$ locus was discovered in two ways. One of these utilized selected kernels on ears of plants of the constitution $a_1{}^{m-5}$ Sh_2/a_1 sh_2 (sh_2, shrunken endosperm; a_1, standard recessive allele of A_1) that had *Spm* located close to $a_1{}^{m-5}$, produced by a cross with plants homozygous for a_1 and

sh_2 and having no *Spm*. Plants were grown from 29 Sh_2 kernels that exhibited uniform anthocyanin pigmentation in the aleurone layer, intense in some kernels and pale in others. The plants were tested for presence or absence of *Spm* and, if it was present, for the number of *Spm* elements and their relative locations in the chromosome complement. When *Spm* was absent, it was introduced by means of a cross into the endosperm nuclei of kernels on the ears produced by these plants, in order to test the expression in its presence of the A_1 gene in the Sh_2–carrying chromosome. The tests indicated that, in 28 of the 29 plants, action of the gene A_1 was no longer under the control of the *Spm* system; the same level of genic expression appeared both in the presence and in the absence of *Spm*. In some of these 28 plants, no *Spm* was present. In others, one or more *Spm* elements were present but were not located close to the A_1 gene. In 2 plants, however, *Spm* was found to be located very close to the gene, even though genic action had been released from the control of the *Spm* system.

The remaining plant of the 29 was derived from a pale-pigmented kernel. Tests for the presence of *Spm* were negative. Nevertheless, the action of the A_1 gene in the Sh_2–carrying chromosome remained under the control of the *Spm* system, as was shown when *Spm* was introduced by a cross of this plant with one carrying *Spm*. The response of the gene to *Spm* was similar to that given by the class II states of a_2^{m-1} described in *Year Book 57*. With this state of a_1^{m-5}, a medium level of A_1 gene action is expressed in plants and kernels that have no active *Spm* in their nuclei, but gene action is suppressed if an active *Spm* is present. Here, then, the *Spm* system continued to control the action of the A_1 gene although *Spm* no longer occupied a position close to it. A typical two-element system of control of gene action had evolved from an apparently one-element system.

The second demonstration that not all transpositions of *Spm* away from the locus of a_1^{m-5} release the genic action from the control of the *Spm* system was provided by an examination of plants derived from other selected Sh_2 kernels from ears produced by the same type of cross as that producing the 29 kernels whose constitutions are described above. Each of these kernels exhibited a markedly altered pattern of pigmented and nonpigmented areas, as compared with that of kernels carrying the original state of a_1^{m-5}. It was suspected that each of these kernels had received an a_1^{m-5} locus whose state had been altered in a cell of the a_1^{m-5}–carrying parent plant. To test this assumption, plants derived from 10 such kernels, each selected from a different ear, were examined, and extensive tests were subsequently conducted with the progeny of 4 of them. All 10 plants carried a modified state of a_1^{m-5}, and also an *Spm* element. In 3 of the plants, the single *Spm* had remained in intimate association with the a_1^{m-5} locus; that is, the event responsible for the alteration of state had not resulted in its removal to a more distant location. In the other 7 plants, however, *Spm* was located elsewhere in the chromosome complement, and in 6 of them it was not linked with a_1^{m-5}; in the seventh, it was located approximately 30 crossover units from a_1^{m-5}. Thus a typical two-element system of control of gene action was operating in each of these 7 plants, and *Spm* was its regulator.

The illustrations given above show that with a_1^{m-5} the three anticipated consequences of different types of transposition of elements of the control system were observed: (1) Release of A_1 gene action from control by the *Spm* system, associated with removal of *Spm* from the immediate vicinity of the gene. (2) Release of such control, not accompanied by transposition of *Spm*. (3) Continued control of A_1 gene action by the *Spm* system, after removal of the *Spm* element from the immediate vicinity of a_1^{m-5}.

Analysis of a_1^{m-2}

The analysis of a_1^{m-5}, just described, proceeded rapidly as soon as plants had been isolated that carried a single *Spm* element located close to the A_1 gene. The types of gene action produced by the stable mutations were not difficult to interpret; they appeared to express different levels of standard A_1 gene action. The behavior of the modified states was also readily interpretable. Those that were associated with the two-element system of control of gene action whose origins are described above followed the same rules that had previously been established for the *Spm* system.

Analysis of a_1^{m-2}, on the other hand, has been complicated. Although the original state is similar to the original state of a_1^{m-5}, in that *Spm* resides close to the A_1 locus and the *Spm* system controls A_1 gene action, the types of expression resulting when the gene is released from the control of the *Spm* system are distinctly different. There are two classes of mutants. The first has a phenotype resembling that produced by the standard A_1 gene. The other class is composed of a series of alleles, distinguished from the first class by the distribution and intensity of pigment in plant and kernel. In the kernel, the intensity of pigmentation in the aleurone layer is not uniform, so that kernels appear somewhat mottled. The different alleles in this class may be distinguished from one another by the degree of intensity of kernel pigmentation, which ranges from very faint to fairly dark. The plants also are pigmented, but the color develops slowly and is markedly affected by sunlight: the parts of a plant exposed to direct sunlight become intensely pigmented, whereas parts not so exposed remain light in color. Although some pigment develops in the mid-rib of the leaf and at its edge, very little or none develops in the leaf blade. The two classes of mutants are thus readily distinguished. The first will be referred to as "A_1" mutants and the second as "mottled" mutants.

Control of gene action at a_1^{m-2} by the *Spm* system is quite different from the control exercised by that system when the *Spm* element is not located near the controlled gene. For example, in the modified loci a_1^{m-1} and a_2^{m-1}, in the above-described derivatives of a_1^{m-5}, and in wx^{m-8}, gene action is suppressed by an active *Spm* element but is expressed in its absence or when it is present but inactive. With a_1^{m-2} (original state), however, the reverse is true: when *Spm* is inactive, the action of the gene is suppressed; when it is active, gene action is expressed. This fact could be determined because it was possible to select some a_1^{m-2}–carrying plants in which *Spm* was in an inactive phase of long duration, and others in which *Spm* was in an active phase of long duration. Some tests conducted with plants having *Spm* in an active phase of long duration will be considered first.

Location of Spm before and after release of control of gene action at a_1^{m-2} *by the Spm system.* With the original state of a_1^{m-2}, the location of *Spm* close to the A_1 gene was established by several types of test, commencing with a cross of plants of the constitution a_1^{m-2} Sh_2/a_1 sh_2 by plants that had no *Spm* and were homozygous for a specially selected state of a_1^{m-1} and also for sh_2. A_1 gene action in these last-named plants was under the control of the *Spm* system, involving an operator element located close to the A_1 gene and an *Spm* element located elsewhere in the chromosome complement. With this selected state of a_1^{m-1}, gene action is expressed in the absence of *Spm* (or when it is present in an inactive phase). Anthocyanin pigment appears in both plant and kernel. In the kernel, pigment of medium intensity is uniformly distributed over the aleurone layer. When an active *Spm* is present somewhere in the chromosome complement, gene action is suppressed until there occurs, in some cells, a response of the operator to *Spm*

that effects a release of gene action from the control of the *Spm* system. These releases occur in a relatively few cells late in the development of plant and kernel, and most of them lead to an expression of A_1 gene action resembling that of the standard A_1 gene. Consequently, they give rise to a distinctive pattern of deeply pigmented dots in the kernel and small pigmented streaks in the plant, both appearing on a nonpigmented background. In plants and kernels carrying the original state of a_1^{m-2}, on the other hand, release of control of gene action by the *Spm* system may occur in many cells, both early and late in development. As is described above, such release may result either in a high level of A_1 gene action, a lower level that produces the "mottled" phenotype, or, rarely, a null expression of the gene. Thus, kernels carrying the original state of a_1^{m-2} and a fully active *Spm* exhibit both large and small pigmented areas of various intensities.

In the above-described cross, nearly all the Sh_2 kernels on an ear receive from the heterozygous parent either unmodified a_1^{m-2} or a modified derivative of it, and nearly all the sh_2 kernels receive the standard a_1 allele. This happens because crossing over between the locus of a_1^{m-2} and that of Sh_2 is very infrequent, not exceeding 0.12 per cent. The presence or absence of active *Spm* can be detected readily in the sh_2 kernels on the ears produced by the cross, and also in Sh_2 kernels that have received a gamete carrying a stable mottled mutant of a_1^{m-2}. If an active *Spm* is present in one such kernel, the distinctive pattern of deeply pigmented dots produced by the response of a_1^{m-1} to *Spm* appears in a mottled background. If *Spm* is absent, these dots are absent and the kernels exhibit only the mottled phenotype. Table 2 lists the phenotypes of kernels that appeared on some ears produced by the cross. The ratios of kernel types were not the same on all these ears. Nevertheless, except on ears of plants whose numbers are printed in italics, there was a direct relationship between the percentage of kernels in the Sh_2 class that received a germinal mutant of a_1^{m-2} and the percentage of kernels in the sh_2 class that received *Spm*. On ears in which all the Sh_2 kernels had received unmodified a_1^{m-2} there were no kernels in the sh_2 class that carried *Spm*. Among the ears bearing kernels that expressed germinal mutations of a_1^{m-2} the percentage of such kernels and the percentage of sh_2 kernels with *Spm* were directly related. (This correlation was exhibited among the kernels on ears having no detectable sectors derived from cells in which a stable mutation of a_1^{m-2} had occurred early in development. Ears with such sectors are not included in the table.) The correlation suggested that *Spm* was located very close to a_1^{m-2} in the heterozygous parents and that its removal from this location was associated with the origin of many of the stable mutations.

This possibility was also suggested by the phenotypes of kernels on ears produced by testcrosses conducted with other plants having the constitution a_1^{m-2} Sh_2/ a_1^{m-1} sh_2. Ears of 33 plants of this constitution were utilized in crosses with plants homozygous for a_1^{m-1} and sh_2 and having no *Spm*, and also with plants homozygous for a_1 and sh_2 and having no *Spm*. Table 3 shows the phenotypes of kernels that appeared on ears produced by the second cross. Again, a direct relationship will be noted between the percentage of kernels that received a germinal mutant of a_1^{m-2} and the percentage of kernels in the sh_2 class that received *Spm*.

The cross that produced the kernels entered in table 2 was conducted after the above-described correlation had been recognized. The ear-bearing parent plants in cultures 7979A and B, 7980A, and 7981A were derived from variegated, Sh_2 kernels on ears of plants 7799B–1, 7799B–6, and 7800A–5 of table 3. Plants were grown from kernels in the underlined classes in table 3 in order to test the conclusion that *Spm* resides close to unmodified a_1^{m-2} and that many of the

TABLE 2. Phenotypes of Kernels on Ears of Plants of the Constitution a_1^{m-2} Sh_2/a_1 sh_2 Produced by a Cross with Plants That Were Homozygous for a_1^{m-1} and sh_2 and Had No *Spm*

Plant Number	Phenotypes of Kernels							
	*Sh*₂ Class*					*sh*₂ Class†		
	Germinal Mutations			Variegated for A_1 and Mottled Spots	Percentage Germinal Mutations	Pale (No *Spm*)	Dots of A_1 in Colorless Background (*Spm*)	Percentage with *Spm*
		Mottled						
	A_1	No A_1 Dots (No *Spm*)	A_1 Dots (*Spm*)					
7979A–7	0	0	0	57	0	73	0	0
A–8	0	0	0	233	0	189	0	0
B–1	0	0	0	239	0	219	0	0
A–6	0	3	1	69	5.4	59	1	1.6
A–3	1	4	1	71	7.7	64	2	3.0
A–12	3	13	11	98	21.6	114	12	9.5
A–1	4	25	15	105	29.5	118	20	14.5
B–4	4	36	13	109	32.7	148	29	16.3
A–2	3	33	25	113	35.0	148	25	14.4
A–13	3	35	22	106	36.1	142	17	10.6
A–10	9	46	47	169	37.6	232	51	18.0
A–11	7	61	57	115	52.0	171	53	23.6
A–9	10	26	62	134	42.2	97	157	61.8
B–3	25	44	82	83	64.5	96	137	58.8
7980A–9	11	32	22	149	30.3	177	31	14.9
A–7	1	5	5	24	31.4	22	6	21.4
A–3	5	20	6	53	36.9	76	13	14.6
A–4	6	38	21	110	37.1	163	30	15.5
A–1	6	62	41	169	39.2	263	64	19.5
A–2	10	55	43	110	49.5	190	49	20.5
A–8	12	33	80	111	52.9	121	129	51.6
7981A–1	3	49	24	151	33.4	183	22	10.7
A–4	5	51	33	114	43.8	108	41	27.5
A–5	10	64	33	136	44.0	199	37	16.1
A–7	6	41	66	68	62.4	157	53	25.2
A–8	9	63	66	59	70.0	144	59	29.0

* In addition there was one pale, Sh_2 kernel.
† In addition there were three sh_2 kernels that received a_1^{m-2}.

germinal mutations arise when *Spm* is transposed to a new location in the chromosome complement. Among the 26 plants listed in table 2, 23 had one *Spm* element in the cells that gave rise to the testcross ear and 3 (whose numbers appear in italics) had two *Spm* elements. There were 4 additional plants, each also derived from an Sh_2 kernel whose endosperm was variegated. A mottled phenotype was expressed in these 4 plants rather than the phenotype produced in plants that commence development with unmodified a_1^{m-2}. The presence of a mottled mutant in these plants was confirmed by the kernel types that appeared on a testcross ear of each (rows 1–4, table 4). Since the endosperm of the kernel from which each of these plants arose started development with unaltered a_1^{m-2}, the event that produced the mottled mutant must have occurred during development of the female gametophyte in the parent plant, or in the kernel early in development of the embryo. All 4 plants had one or more active *Spm* elements. In 2 of them (7981A–3 and 7981A–6), one *Spm*, not

TABLE 3. Phenotypes of Kernels on Ears of Plants of the Constitution a_1^{m-2} Sh_2/a_1^{m-1} sh_2 That Had One Active *Spm*, Produced by a Cross with Plants Homozygous for a_1 and sh_2 and Having No *Spm*

Plant Number	Phenotypes of Kernels							
	Sh_2 Class				sh_2 Class			
	Germinal Mutations		Variegated for A_1 and Mottled Spots	Percentage Germinal Mutations	A_1	Pale (No *Spm*)	Dots of A_1 in Colorless Background (*Spm*)	Percentage with *Spm*
	A_1	Mottled						
7799B–1	0	0	201	0	0	214	1	0.46
7799B–6*	2	50	176	22.8	1	216	28	11.4
7800A–5	6	60	153	30.1	1	180	21	10.4
7984 –7	6	64	131	34.8	0	172	31	15.2
7984 –4	2	60	109	36.2	0	150	27	15.2
7984 –3	4	62	99	40.0	1	131	30	18.5
7799A	14	95	119	47.8	2	160	44	21.3

* In addition there was one colorless, sh_2 kernel on this ear. The plant derived from it had no *Spm*.

linked to the mutant locus, was present in the cells that produced the testcross ear. In the other 2, two *Spm* were present in the cells giving rise to the testcross ear—neither element linked with the mutant locus in plant 7980A–6, but one linked with it in plant 7981A–2.

The plant grown from the single sh_2 kernel containing *Spm* on the ear of plant 7799B–1 (row 1, table 3) proved to be a_1^{m-1} sh_2/a_1 sh_2 in constitution and had two independently located *Spm* elements in the cells that produced each of its tested ears.

Nine plants derived from the mottled Sh_2 class of kernels on the ear of plant 7799B–6 (row 2, table 3) were also tested for *Spm* constitution. No evidence of its

TABLE 4. Phenotypes of Kernels on Ears of Plants That Were Mottled-Mutant Sh_2/a_1 sh_2 in Constitution, Produced by a Cross with Plants Homozygous for a_1^{m-1} and sh_2 and Having No *Spm*

Plant Number	Phenotypes of Kernels			
	Mottled Sh_2 Class		sh_2 Class	
	No Dots of Deep Pigmentation (No *Spm*)	Dots of Deep Pigmentation (*Spm*)	Pale (No *Spm*)	Deep-Pigmented Dots in Colorless Background (*Spm*)
7980A–6	86	162	73	149
7981A–3	120	107	120	111
7981A–6	82	81	74	82
7981A–2	92	125	111	55
7980B–3	91	85	103	80
7980C–2	60	141	166	45
7980B–4	30	211	95	127
7981B–1	20	219	254	22
7981B–6	0	274	255	1
7981B–8	80	126	149	71
7981C–3	47	175	51	180

presence was shown by the kernels on the ears of 6 of these 9 plants; but it was present in the cells that gave rise to the testcross ear in the remaining 3 plants (7980B and C, table 4). Plant 7980B–3 had one *Spm*, not linked with Sh_2; plant 7980C–2 had one *Spm*, linked with Sh_2; and plant 7980B–4 had two *Spm*, one linked with Sh_2. Testcrosses conducted with 8 of the 12 plants derived from mottled Sh_2 kernels on the ear of plant 7800A–5 (row 3, table 3) produced no evidence of the presence of *Spm*. It was present, however, in the remaining 4 plants, as indicated in table 4. Very close linkage of *Spm* with the locus of the mottled mutant was exhibited by plant 7981B–6. Linkage of *Spm* with the locus of the mutant was expressed in plants B–1 and B–8. The ratio of kernel types on the ear of plant 7981C–3 (355 with *Spm*/98 with no *Spm*) suggests the presence of at least two *Spm* elements, not linked with Sh_2, in the cells that gave rise to this ear.

All together, 42 plants derived from mottled Sh_2 kernels on ears of a_1^{m-2} plants that had one *Spm*, located close to a_1^{m-2}, have been tested for *Spm* constitution and location. Twenty-six of the plants showed no evidence of the presence of *Spm*. Twelve plants had one *Spm*: in 2 of them it was situated very close to the locus of the mutant; in 2 others it was linked with the mutant locus; and in the remaining 8 there was no evidence of such linkage. Three plants had two *Spm*; neither was linked with the locus of the mutant in 2 of the plants but one *Spm* was linked with it in the third. The remaining plant of the 42 had three *Spm* elements, none of them linked with the mutant locus. Thus, *Spm* was present in only 16 (38 per cent) of the 42 plants derived from kernels in which a chromosome carrying a germinal mutation was received by both the endosperm and the zygote nuclei. Among the 1288 mottled Sh_2 kernels in table 2 that appeared on ears of plants having one *Spm*, 552 (43 per cent) carried *Spm* and 736 had no *Spm*. The agreement in distribution of *Spm* to the mutant class, demonstrated by these two types of test, is good.

Further confirmation that *Spm* was located close to a_1^{m-2}, and that its removal from that location was related to the origin of the stable mutants, was provided by tests of the progeny of an a_1^{m-2} Sh_2/a_1 sh_2 plant produced by crossing this plant with one that was homozygous for a_1^{m-1} and sh_2 and had no *Spm*. A very large sector, present in the a_1^{m-2}–carrying plant, was derived from a cell in which a mutation to a stable mottled allele had occurred. On the ear of the described testcross there were 306 mottled, Sh_2 kernels, of which 150 carried *Spm* and 156 had no *Spm*. Only 18 Sh_2 kernels on this ear had received unmodified a_1^{m-2}. Among the 315 sh_2 kernels on this ear, 177 were uniformly pigmented (no *Spm*) and 138 had dots of deep pigmentation in a colorless background (*Spm* present). Ten plants grown from mottled Sh_2 kernels carrying *Spm*, and 10 plants from Sh_2 kernels that had received unmodified a_1^{m-2}, were tested for *Spm* number and location. On testcross ears produced by the 10 plants derived from the mottled kernels the ratio of kernel types indicated that 9 of them had one *Spm*, not linked with Sh_2, and that two *Spm* elements, not linked with Sh_2, were present in the cells that produced the ear on the tenth plant. On testcross ears of the 10 plants derived from kernels having unmodified a_1^{m-2}, the ratios of kernel types were similar to those entered in table 3: one *Spm* was present in each plant, and it was located close to a_1^{m-2}. It may be concluded, then, that the a_1^{m-2}–carrying parent of these 20 plants commenced development with a single *Spm*, located close to a_1^{m-2}. Early in development of that plant, transposition of *Spm* to a new location, occurring in one cell, led to the origin of the stable mottled mutant that was present in all descendants of the cell.

From the above-described series of tests, it is evident that the origin of many

of the stable mutants of a_1^{m-2} is associated with transposition of *Spm* to a new location in the chromosome complement. In some of the stable a_1^{m-2} mutants, on the other hand, *Spm* continues to occupy a position close to the locus of the modified A_1 gene. Thus, in these respects, a_1^{m-2} and a_1^{m-5} are comparable.

Origin of a two-element system of control of gene action at a_1^{m-2}. Although the tests aimed at identifying events in which a clearly expressed two-element system of control of gene action arises from a_1^{m-2} have not yet been completed, one example may have been found. A kernel with a distinctive phenotype appeared on an ear of a plant that was a_1^{m-2} Sh_2/a_1 sh_2 in constitution and had one *Spm* located close to a_1^{m-2}, after it was crossed with a plant of similar constitution. The selected kernel was weakly and irregularly pigmented, with no spots of deep pigmentation in its aleurone layer. The phenotype of the plant grown from this kernel was similar to that expressed by many of the stable mottled mutants of a_1^{m-2}, since anthocyanin pigment appeared in the same regions of the plant. Testcrosses conducted with this plant gave no evidence of the presence of *Spm*. Its constitution, however, proved to be a_1^{m-2} Sh_2/a_1 sh_2. The kernels on one of its ears were produced by a cross with a plant homozygous for a_1 and sh_2 and having no *Spm*. All the Sh_2 kernels on this ear exhibited the same phenotype as that shown by the kernel that gave rise to the plant. The kernels on a second ear of the plant were produced by a cross with a plant that was homozygous for a_1 and sh_2 and carried one *Spm* closely linked with the *Pr* marker in chromosome 5 (*Pr*, purple aleurone; *pr*, recessive allele, red aleurone). Of the 217 Sh_2 kernels on this ear, 102 were variegated for pigmented areas of different intensities, in a pattern resembling that produced by unmodified a_1^{m-2}. The phenotype of the remaining 115 Sh_2 kernels was similar to that on the first ear, just described. From the close linkage of the variegated phenotype with *Pr* and the nonvariegated phenotype with *pr*, it is concluded that gene action at this modified a_1^{m-2} locus is under the control of the *Spm* system, although *Spm* no longer resides close to the locus.

The modified a_1^{m-2} locus present in the plant just described could have arisen from removal of only the *Spm* element from the vicinity of the a_1^{m-2} locus, the operator element remaining in location. That inactivation of the *Spm* element was responsible for the modification is not probable, as it is well established that such inactivation results in suppression of the action of the A_1 gene (see below); much pigment appeared in the plant having this modified a_1^{m-2} locus, and some pigment appeared in the aleurone layer of the kernels.

Inactive Spm at the locus of a_1^{m-2}. Testcrosses conducted with plants in which *Spm* was in an inactive phase, changing to an active phase only in a few cells very late in development, also served to place *Spm* close to the locus of unmodified a_1^{m-2}. Two types of testcross were performed. When plants with inactive *Spm*, a_1^{m-2} Sh_2/a_1 sh_2 in constitution, were crossed with plants homozygous for a_1 and sh_2 and having no *Spm*, all the kernels on some ears were colorless. On other ears, however, a few kernels in the Sh_2 class had a sector containing pigment of light intensity, and some of these sectors, in turn, also displayed small dots of deep pigmentation. Occasionally, the entire aleurone layer of an Sh_2 kernel exhibited such dots on a lightly pigmented background. Also, an occasional Sh_2 kernel was variegated throughout its aleurone layer, with large as well as small pigmented areas of various intensities. Tests conducted with plants derived from such variegated kernels indicated that the phenotype of the kernels was produced by a change in phase of activity of *Spm*, from inactive to active, occurring in a cell late in the development of the a_1^{m-2}–carrying plant.

The second type of test utilized the same plants as those described above, as

ear-bearing parents in crosses with plants that were homozygous for a_1^{m-1} and sh_2 and had no *Spm*. On some of the resulting ears, all kernels in both the Sh_2 and sh_2 classes were uniformly pigmented and showed no evidence of the presence of *Spm*. On other ears, a few of the kernels in the Sh_2 class exhibited sectors of much lower pigment intensity, and some of these, in turn, had small deeply pigmented spots. Occasionally, the whole aleurone layer of an Sh_2 kernel exhibited this small-spotted phenotype, or one with large as well as small areas of different grades of pigment intensity. Kernels having these last two phenotypes would be expected to appear if, in the a_1^{m-2}–carrying plant, the inactive *Spm* underwent a change to the active phase in some cells, late during development of the ovule or in the female gametophyte.

When plants of the constitution a_1^{m-2} Sh_2/a_1 sh_2 that carried an inactive *Spm* were crossed with plants homozygous for a_1 and sh_2 that carried one or more active *Spm* elements, all or nearly all kernels that received a_1^{m-2} from the heterozygous parent and no *Spm* from the homozygous parent were colorless. In contrast, all those that received active *Spm* from the homozygous parent exhibited many mutant areas, in a pattern resembling that produced by a_1^{m-2} when the *Spm* adjacent to it is in an active phase.

Conclusions derived from the study of a_1^{m-2}. Results of the described tests with plants having unmodified a_1^{m-2}, and with others having modified derivatives of the locus, indicate that certainly two and probably all three of the predicted consequences of transpositional events, outlined early in this report, have been observed.

In this report, some aspects of the analysis of a_1^{m-2} have been considered in detail, not only in order to develop the thesis stated earlier but also to indicate the nature of the evidence that makes it possible to relate the mode of control of the *Spm* system to that controlling the alternate action of the duplicate genes, H_1 and H_2, associated with flagella antigen formation in the bacterium *Salmonella*. In maize plants that are a_1^{m-2}/a_1^{m-1} in constitution, with an *Spm* element located close to a_1^{m-2}, which of the alleles will be active and which inactive is determined by the phase of activity of the *Spm* element. In *Salmonella*, which of the two duplicate genes will be active and which inactive is determined by the phase of activity of the controlling element *Vh*, located close to the H_2 gene.

The Derivatives of bz^{m-2}

Early studies of bz^{m-2} were reported in *Year Books 54* and *55*. It was shown that *Ac*, the regulator of the *Ac* control system, resides close to the locus of the bronze gene in chromosome 9, and also that the *Ac* system controls the action of this gene. The behavior of unmodified bz^{m-2} was examined, initially, in 172 plants carrying bz^{m-2} in one chromosome 9 and the standard stable recessive, *bz*, in the homologue, as well as in 13 plants homozygous for unmodified bz^{m-2}. Subsequent studies were conducted with plants carrying modified derivatives of bz^{m-2}. Since the information obtained is both diverse and extensive, the present report will be confined to summary statements pertinent to the topic in hand.

In a cross of bz^{m-2}–carrying plants to plants homozygous for standard *bz*, kernels that had received a modified derivative of bz^{m-2} appeared on some ears. Most of these kernels exhibited either a null level or a high level of gene action at the bronze locus. It was suspected that in them the action of the bronze gene, derived from bz^{m-2}, had been released from the control of the *Ac* system. To test this conjecture, selections were made of 35 independently occurring examples of change of bz^{m-2} to an apparently stable null-expression allele, and of 14 independently occurring changes to an allele expressing a high level of gene action. It could be determined readily that, in 33 of the 35

selected examples, release of gene action from control by the *Ac* system was associated with the origin of a stable null expression of the bronze gene. In 19 of these 33, *Ac* was absent from chromosome 9 in the original plant carrying the modified bz^{m-2} locus, although it was present elsewhere in the chromosome complement in 6 of the 19 plants. In the rest of the 33 plants (14), *Ac* was present in chromosome 9. It was located close to the bronze gene in 2 of them, and at positions away from the locus in 3 others; but its exact location was not determined in the remaining 9 plants, 1 of which had two *Ac* elements, one in chromosome 9 and one elsewhere.

The 2 remaining kernels of the 35 that were selected for a stable, null expression of the bronze gene produced plants in which no *Ac* was present; both plants were totally bronze in phenotype. When they were crossed with plants carrying *Ac*, it was learned that the bronze gene, derived from bz^{m-2}, was under the control of the *Ac* system. The manner of its response to *Ac* was similar to that observed in the many other examples of two-element control systems in which *Ac* is the regulator element. In both these plants, and in a third plant derived from a kernel selected in a different manner, a two-element system of control of gene action had arisen from bz^{m-2}. Although *Ac* was no longer located close to the bronze gene, it continued to be the regulator of the system controlling its action.

The 14 original kernels selected for the presence of a derivative of bz^{m-2} that expressed a high level of gene action gave rise to 8 plants having *Ac* and 6 having no *Ac* in their nuclei. Seven of the 8 *Ac*-carrying plants had one *Ac* element. In 4 plants it was not linked with markers in the short arm of chromosome 9; in the other 3 it was linked with such markers, being located close to the *Bz*-expressing gene in 2 of them and proximal to the locus of *Wx* in the third. The eighth *Ac*-carrying plant had two *Ac*, one located close to the *Bz* gene and one not linked with markers in the short arm of chromosome 9. Tests of all 14 plants and their progenies indicated that the presence of an *Ac* element in the nucleus did not effect a modification in action of the *Bz*-expressing gene derived from bz^{m-2}. Action of this gene appeared to have been released from the control of the *Ac* system.

A modified state of bz^{m-2} was recognized early in the study of that locus. The alteration at the bronze locus that produced this state did not remove *Ac*, which remained close to the locus of the gene, and the *Ac* system continued to control gene action. In contrast to the original state of bz^{m-2}, this altered state is characterized by a high level of bronze gene action. Some of its *Ac*-controlled modifications result in recognizable changes in level of gene action. Others result in release of the gene from control by the *Ac* system, and such release is often associated with maintenance of a high level of gene action. Still other modifications give rise to further altered states. One of these resembles the initial state of bz^{m-2}, and another has proved to be instructive for the thesis of this report, for it allows ready selection of kernels produced from cells in which *Ac* no longer occupies a position close to the bronze gene. This state was recognized, initially, in a single kernel on an ear. The aleurone layer of the kernel exhibited many deeply pigmented spots in a lightly pigmented background.

Tests of the plant derived from this kernel, and of its progeny, showed that *Ac* occupied a position close to the locus of the modified bronze gene and that the observed changes in action of the gene were expressions of control by the *Ac* system. On ears produced by a cross of plants carrying this state with plants that were homozygous for the standard recessive *bz* and had no *Ac*, kernels that received the state exhibited deeply pigmented areas in a lightly pigmented background. A few kernels on some ears,

however, showed only the light background pigmentation, with no deep-colored spots. Plants derived from 5 such kernels were examined. Tests were made for the presence or absence of *Ac* in them, and for the expression of the weak bronze allele in their progeny produced by a cross with plants that were homozygous for standard *bz* and had either no *Ac* or one or more *Ac*. It was learned that no *Ac* was present in these plants. In the absence of *Ac*, the expression of the bronze gene is constant, and it behaves as a weak allele of *Bz*. When *Ac* is present, however, deeply pigmented spots appear in a lightly pigmented background. It could be determined readily that these spots arise through the response of an operator element at the locus of the weak *Bz* allele to the presence of *Ac*. Thus, a two-element system of control of gene action was expressed in each of these 5 selected examples, and *Ac* was the regulator of the system.

The above-described sequence of events affecting bz^{m-2} may be interpreted in the following manner. Insertion of the operator and regulator elements of the *Ac* system close to the locus of the standard *Bz* gene gave rise to bz^{m-2}, which exhibits a null base level of gene action. Thus, the original bz^{m-2} locus may be symbolized as *bz(op)Ac*. Since only the *Ac* element at the locus of the gene has been determined, the symbol for the operator element (*op*) is shown in parentheses. Removal of both the operator and the regulator, *Ac*, from the locus of the gene, or removal of the operator alone, releases the gene from control by the *Ac* system. Removal of *Ac* alone allows the presence of the operator to be recognized, and the locus can be given the symbol *bz-op*. A change at bz^{m-2}, assumed to be induced by the operator in response to the regulator *Ac*, produces a new state characterized by a high level of gene action, which is symbolized $Bz^{s}(op)Ac$. A subsequent modification, again assumed to be induced by the operator, gives rise to another state characterized by an intermediate level of gene action, which is symbolized

TABLE 5. Response of the Bronze Gene to *Ac* in Selected Derivatives of *bz(op)Ac*, the Original State of bz^{m-2} (Part I), and in Two of Its Modified States, $Bz^{s}(op)Ac$ (Part II) and $Bz^{w}(op)Ac$ (Part III)

Expression of Bronze Gene in Selected Kernel	Response of Bronze Gene to *Ac*	Symbol for Bronze Gene	No. Cases Examined
	Part I		
Null expression; stable	Negative	*bz*	22
	Negative	*bz–Ac*	2
	Negative	*bz*; *Ac* in chromosome 9; position not determined	9
	Positive	*bz–op*	3
High level of gene action; stable	Negative	Bz^{s}	11
	Negative	Bz^{s}-*Ac*	3
High level of gene action; unstable	Positive	$Bz^{s}(op)Ac$	1
	Part II		
High level of gene action; stable	Negative	Bz^{s}	5
	Negative	Bz^{s}-*Ac*	7
Return to bz^{m-2} expression	Positive	*bz(op)Ac*	10
Weak expression of gene; unstable	Positive	$Bz^{w}(op)Ac$	1
	Part III		
High level of gene action; stable	Negative	Bz^{s}	3
Weak expression of gene; stable	Positive	Bz^{w}-*op*	5
Return to high level of gene action; unstable	Positive	$Bz^{s}(op)Ac$	1

Bz^{w}(*op*)*Ac*. Removal of only *Ac* allows the presence of the operator to be recognized, and in this event the gene locus is given the symbol *Bz*^{w}-*op*.

Table 5 summarizes the evidence for the considerations outlined in this section. The symbols in the table are the same as those just described. A symbol that does not include *op* or *Ac* indicates that no evidence has been obtained of the presence of either element at the locus of the gene.

The findings presented in this report are sufficiently extensive to leave no doubt that a two-element system of control of gene action, composed of an operator element at the locus of the gene and a regulator element located elsewhere, may arise at a gene locus that initially carried the regulator of the system. Although in the examples studied the origin of the operator element has not been determined directly, it is nevertheless evident that the predicted consequences of removal of either or both elements from the locus of the gene, on the assumption that both were present initially, have been confirmed.

ENZYMOLOGY

Margaret R. McDonald and Anne K. Carhart

Since 1945 we have intermittently attacked, in a joint endeavor with Drs. Berwind P. Kaufmann and Helen Gay, the problem of the "submicroscopic" organization of the chromosome by means of "enzymatic dissection." It was apparent from the beginning of this program (*Year Book 45*) that to obtain decisive results we must work with enzymes that were pure, were specific in their action, and had access to their substrates; and much effort has been expended during the intervening years in the fulfillment of these requirements. In addition, the biological materials to be tested must have the enzyme's substrate in a proper condition to be acted on, and must be free of endogenous enzymes capable of acting before or after the exogenous one and thus confusing the results.

An excellent example of such confusion was noted in 1950, when it was observed that RNase, although freed of all measurable traces of DNase, appeared under certain conditions to degrade DNA in fixed tissues, as evidenced by reduction in Feulgen stainability. This anomaly was eventually traced to the presence in the tissues of a DNase capable of rapidly depolymerizing isolated DNA but unable to depolymerize intracellular DNA unless the cells had been treated previously with RNase (*Year Book 51*). Several other unexpected results had presented themselves throughout the years, which, although investigated intensively as they were noted, had remained mysteries.

Cognizant of Dr. Kaufmann's inevitable retirement in July 1962, and of the contemplated changes in the organization of genetics research, we decided, in the time left at our joint disposal, to reexamine these unexplained phenomena by means of new tools acquired last year. The results of the investigations are described below, together with a few new findings in our studies of DNase II, which have been pursued simultaneously. We are indebted to Dr. Helen Gay and Miss Ann Weingart for all the cytochemical analyses, details of which can be found in the report by Kaufmann et al. in this volume.

Ribonuclease

The location of DNA in fixed tissues can be determined cytochemically by means of two color reactions: that produced by the Feulgen test, which is regarded as specific for deoxyribose; and that produced by purified methyl green, which colors highly polymerized, but not depolymerized, DNA. Treatment of tissue sections with either DNase I or DNase II

prevents both Feulgen and methyl green staining of the chromosomes, as would be anticipated from the known specificities of these enzymes. An unexpected result was observed, however, when fixed tissue sections were treated with high concentrations of crystalline RNase free of all measurable traces of DNase. Such treatment rendered the chromosomes nonstainable with methyl green without reducing the intensity of the Feulgen reaction (*Year Book 49*). This failure to stain with methyl green did not appear to be due to combination of RNase as a basic protein with the DNA of the chromosomes (although such combination undoubtedly occurs), since other basic proteins, such as lysozyme, cytochrome *c*, and chymotrypsinogen, tested under identical conditions, did not alter methyl green colorability (*Year Books 54, 60*). Furthermore, the reduction in methyl green stainability of fixed sections treated with RNase was dependent on time, temperature, and concentration (*Year Book 60*) and thus definitely suggestive of an enzymatic reaction.

These results were explainable on the supposition that chromosomes contain a complex nucleic acid composed of both deoxyribo- and ribonucleotides. If the ribonucleotides occupied intercalary positions, and the number of adjacent deoxyribonucleotides was large, removal of the ribonucleotides by the specific action of RNase would depolymerize the DNA sufficiently to impair its ability to stain with methyl green without impairing its Feulgen stainability. Such an explanation was offered in *Year Book 49*, and subsequent developments in other laboratories regarding RNA-DNA complexes tended to support such a hypothesis.

Nevertheless, the large amounts of RNase necessary to reduce methyl green stainability, and the variations in relative effectiveness more recently noted among different samples of RNase, were suggestive of an impurity of unknown enzymatic behavior, which we were unable to assay chemically because of lack of knowledge of its substrate, essential cofactors, and so forth. Therefore, when "chromatographically pure" RNase became available commercially last summer, we immediately tested it for action on methyl green stainability and found it to be completely ineffective. But chromatography of crystalline RNase yields several fractions capable of degrading RNA, only one of which was available commercially. So it became necessary for us to chromatograph crystalline RNase and analyze the various fractions for ability to render chromosomes unstainable with methyl green.

Several examples of crystalline RNase (chosen to represent the extreme variations among our samples) were chromatographed on the carboxylic acid cation exchange resin Amberlite IRC–50, according to the method of Hirs, Moore, and Stein, with sodium phosphate buffer (0.2 *M*, *p*H 6.47) as the eluant, or on carboxymethyl cellulose ion-exchange columns buffered with tris(hydroxymethyl)aminomethane–hydrochloric acid at

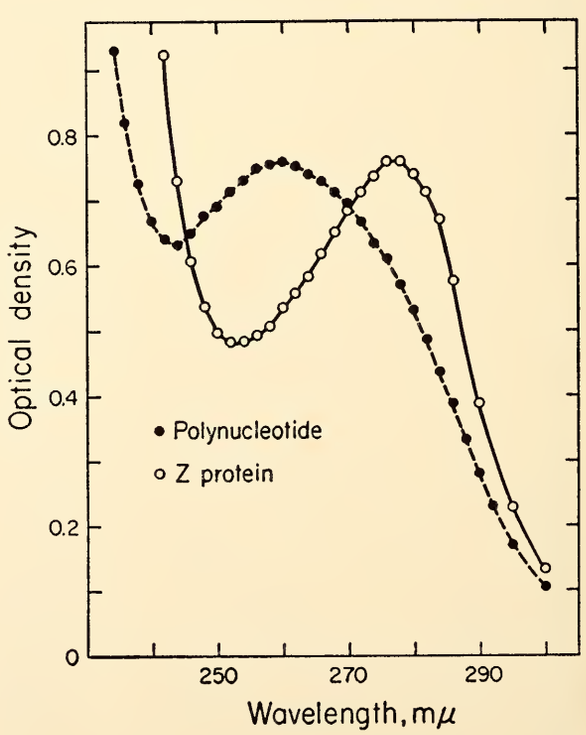


Fig. 2. Ultraviolet absorption spectra of two impurities present in crystalline ribonuclease.

*p*H 8.0, according to the method of Taborsky, with 0–0.1 *M* sodium chloride as the eluant. Each sample yielded polynucleotide material and three protein fractions having RNase activity. Cytochemical analyses of these fractions, singly and in all possible combinations, proved that none of them was capable of effacing methyl green stainability. It was apparent that the material in crystalline RNase responsible for that activity either (*a*) was present in amounts too small to be detected with the monitoring system employed, (*b*) had been inactivated by the fractionation procedures, or (*c*) had remained on the column. Tests of these three possibilities proved the last to be true.

By increasing the sodium chloride concentration of the eluting medium in Taborsky's procedure to 1 *M*, we obtained a fourth protein (fraction Z, fig. 2), the concentration of which could be correlated with the ability of the sample of RNase to eliminate methyl green stainability (table 6); and treatment of fixed sections of onion root tips with this protein did indeed render them nonstainable with methyl green without reducing their capacity to stain with the Feulgen reagent. The reduction in methyl green stainability effected by fraction Z is dependent on both time and concentration. The new protein shows slight traces of RNase activity; its specific activity is approximately 1/25 that of chromato-

TABLE 6. Summary of Assays of Various Samples of Crystalline Ribonuclease

Assays: I. Relative RNase activities, with the most active (chromatographed RNase, A, according to Hirs, Moore, and Stein) as 100.

II. Total phosphorus, expressed as per cent of weight of air-dried enzyme. Essentially no inorganic phosphorus was detected.

III. Deoxyribose, measured in terms of DNA–P and expressed as per cent of weight of air-dried enzyme.

IV. Effect on methyl green stainability of fixed onion-root-tip chromosomes. No measurable effect = 0; maximum effect = +++++.

V. Z protein, expressed as the percentage of the total optical density at 278 mμ placed on column recovered in 1 *M* sodium chloride fraction.

Sample	I RNase Activity	II Phosphorus Content	III Deoxyribose Content	IV Effect on Methyl Green Stainability	V Amount of Z Protein
A	100			0	
III*	99	0.007		0	
1	95	0.020	0.005	+	0.05
2	94	0.021	0.003	+	
E	90	0.062	0.028	++(+)	
W†	90	0.037		++	
G	86	0.045	0.020	++++	
51	86	0.016	0.009	++	
D	82	0.058	0.023	++	
7F	73	0.071	0.028	++++	
H	72	0.063	0.031	+++(+)	
8F	69	0.099	0.049	++	2.3
11F	69	0.063	0.025	++++	
60	66	0.058	0.020	++++	
4F	60	0.116	0.051	+++++	11.7
70	56	0.029	0.007	+++++	14.6

* Chromatographed according to Taborsky, type III. Purchased from Sigma Chemical Company: lot R22B–70.

† Purchased from Worthington Biochemical Corporation: lot 597–L.

graphed RNase. This activity is probably due to contamination with residual RNase from the column, but a definitive answer has not yet been obtained. So far we have been unable to demonstrate any enzymatic reaction between isolated highly polymerized DNA and fraction Z, as measured either by increase in ultraviolet absorption of the DNA or by its ability to combine with methyl green. Tests are still being made by adding various activators and coenzymes that could be present in fixed biological materials but absent from isolated DNA. Until they are completed it seems futile to speculate about the mechanisms whereby this protein reduces methyl green stainability, or even about the mechanics of methyl green staining.

Chromatography of crystalline RNase in other laboratories has usually shown a leading, enzymatically inactive band, composed of material believed to be polynucleotide on the basis of its ultraviolet absorption spectrum and its retention within the membrane sac on dialysis. Our results have confirmed these findings (fig. 2) and have shown that the amount of this contaminant, as judged from phosphorus analysis, varies enormously in different preparations (table 6). We have analyzed the material for ribose by the orcinol and phloroglucinol procedures, for deoxyribose by Burton's modification of the diphenylamine reaction, and for deoxyribonucleosides by the microbiological assay procedure of Hoff-Jørgensen. The results indicate that the polynucleotide fraction is essentially polydeoxynucleotide rather than polyribonucleotide. But it should be noted that not all the phosphorus is accountable as DNA-phosphorus (table 6). Further analysis of this fraction awaits stockpiling of a supply.

Cytochrome c

An enigmatic action of cytochrome *c* (and other compounds containing the heme molecule), namely, a simulation of RNase in reducing cellular basophilia in fixed tissue sections, was reported in *Year Books 53* and *54*. Concurrent with the reduction in basophilia was a reduction in absorption of light in the ultraviolet range (wavelength, about 260 mμ), corresponding to "loss" of purines and pyrimidines. There was also an increased absorption of 404 mμ, indicating combination of cytochrome *c*, or at least its heme component, with the materials of the sections, and an increase in stainability with acidic dyes such as fast green. Similar studies with other proteins (lysozyme, chymotrypsinogen, hemoglobin, egg albumin, and serum albumin) indicated that combination of basic proteins per se with cellular RNA was not the primary factor in reducing basophilia; it could account, however, for the increased stainability with acid dyes of tissue sections treated with basic proteins. The results with hemoglobin were essentially similar to those obtained with cytochrome *c*.

Since chromatography of cytochrome *c* had been reported to yield a rapidly moving component containing no heme (probably globin from myoglobin), as well as reduced cytochrome *c*, oxidized cytochrome *c*, and a firmly bound oxidized cytochrome *c* that could be removed only at high *p*H values, and since we had found that chromatographed RNase did not have the same unusual effect on methyl green stainability as the nonchromatographed enzyme, we decided to investigate the possibility that the unexpected effect of cytochrome *c* in reducing basophilic staining might also be due to an impurity. Chromatography of the commercial sample of cytochrome *c* that had been used in our earlier work, on Amberlite IRC–50, according to Paléus and Neilands, yielded the expected four fractions. The nonheme protein was found to have no effect on the pyronin stainability of fixed tissue sections; the three cytochrome fractions seemed to be equally effective in reducing it. Apparently, therefore, the simulation of ribonuclease by cytochrome *c* in effacing

TABLE 7. Comparison of Rates of Hydrolysis of Native and Heat-Denatured Deoxyribonucleic Acid by Salmon-Testis Deoxyribonuclease of Various Degrees of Purity

Experimental procedure: Denatured DNA was prepared by heating a 0.036 per cent solution of native calf-thymus DNA in 0.15 *M* acetate buffer, *p*H 5.1, for 15 minutes at 98°C, then cooling it rapidly to 1°C. The preparation of the DNase samples is described in *Year Book 60*. A and B signify the rates of formation of acid-soluble split products from native and from heated DNA, respectively.

Fraction	Specific Activity	B/A
G	59	0.103
H	86	0.100
K	270	0.103
N	580	0.107
16*	700	0.103
NH†	116	0.104

* Fraction 16, plate 2B, Department of Genetics, *Year Book 60*.
† Fraction N, after standing for 10 minutes at 75°C, *p*H 4.5.

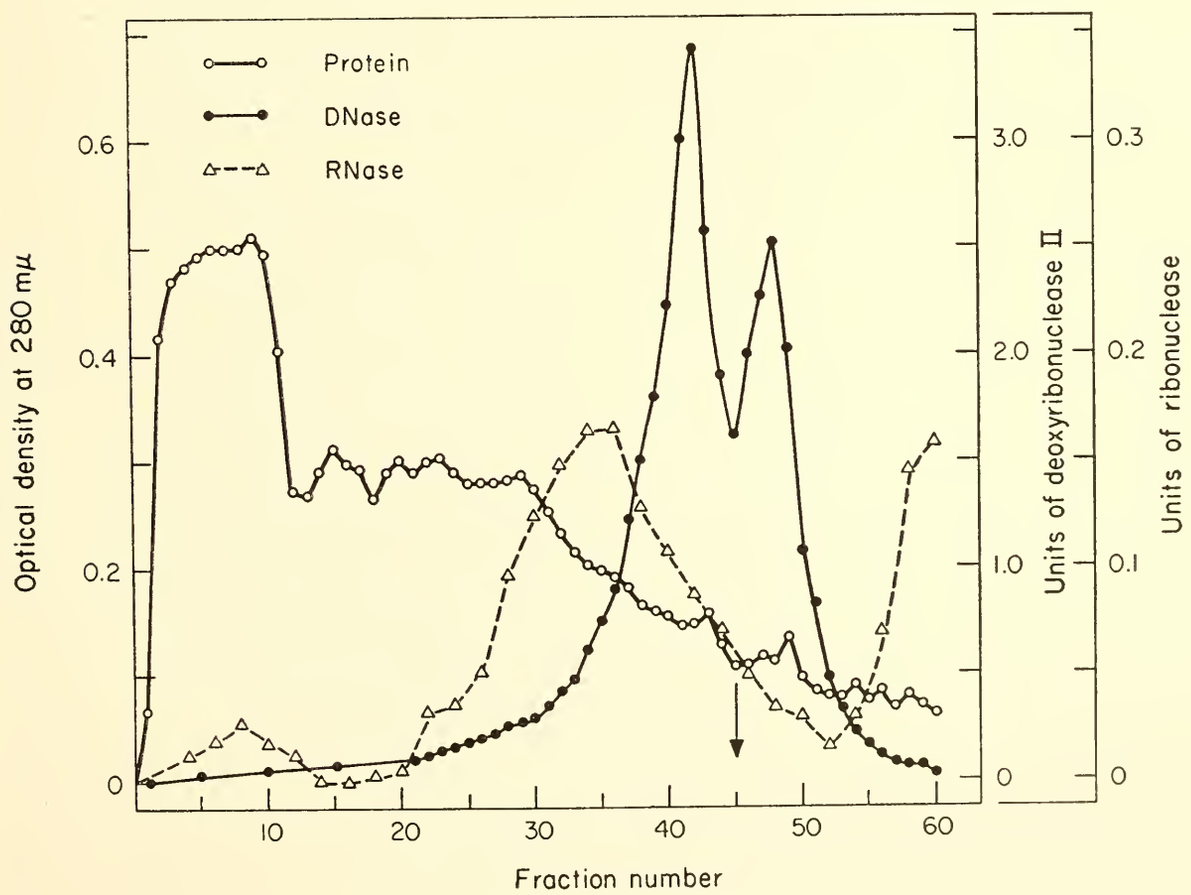


Fig. 3. Electrophorogram of partly purified calf-spleen deoxyribonuclease II. Plot of 5-ml fractions from descending electrophoresis of 40 mg of heated fraction of crude spleen deoxyribonuclease (*Year Book 53*) in a 0 to 50 per cent sucrose gradient. Buffer, 0.03 *M* borate, *p*H 8.5; time, 21 hours; temperature, 3°C; current, 600–625 volts, 12–14 milliamperes. Arrow indicates the origin.

pyronin stainability is indeed due to cytochrome and not to an impurity in the preparation. The mechanism of the action remains unsolved.

Deoxyribonuclease II

Methods for the preparation of highly purified DNase II from salmon testes, and some properties of the enzyme, were described in *Year Book 60*. As reported there, it degrades heat-denatured DNA only one-tenth as fast as native DNA, whereas pancreatic DNase I degrades heat-denatured DNA at a rate only slightly slower than that of its degradation of native DNA. We suggested that the testis preparation might contain two enzymes, one attacking only native, the other only heat-denatured, DNA. Results of experiments conducted this year to test that hypothesis appear to negate it. As is evident from the data in table 7, the ratio of the two rates of degradation remains constant throughout the later stages of the fractionation procedure. Furthermore, the ratio of the two activities remains unchanged on heat denaturation of the enzyme.

Preliminary determinations of the molecular weight of salmon-testis DNase, based on ultracentrifugation data supplied by Dr. A. D. Hershey, indicate a value of about 52,000.

To judge by our results with salmon-testis DNase, zonal density gradient electrophoresis can provide both useful analytical information and preparative methods for purification of enzymes (*Year Book 60*). The results of application of this technique to partly purified samples of DNase II from calf spleen (of which we had accumulated a stock during earlier attempts to purify that enzyme) are shown in figure 3. They strongly suggest that spleen contains several RNase's, and at least two DNase II's (cf. *Year Book 54*) with similar specific activities. Modifications of the technique will undoubtedly enable us to separate these activities further, should it prove necessary to add to the battery of DNase's available for dissecting DNA in studies of its structure.

ORGANIZATION OF CELLULAR MATERIALS

B. P. Kaufmann, Helen Gay, Jennie Buchanan, Ann Weingart, Keizo Maruyama, and Alice Akey

Much of the work of the past year has been devoted to completion of projects already under way, rather than the initiation of new ones, in preparation for the dissolution on June 30 of the research group named above. The rationale of the program in its entirety and the significance of the individual experimental components were stated in *Year Book 60* and will not be repeated here.

In studies of the organization of chromosomal and cytoplasmic organelles, which have represented our primary endeavors over the years, we have been aided since 1946 by a research grant (RG–149) from the National Institutes of Health, U. S. Public Health Service, and since 1957 by an additional grant (RG–5336) from the same agency, this one sponsored by the Long Island Biological Association. Grant RG–5336 has supported during the past year the work of Mrs. Gloria Gillies and Dr. C. C. Das.

Miss Myrna Thomas, a graduate student at Adelphi College, completed a thesis project in the Department under the supervision of Dr. Gay, and received her M.S. degree in June 1962. The work is summarized later in this report.

Drs. Gay and Kaufmann continued as guest investigators at Brookhaven National Laboratory and as editors of *Biological Abstracts*. On June 30, Kaufmann completed a term of six years as a member of the Executive Committee of the Division of Biology and Agriculture of the National Research Council. As president of the Genetics Society of

America during 1961, and since then as past-president, he has served on the Executive Committee of that Society. He has also continued as an associate editor of *The Nucleus* and of the *International Journal of Radiation Biology*.

By the time this report is published, most of the individuals named above will have moved away from Cold Spring Harbor. Miss Akey has joined the research group of Dr. Philip Woods at the University of Delaware. Miss Thomas will continue her graduate studies at Temple University in Philadelphia. Miss Weingart plans to move to the University of Colorado Medical School in Denver to work with Dr. Richard Franklin, a summer associate at the Biological Laboratory. Dr. Gay and Mr. Maruyama will transfer their program in cytogenetics to the University of Michigan, where Dr. Gay will continue as a Staff Member of the Institution and has been accorded the title Professor of Zoology by the University. Dr. Kaufmann also has been appointed to a professorship in that University.

Mutagenicity of Agents Interacting with DNA

Experiments designed to determine the mutagenic properties of agents capable of modifying specifically the deoxyribonucleic acid (DNA) of spermatogenous cells of *Drosophila* have now largely been completed.

The data reported in *Year Book 60* showed that pancreatic deoxyribonuclease (DNase I), when injected into the abdomens of adult *D. melanogaster* males, is an effective mutagenic agent, capable of inducing both lethal mutations and chromosomal rearrangements. During the past year Kaufmann and Buchanan, with the assistance of Mrs. Gillies, have tested extensively a sample of deoxyribonuclease extracted by Dr. Margaret McDonald from salmon testes (DNase II). This enzyme also has proved to be a mild but effective mutagen. Viewed chemically, these results are not surprising, since both DNase I and DNase II act specifically to depolymerize DNA, the first to yield polynucleotides with 5′-monoesterified phosphate end groups, the second to yield polynucleotides with 3′-monoesterified phosphate end groups. Thus, at face value, the tests for mutagenic activity would suggest that the direct action of the enzyme on DNA causes both point mutations and gross chromosomal alterations. From a biological viewpoint, however, the results are less meaningful, since our experiments have shown that another protein of about the same molecular weight as DNase I but lacking enzymatic activity, namely, bovine plasma albumin, also acts as a mild but effective mutagen.

In the light of these observations, we directed our efforts toward an assay of the mutagenic potential of an agent that is capable of altering DNA, not by disrupting the phosphate-sugar helices but by effecting modification of base sequences. The agent tested, 5-bromodeoxyuridine, is selectively incorporated in DNA as a substitute for thymine. When injected into adult males, it induced sex-linked lethals but, in contrast to the DNases, no chromosomal rearrangements. The viable types detected in these studies represented but a residue of the changes originally induced. Only about one-third of the males injected with 5-bromodeoxyuridine survived the treatment, and they had low fertility. Moreover, the F_1 progeny had poor viability. Presumably these responses reflected a high frequency of damage to the germ cells of the injected individuals.

To obtain a more direct measure of the effect of 5-bromodeoxyuridine on chromosomes, a cytological analysis of its action on mitotic chromosomes of the meristem of *Tradescantia* root tips was undertaken by Miss Akey and Dr. Gay. Preliminary results show that, in the first anaphase or telophase after administration of the analogue, chromosomal fragments and micronuclei are present—an indication that direct chromosomal breakage does

occur. The complete analysis of this experiment will include scoring of fragments found in the second mitosis following treatment, as well as a comparison of the effects of several concentrations of the agent.

Effect of "Near-Infrared" Radiation on Crossing Over

A study, reported in *Year Book 57*, of the effect of near-infrared radiation on crossing over suggested that the part of the electromagnetic spectrum around 1 μ was effective in increasing double crossovers by reducing or eliminating interference. In subsequent experiments we attempted to measure the effects of more restricted regions of the spectrum. The results presented in *Year Book 60* indicated that radiation passing through a Corning Glass filter (C.S. 7–57, whose maximum transmission zone falls between 1 and 2.5 μ) did not significantly increase the frequencies of double crossovers as compared with those in the unirradiated controls. We stated in that report our intention to test a Corning Glass filter (C.S. 7–69) that covers the range from 0.72 to 1.0 μ but affords over 75 per cent transmission at 0.8 μ.

Those tests have now been completed in collaboration with Miss Weingart. The analysis utilized a series of six markers spread out along the euchromatic part of the X chromosome of *D. melanogaster*, namely, yellow, apricot, crossveinless, vermilion, forked, and Bar. Some 13,000 flies were examined in the "infrared-treated" and control series. The frequency of double crossovers was higher in the treated than in the control, and statistical evaluation indicates that the difference is significant at the 2 per cent level.

The results suggest that the part of the spectrum around 0.8 μ can modify intracellular phenomena. Our original investigation (*Year Book 44*) showed that pretreatment with near infrared increased by about 50 per cent the frequency of chromosomal rearrangements induced in mature spermatozoa by a given dose of X rays. The filters we were then employing assumedly afforded maximum transmission in the 1 μ region; but more recent findings of Withrow and Mow, and the Kleins, suggest that the "far red," with peak wavelengths in the 0.76–0.78 μ range, is probably the more effective component. Our recent data tend to support the findings of those investigators, although it should be emphasized that we have not tested the potentiating effect of far-red light on the action of another type of radiation, but rather its direct influence on crossing over, which occurs under normal conditions in females of *D. melanogaster*. Apart from this, we believe that our findings now give us an important lead in efforts to elucidate the mechanisms of recombination in higher plants and animals. Many lines of evidence suggest that ribonucleic acid may be implicated in processes of structural rearrangement, as we noted in *Year Book 57*. The definitive experiments necessary to test the validity of that assumption can now be fruitfully undertaken.

Chromosomal Changes during Cleavage Mitoses in Drosophila

In our efforts to define normal and aberrant cell function in terms of specific chromosomal materials and gene loci, we have carried the analysis in recent years to the level of resolution afforded by the electron microscope. Electron micrographs show that chromosomes contain a multitude of fine fibrillar elements, but the chemical nature and the patterns of association of the discernible units have not been characterized satisfactorily. What relationship, for example, do the helically disposed 100 A strands, apparent in an electron micrograph of a somatic prophase chromosome, bear to the half-chromatids, whose existence was discovered by cytological observation and confirmed experimentally? How are nucleic acids and proteins associated in

these strands, and what changes occur in their patterns of organization during somatic and meiotic mitoses?

In the search for answers to these and related questions, a combined cytochemical and electron-microscopical study of the chromosomes of *D. melanogaster* has been undertaken by Dr. C. C. Das, working in collaboration with Kaufmann. In the first phases of this work, attention has been directed to analysis of the cleavage mitoses up to the time of blastoderm formation. A stock carrying a ring-X chromosome was chosen, primarily for the advantages to be gained in studying problems of replication, since the chromatids of the ring X appear to separate during mitosis while maintaining the form of a closed circle. This aspect of the study remains to be explored; our initial efforts have been concerned with cytochemical determinations of the amounts of DNA and proteins in the chromosomes during early embryonic development.

Amounts of DNA have been determined spectrophotometrically by the two-wavelength method, from Feulgen-stained sections of material fixed in either acetic acid–alcohol or formalin. Since the nuclei divide synchronously, and cells are not delimited until the onset of blastoderm formation after the eleventh cleavage, it has been possible to determine cytologically with great precision the exact stage of development. The results of the measurements indicate clearly that there is no significant change in the amount of DNA per diploid set of chromosomes from the first to the eleventh cleavage mitosis. The amount of DNA also corresponds closely, as would be anticipated, with that present in the diploid nucleus of the larval neuroblast. All these observations are in harmony with the tenets of the constancy hypothesis.

To determine the distribution of proteins and possible changes occurring therein, sections of formalin-fixed eggs have been stained with alkaline fast green (at *p*H 8.0–8.2) or acidic bromphenol blue (at *p*H 2.3). Before the tenth cleavage the nuclei do not stain with fast green, although they color very faintly with bromphenol blue. They begin to stain at the tenth division, when the nuclei have moved to the periphery of the egg, and they stain even more deeply during the eleventh cleavage and thereafter. In the light of available information these results suggest that a type of protein appears during the tenth and eleventh cleavages that was not present at earlier stages. The known specificity of the alkaline fast green method indicates that this protein is a histone (the somatic adult histone); and its synthesis does not appear to be restricted to the nucleus, inasmuch as a comparable increase in stainability occurs in the yolk spherules, located in the cytosome. Stainability of the chromosomes with bromphenol blue during early cleavages is assumedly due to the presence of another basic protein (the so-called cleavage histone), which is neither a protamine nor an arginine-containing protein, since stainability is lost after deamination.

The significance of these findings with respect to problems of differentiation, particularly as related to the appearance of nucleoli (which are not produced during early cleavages), the migration of nuclei, and the formation of independent cells (replacing the syncytial state), cannot be discussed at this time but will be considered when the complete data are published.

Cytochemical Studies

Modification of chromosomal stainability with ribonuclease. Last year (*Year Book 60*) we described experiments designed to test whether the reduction of methyl green stainability in chromosomes effected by ribonuclease—a change implying modification of DNA but not loss of this nucleic acid, since the Feulgen reaction remains unimpaired—is indeed dependent on the enzymatic activity of ribonuclease. We concluded that the loss of colorability

is not due to a nonspecific effect of ribonuclease, acting as a protein, but does depend on the concentration, temperature, and *p*H of the enzyme solutions and on the length of the reaction time. It seemed, therefore, that hydrolysis by ribonuclease degrades an RNA which is intercalated in chains of DNA, so that it becomes depolymerized. Since these findings offered a basis for an interpretation of considerable interest in the present era of DNA coding, that is, the cytochemical demonstration of a DNA-RNA complex in chromosomes, it was thought advisable to pursue the study with the purest ribonuclease available.

Until recently, crystalline ribonuclease free of proteolytic and deoxyribonuclease activity, prepared by Dr. Margaret McDonald, had been the most rigidly analyzed and purified sample of this enzyme available to us. With the advent of column chromatography, however, ribonuclease has been separated into several fractions, and as of last summer we have been able to obtain commercially from Sigma Chemical Company a chromatographed, "essentially homogeneous" ribonuclease. When this enzyme was tested cytochemically under the conditions of our former experiments (6 mg/ml in water at *p*H 6.0 for 2, 4, or 8 hours), no reduction of methyl green stainability of chromosomes occurred.

Although this finding raised serious questions about the validity of our conclusion that an RNA-DNA complex exists in chromosomes, it also brought into question the contention of our critics that the ribonuclease reduction of methyl green stainability in our earlier experiments had been due to blocking of the stainable groups on the DNA molecule through formation of a nonstainable DNA-ribonuclease complex. As a result, we became interested in determining what was present in the crystalline, protease-free ribonuclease samples that could effect a change in methyl green staining of chromosomes. Up to the moment the puzzle has not been solved, but a brief description of our progress in analyzing the problem is presented below. The chemical aspects of analyses of the ribonucleases are described elsewhere in this volume, in the report of Dr. Margaret McDonald.

Fourteen different samples of crystalline ribonuclease prepared over a period of years by Dr. McDonald were tested cytochemically by Dr. Gay and Miss Weingart to determine their effect on the stainability of chromosomes by methyl green. The samples reacted differently when tested under the same conditions: some caused very little reduction in colorability, whereas others completely eliminated it. Chromatographic and chemical analyses of the samples showed that they contained different amounts of a nucleotide fraction (eluted from the column before the enzyme); but the amount of this fraction present in a sample could not be correlated with the degree of methyl green reduction it produced.

Further study of the chromatographed ribonucleases showed that none of the fractions, either separately or combined—and not even a combination of all the fractions to reconstitute the "whole" enzyme—would cause the cytochemical reduction of chromosomal methyl green stainability. Dr. McDonald concluded that the factor responsible for modification of chromosomal staining might have remained on the chromatographic column. Elution with 1 *M* NaCl released a protein-containing fraction from the column, and cytochemical tests showed that it did impair methyl green stainability. This fraction is currently being analyzed by Dr. McDonald to determine whether the reduction is referable to enzymatic activity and, if so, what the substrate is, whether DNA or RNA.

Pending receipt of this definitive information, we must suspend efforts to interpret elimination of methyl green stainability by ribonuclease in terms of organizational patterns of chromosomal RNA and DNA.

DNA content during microsporogenesis in Tradescantia. Miss Myrna Thomas, who received the M.S. degree from Adelphi College in June 1962, had completed her research problem in our laboratory during the summer and fall of 1961 under the guidance of Dr. Gay. By cytospectrophotometric methods, she determined the DNA content of the nuclei during microspore development in *Tradescantia paludosa.* Since we had been investigating by electron microscopy the ultrastructural modifications undergone during microsporogenesis, a clear understanding of the behavior of nuclear DNA in the course of that process was needed.

Several previous studies had shown that in general, throughout meiosis and the postmeiotic development of the gametophyte, the amount of DNA conforms with that expected according to the "constancy hypothesis." Some discrepancies among the results of various investigations had been reported, however, particularly with respect to the quantity of DNA in the generative and vegetative nuclei just before germination of the pollen grain. Moses and Taylor, in 1955, found a slow and incomplete synthesis of DNA in the vegetative nucleus, although that nucleus never divides. Earlier, however, one of these authors suggested, on the basis of P^{32}-incorporation experiments, that the tube nucleus does not take up radioactive phosphorus and therefore does not synthesize DNA. Results of still another spectrophotometric analysis, by Woodard, hinted at an increase in DNA in the vegetative nucleus, but these data were inconclusive. Since differences in methods of preparation and microspectrophotometry made comparisons among the several *Tradescantia* studies difficult, a reinvestigation of the problem seemed warranted.

Amounts of DNA were determined by the two-wavelength method, which facilitates measurement of irregularly shaped nuclei. Feulgen-stained smear preparations ensured the inclusion of whole nuclei in the determinations. The *Tradescantia* plants, grown in the greenhouse, were clonal descendants of a single plant. Measurements of DNA in microspores before, during, and after the postmeiotic mitosis, which leads to production of the generative and vegetative nuclei, were made in cells from buds of a single inflorescence. These values were then compared with the haploid DNA value, C, which had been determined by measurement of late-anaphase or telophase chromosomal groups of the quartet or tetrad (four-microspore) stage.

The amounts of DNA measured in nuclei of the two- and four-cell stages and the pollen-grain mitosis all conform with values expected on the basis of the DNA-constancy hypothesis, which predicts a doubling of DNA amount during the interphase before each mitosis, except during interkinesis, which is the phase between the two meiotic divisions when reduction takes place. Miss Thomas found that in the generative nucleus of the binucleate pollen grain, very soon after its formation, DNA is synthesized to the 2C value. The generative nucleus is thus prepared well in advance for the mitosis that will form the two sperm nuclei in the pollen tube. Contrary to expectation, she found that during this same period the vegetative nucleus, which will not divide again, shows a slow increase in amount of DNA. Although the observed increase was not so great as that reported earlier by Moses and Taylor, leading only to a 1.3C value as compared with their 1.8C, it was statistically significant.

We conclude that DNA synthesis in *T. paludosa,* throughout meiosis and during postmeiotic pollen-grain development, is consistent with expectation, except that synthesis occurs in the vegetative nucleus at the time when it is no longer undergoing mitosis. Because this phenomenon has been observed in other studies and is statistically significant in the present analysis, it is suggested that the amoeboid nucleus may form some DNA whose function is concerned

with differentiation rather than with replication. We must now investigate the exact nature of this DNA increase, its variability in different inflorescences, and its relation to metabolic cellular changes.

Electron-Microscope Studies

Electron-microscope investigations during the year have focused on the modifications in cytoplasmic organelles throughout differentiation and growth of two types of *Tradescantia* cells. Mr. Maruyama, in collaboration with Dr. Gay, has completed a study of ultrastructural changes in the stigma cell and is currently engaged in observing changes that occur during microsporogenesis. Preliminary results of the stigma-cell study were described in *Year Book 60;* the findings are brought up to date in the following paragraphs. The initial studies of changes in fine structure of the pollen grain, after the differential mitosis which produces the vegetative and generative nuclei, were concerned mainly with formation of the wall between the two cells. Analysis of the changes in cytoplasmic organelles during this period has now been completed. Observations have also been made of the earlier developmental changes during the two meiotic divisions leading to formation of the microspore.

Fine structure of the developing stigma cell. At certain stages in the growth of the *Tradescantia* stigma cell, ellipsoidal or irregularly shaped electron-dense bodies, limited by a single membrane, appear in the cytoplasm. As the cell grows, these "dense bodies" gradually increase in size, become less dense, and fuse to form a single large vacuole. Observations reported last year suggested that the dense bodies arise from the endoplasmic reticulum of the cell, and therefore that the vacuole itself originates from this cytoplasmic membrane system.

Better-preserved potassium permanganate–fixed stigma cells have now been obtained by imbedding in an epoxy resin (Epon 812). Electron micrographs of sections of this material give a clear picture of the single membrane bounding the dense bodies and of its direct continuity with the endoplasmic reticulum. They leave little doubt that the dense bodies are formed by accumulation of material within the cisternae of the endoplasmic reticulum, and that they fuse to produce the large vacuole.

The origin of vacuoles from the endoplasmic reticulum has been reported by the French cytologist Buvat and his co-workers, and by Whaley et al. The French workers have shown that small vacuoles arise as swellings of the endoplasmic reticulum in *Elodea* buds and *Triticum* roots. Whaley's group, observing dense "lipid bodies" as well as vacuoles with contents of low electron density in maize roots, postulated that the dense bodies were precursor vacuoles containing substances in high concentration. Our observations of *Tradescantia* stigma cells confirm the conclusions of these workers and extend their findings.

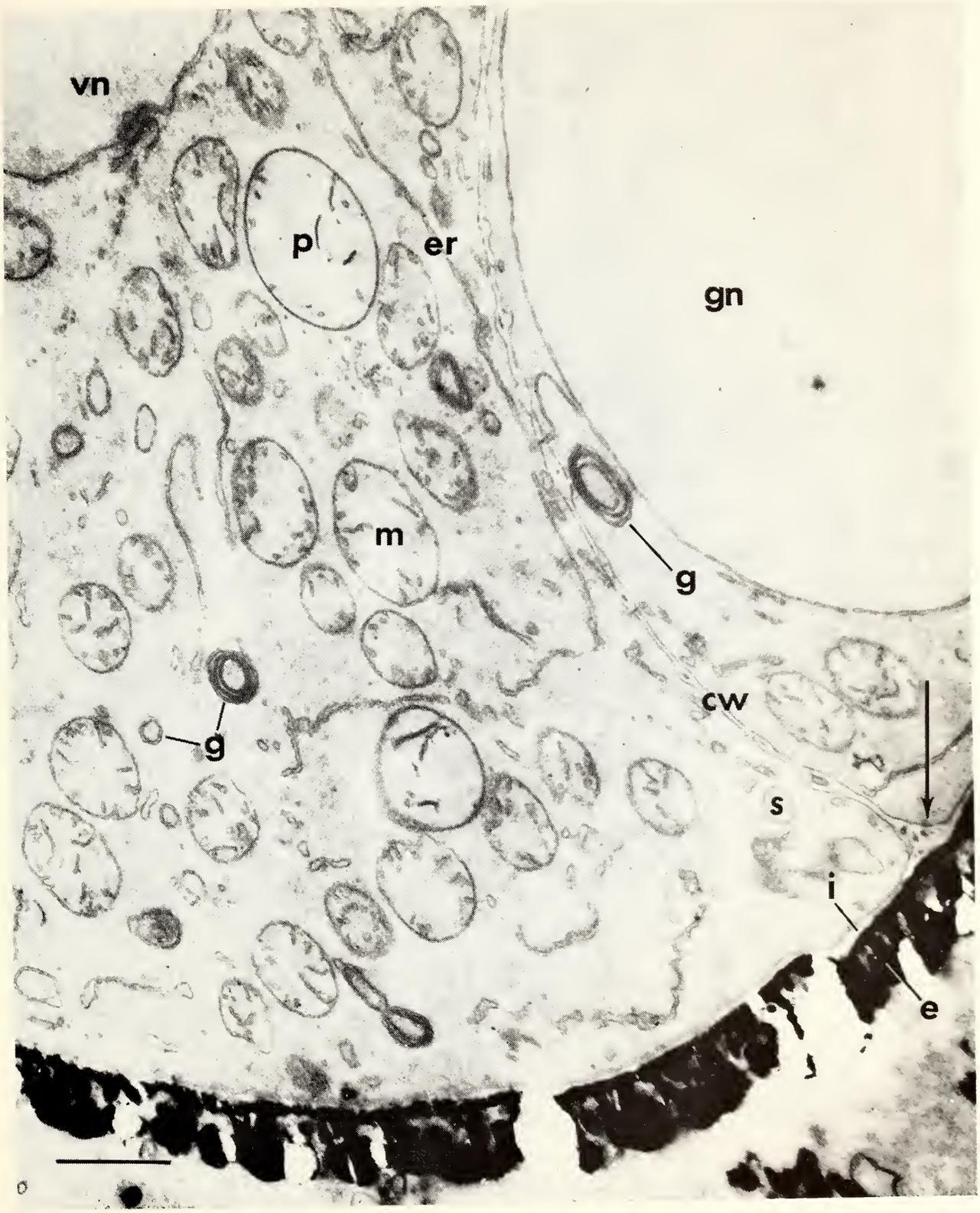
In *Tradescantia* stigma cells the origin of the vacuolar system from the endoplasmic reticulum can be more clearly visualized than in the types of cells studied by others. In the first place, the vacuole arises in a cytoplasm devoid of any vacuoles. Second, the cisternae of the endoplasmic reticulum that are destined to become vacuole precursors develop more or less synchronously, so that the sequence of events in vacuole formation—that is, swelling, accumulation of dense material, subsequent dilution of contents, and fusion into a large vacuole—is very noticeable. Last, these changes can be clearly associated with cellular growth, since the age of the cell being studied is determined on the basis of cytological or morphological characteristics of the bud or flower, not merely by its own appearance. Thus, the origin of the large vacuole from its earliest precursors can be unequivocally traced.

Unfortunately, we have not been able to discover the nature of the electron-

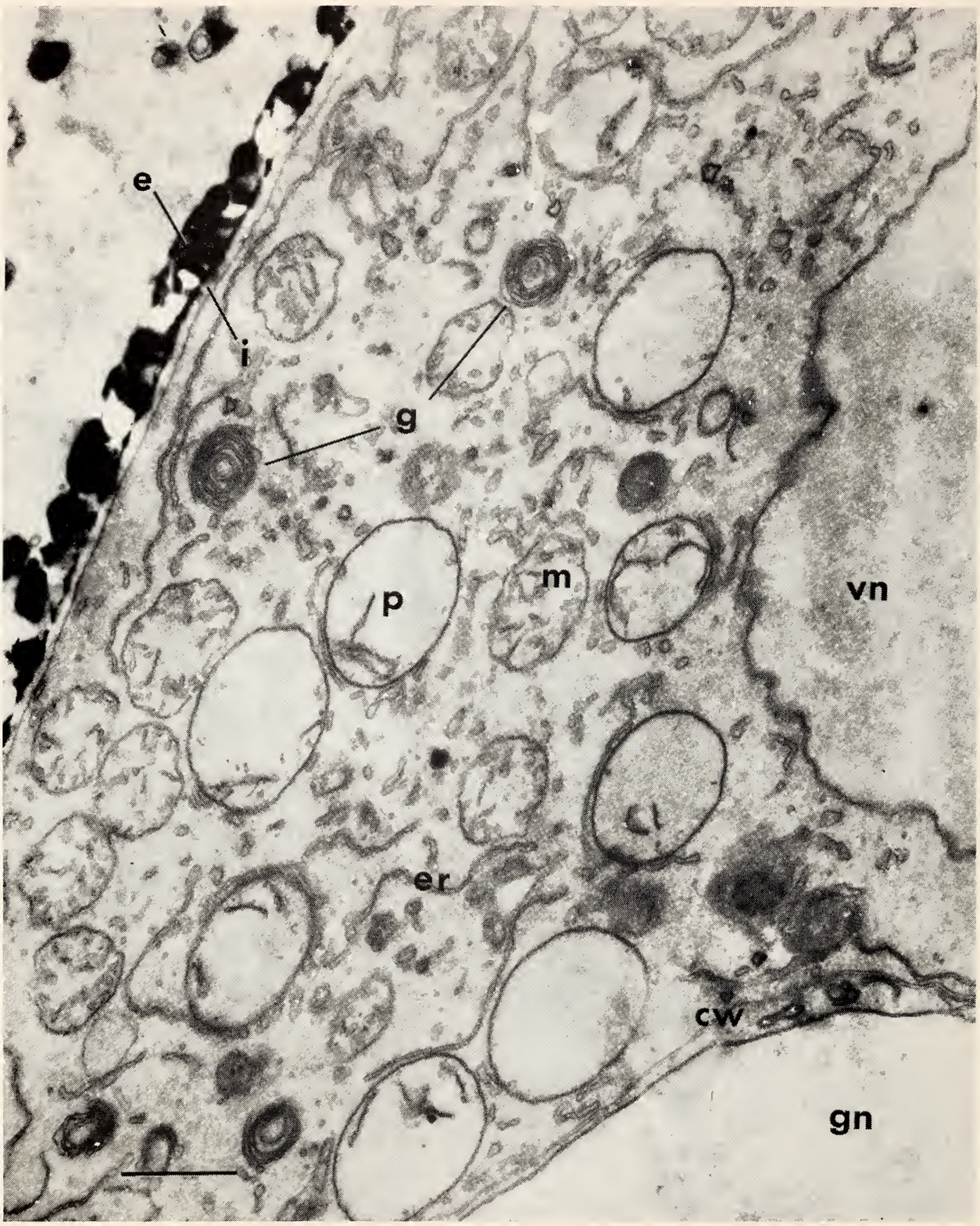
Abbreviations on plates: *gn*, generative nucleus; *vn*, vegetative nucleus; *g*, Golgi bodies; *p*, plastid; *m*, mitochondrion; *er*, endoplasmic reticulum; *s*, spherosome; *cw*, cell wall between the generative and vegetative cells; *i*, intine, and *e*, extine, of pollen wall. Solid-line marker indicates 1 μ.

Plate 1

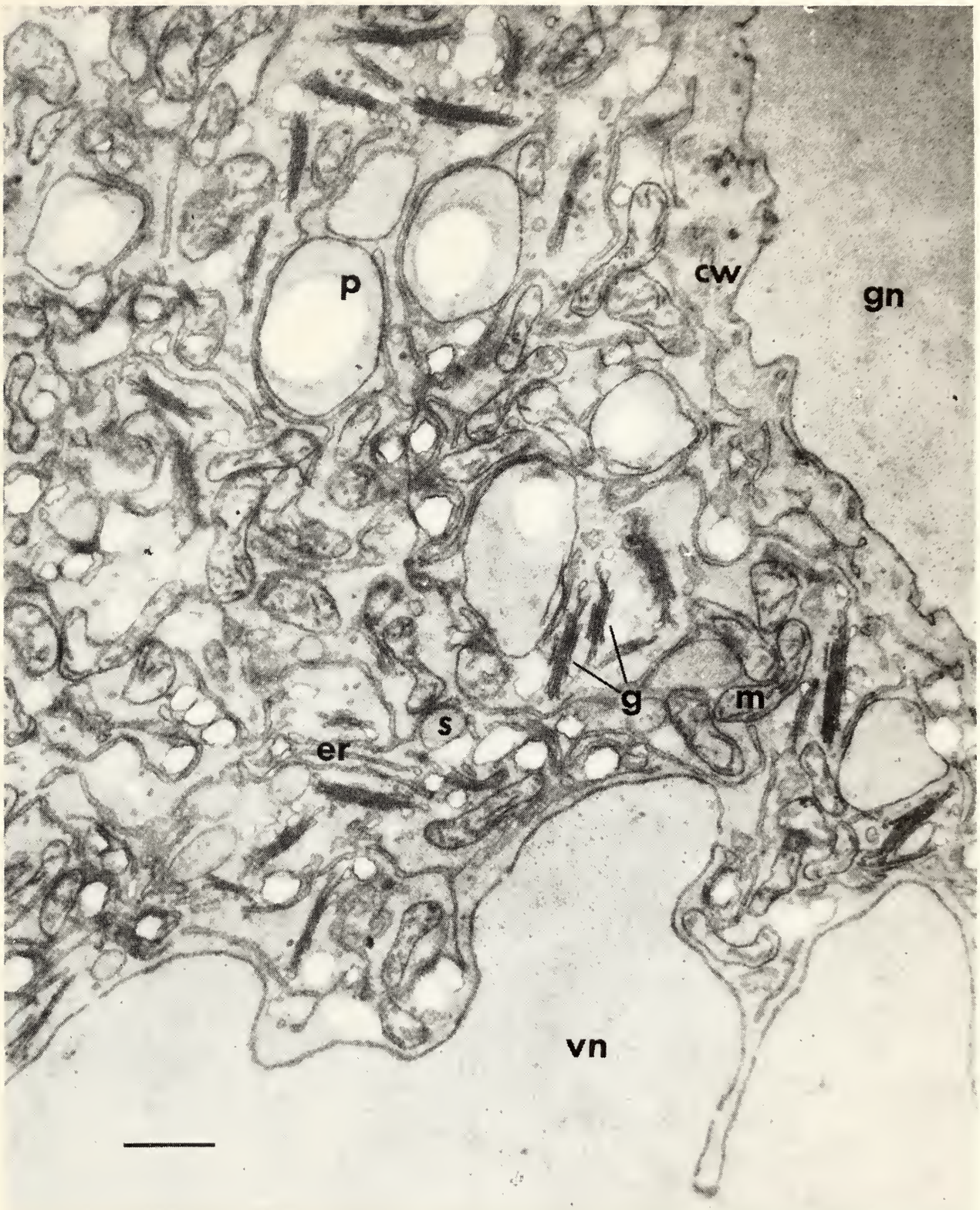
Department of Genetics



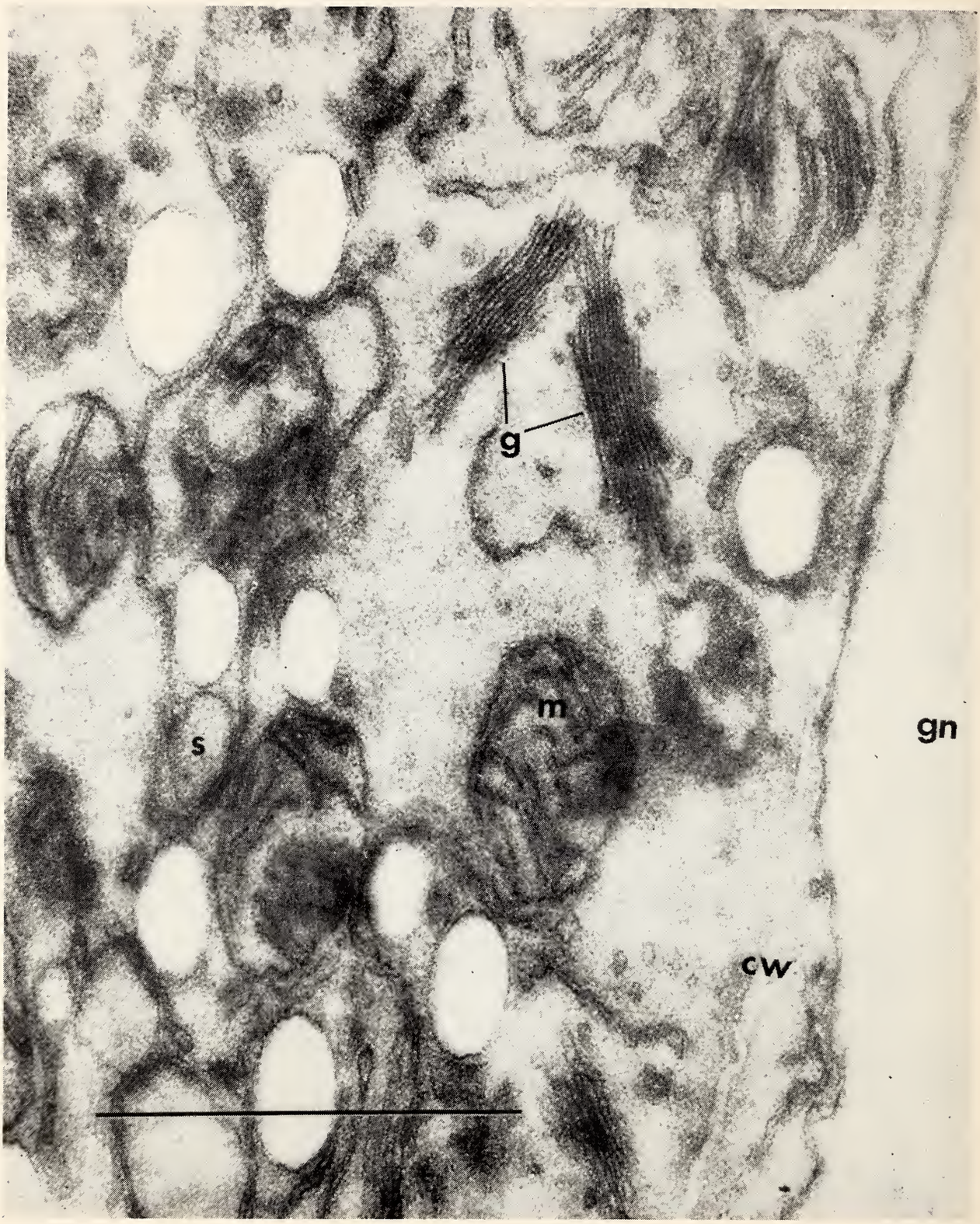
Pollen grain of *Tradescantia paludosa*, just after formation of generative and vegetative nuclei. Cell wall between the two cells is continuous with the intine of the pollen wall (arrow). The two dense lines bordering the cell wall are continuous with the line that borders the intine; each line may represent the plasma membrane of one of the two cells. Golgi bodies are composed of one or a few concentric cisternae.



Pollen grain of *Tradescantia* at a somewhat later stage than that shown in plate 1. Golgi bodies have larger numbers of paired concentric layers than those in previous micrograph. Apparently, growth of the pro-Golgi bodies occurs through an increase in number of concentric cisternae.



Pollen grain whose generative nucleus is crescent shaped. Golgi bodies have assumed their typical form, probably by an opening-out of the concentric cisternae of the pro-Golgi apparatus.



Golgi bodies of adult form, at a higher magnification. They are now composed of stacks of straight, parallel cisternae.

dense vacuolar substance of the *Tradescantia* stigma cell. Our studies show that fixation with osmium tetroxide or formalin does not preserve this material. Identification of the vacuolar contents would enable us to determine objectively whether the substance in young and old vacuoles is the same. It might also help us understand more fully the functional significance of these special cisternae of the endoplasmic reticulum.

Our findings indicate, then, that the vacuolar system in plants originates from the endoplasmic reticulum and consequently that the tonoplast of the *Tradescantia* stigma cell consists of a single membrane. The real challenge of these findings consists in the problem they raise as to the predisposing factors responsible for the differentiation of parts of an apparently morphologically homogeneous cytoplasmic membrane system into a vacuolar system.

Fine structure and development of the pollen grain. 1. The cell wall. Last year, when studies of the ultrastructural changes during microsporogenesis in *Tradescantia* were just getting under way, we reported our early findings about the nature of the thin cell wall that separates the generative and vegetative cells. We noted a continuity between the newly formed wall and the intine (the innermost layer of the pollen wall), and a similarity in structure and density, observable shortly after formation of the vegetative and generative nuclei (pl. 1). Because of these relationships it was assumed that the cell wall, like the PAS-positive intine, is carbohydrate and probably made up of cellulose and pectin, which have low electron-scattering power. In a recent study, Bopp-Hassenkamp notes the two dense membranes lying on either side of the lighter middle layer (see pl. 1), and suggests that together they form the plasmalemma of the generative cell rather than a true cell wall. Our interpretation is that the dense membranes are the plasma membranes of the generative and vegetative cells, which are separated by the true cell wall, the lighter middle layer.

During the past months we have been analyzing the formation of the cell wall when the cell plate is laid down at the telophase of the microspore mitosis. Endoplasmic reticulum cisternae are very closely involved in this process, as other workers also have noted. Vesicles, supposedly pectin containing, with an internal content whose density is similar to that of the thin separating cell wall described above, are contiguous with endoplasmic reticulum vesicles in this region. Mr. Maruyama is currently investigating the extent to which endoplasmic reticulum cisternae contribute to pectin vesicle production and plasma membrane formation.

2. The Golgi apparatus. A study has been made of the submicroscopic structure of the pollen grain from the time of the microspore mitosis until the time of pollen-grain maturity, which occurs in *Tradescantia* as the flower opens. The analysis has been restricted to changes in the cytoplasm of the vegetative cell, since the differential mitosis leaves virtually no cytoplasm around the generative nucleus. Development and multiplication of cytoplasmic organelles proceeds throughout the period studied, reflecting a high metabolic activity. The endoplasmic reticulum increases in amount, particularly just before the flower opens, when the lamellae become arranged in more or less parallel bundles. Plastids multiply, enlarge, and accumulate starch. Mitochondria also multiply, and in later stages of development become longer. Their internal organization is modified so that cristae, which were previously short and perpendicular to the outer membrane, become long and parallel to the long axis of the organelle. These findings and their implications will be discussed in a future publication. We shall restrict the discussion here to changes in the Golgi apparatus, since they represent new observations not described by others.

Electron microscopy in the past few

years has revealed that a Golgi apparatus, similar in structure to that first demonstrated in animal cells, occurs in many kinds of plant cells. The Golgi bodies are composed of stacks of cisternae with associated small vesicles, and are usually dispersed at random within the cytoplasm. Most published reports suggest that there is no very great change in these structures as differentiation proceeds. Some workers have observed increased dilation of the ends of the cisternae, and others have noted an increased number of small vesicles, but no one has reported any major modifications of the whole organelle.

We find that in *Tradescantia*, during the period from microspore mitosis until shortly after pollen maturity, all the Golgi bodies in the vegetative cells undergo structural changes more or less synchronously. Because of this synchrony, all Golgi bodies within a cell look approximately similar, and their morphological modifications as they change together become conspicuous. The sequence of developmental changes can readily be reconstructed, since the stages of cell growth are identified by well known nuclear events. Just after microspore mitosis, no Golgi bodies of the characteristic type (i.e., stacks of cisternae) are found. Instead, seen in micrographs of ultrathin sections, are concentric multilayered ring structures (pl. 1). The spacing of the rings makes them appear to be paired. The number of paired rings increases as the cell grows (pl. 2), a maximum of seven pairs constituting the largest complex, which is about 1 μ in diameter. Sometimes the last two or four outer layers do not form complete rings but are continuous with each other, thus forming apparent cisternae.

At a later stage of development, when the generative nucleus is crescent shaped, the Golgi bodies assume their typical form, stacks of straight or slightly curved parallel cisternae, seven to twelve in number (pls. 3 and 4). Finally, in the pollen grain of the open flower, the number of flattened cisternae is reduced and the ends of some of the sacs are prominently dilated. These observations suggest that the structural modifications we have seen may be related to growth of the Golgi apparatus. The pro-Golgi appears in cross section as one or two pairs of concentric rings. Although we have not yet reconstructed a model from serial sections, the spacing and shape of the rings suggest that the young Golgi apparatus must be either spherical or disclike. Since thin sections of these bodies always show circular rings, we are inclined to favor a spherical shape. In thick sections, on the other hand, the appearance of these bodies is reminiscent of the "osmiophilic platelets" described by Bowen and postulated by him to be the Golgi of plants. Whatever its three-dimensional form, the organelle appears to increase in size by the addition of concentric cisternae. We believe that these arrays open out to form the stacks of parallel straight cisternae, which then decrease in number by vesiculation of the cisternal elements themselves.

We shall not discuss here the mechanisms proposed by others to explain multiplication of the stacks of cisternae in Golgi bodies. If our observations concerning the growth of this organelle are correct, it must be concluded that the Golgi body does arise and develop from a simple structure, circular in cross section, into a multilayered concentric structure, and finally into the familiar stacked form. If other mechanisms do obtain, we must conclude that this cytoplasmic organelle may reproduce in several different ways. In our preliminary study of the earlier stages of microsporogenesis, that is, premeiotic mitosis and meiosis, the same series of changes in the Golgi apparatus has been noted.

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Hershey, A. D., *see* Burgi, E.; Rubenstein, I.

Kaufmann, B. P., *see* Maruyama, K.

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PERSONNEL

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Kaufmann, Berwind P.,[2] Director

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[2] Retired June 30, 1962.

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McDonald, Margaret R., Chemist
McDonald, William T., Janitor
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Weingart, Eleanor Ann, Research Assistant
White, Harry S., Superintendent of Buildings and Grounds; Chief Mechanic
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[2] Retired June 30, 1962.

Temporary and Part-Time

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[3] Biological Laboratory Undergraduate Research Participation Program, sponsored by the National Science Foundation, summer 1961.

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July 1, 1961 – June 30, 1962

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Part 2. Tatiana Proskouriakoff. Civic and religious structures of Mayapan. Pages 87–163, frontispiece, figures 1–12.
Part 3. A. Ledyard Smith. Residential and associated structures at Mayapan. Pages 165–320, figures 1–23.
Part 4. Tatiana Proskouriakoff. The artifacts of Mayapan. Pages 321–515, figures 1–53.

621. *Contributions to Embryology,* volume xxxvii. Quarto, iv + 129 pages, 94 plates, 20 text figures. March 1962.
252. George W. Bartelmez. The proliferation of neural crest from forebrain levels in the rat. Pages 1–12, 8 plates, 7 text figures.
253. George W. Bartelmez and A. S. Dekaban. The early development of the human brain. Pages 13–32, 30 plates.
254. Bent G. Böving. Anatomical analysis of rabbit trophoblast invasion. Pages 33–55, 15 plates, 1 text figure.
255. L. E. DeLanney and J. D. Ebert in collaboration with C. M. Coffman and A. M. Mun. On the chick spleen: origin; patterns of normal development and their experimental modification. Pages 57–85, 14 plates, 1 text figure.
256. Pieter A. de Vries and John B. de C. M. Saunders. Development of the ventricles and spiral outflow tract in the human heart. Pages 87–114, 9 plates, 9 text figures.
257. Roberto Narbaitz. The primordial germ cells in the male human embryo. Pages 115–119, 5 plates.
258. John McKenzie. The development of the sternomastoid and trapezius muscles. Pages 121–129, 13 plates, 2 text figures.

622. Steinhart, John S., and Robert P. Meyer, with contributions by William E. Bonini, T. Jefferson Smith, and George P. Woollard. *Explosion Studies of Continental Structure.* Quarto, xiii + 409 pages, 142 figures, 2 plates in back cover pocket. July 1961.

PUBLICATIONS BY THE PRESIDENT OF THE INSTITUTION

Caryl P. Haskins
(With Edna F. Haskins, John J. A. McLaughlin, and Richard E. Hewitt) Polymorphism and population structure in *Lebistes reticulatus,* an ecological study. Pages 320–395 in *Vertebrate Speciation,* a University of Texas Symposium, edited by W. Frank Blair. University of Texas Press, Austin. August 1961.

A flower where the roads divide. *Proceedings of the American Philosophical Society,* volume 105, number 4, pages 452–458, August 1961.

Report of the President. *Carnegie Institution of Washington Year Book 60,* pages 1–54. Carnegie Institution of Washington, Washington, D. C. December 11, 1961. Excerpts reprinted under title Indigenous science for new nations, in *Current,* March 1962, pages 27–29.

Technology, science and American foreign policy. *Foreign Affairs,* volume 40, number 2, pages 225–243. January 1962.

PUBLICATIONS BY THE EXECUTIVE OFFICER OF THE INSTITUTION

Edward A. Ackerman

Reasons for research and development on water desalting. Statement prepared for the Desalination Research Conference, June 19 to July 14, 1961, National Academy of Sciences—National Research Council and Office of Saline Water, Woods Hole, Mass. Included in *Saline Water Research and Development Program,* Hearings before the Subcommittee on Irrigation and Reclamation of the Committee on Interior and Insular Affairs, House of Representatives, 87th Congress, 1st Session, on H. R. 152, H. R. 431, H. R. 949, and H. R. 2991; H. R. 595 and H. R. 5883; H. R. 3089; H. R. 4721, H. R. 4757, and H. R. 4759; and H. R. 7916. March 17, June 26 and 27, and July 17 and 18, 1961. Serial no. 7. Pages 253–261. Printed for the use of the Committee on Interior and Insular Affairs. U. S. Government Printing Office, Washington, 1961.

(With Marion Clawson) Toward a permanent farm policy. Pages 65–71 in *Man and His Environment, Proceedings of the Second National Congress on Environmental Health,* Ann Arbor, Michigan, June 6–8, 1961. Continued Education Series 95, distributed by Continued Education Service, School of Public Health, University of Michigan.

Administrative Reports

Report of the Executive Committee

To the Trustees of the Carnegie Institution of Washington:

Gentlemen: In accordance with the provisions of the By-Laws, the Executive Committee submits this report to the annual meeting of the Board of Trustees.

During the fiscal year ending June 30, 1962, the Executive Committee held four meetings. Printed accounts of these meetings have been or will be mailed to each Trustee.

The estimate of expenditures for the fiscal year beginning July 1, 1962, has been reviewed by the Executive Committee.

Two vacancies exist in the membership of the Board of Trustees, resulting from the deaths of James F. Bell in May 1961 and of Robert Woods Bliss in April 1962. The office of Secretary of the Board is vacant because of Mr. Bliss's death.

The terms of office of the Chairmen of all Committees of the Board expire on May 11, 1962. A vacancy has occurred in the Executive Committee by reason of the resignation of Henry R. Shepley. The terms of the following members of Committees also expire on May 11, 1962:

Executive Committee
Robert A. Lovett
James N. White

Finance Committee
Richard S. Perkins
Elihu Root, Jr.
James N. White

Retirement Committee
Omar N. Bradley
Henry S. Morgan

Nominating Committee
Walter S. Gifford
Henry S. Morgan

HENRY S. MORGAN, *Chairman*

May 11, 1962

Report of Auditors

LYBRAND, ROSS BROS. & MONTGOMERY

To the Auditing Committee of Carnegie Institution of Washington:

We have examined the statement of assets, liabilities and fund balances of Carnegie Institution of Washington as of June 30, 1962, and the related summary statement of changes in funds for the year then ended and the supporting exhibits and schedules, which have been prepared on the general basis of cash receipts and disbursements and accordingly do not reflect accrued income, accounts payable nor provision for depreciation. Our examination was made in accordance with generally accepted auditing standards, and accordingly included confirmation from the custodian of securities owned at June 30, 1962, and such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying financial statements and supporting exhibits and schedules present fairly the assets, liabilities and fund balances of Carnegie Institution of Washington at June 30, 1962, and the changes in funds for the year then ended on a basis consistent with that of the preceding year.

Lybrand, Ross Bros. & Montgomery

Washington, D. C.
August 9, 1962

STATEMENT A

ASSETS, LIABILITIES, AND FUND BALANCES
JUNE 30, 1962 AND 1961

	JUNE 30	
	1962	1961
ASSETS		
Operating Funds:		
Cash	$ 512,904.28	$ 477,300.80
Advances	17,800.78	28,920.45
Securities - Schedule 2 (See Note)	490,865.29	442,501.55
Prepaid insurance	38,162.66	51,858.57
	$ 1,059,733.01	$ 1,000,581.37
Restricted Grants:		
Cash	$ 390,903.30	$ 409,578.63
Endowment, General Reserve, and Special Funds:		
Cash awaiting investment	$ 96,925.10	$ 318,121.47
Advances - Building program	5,089.56	266,171.11
Investments:		
Savings account	1,477,156.66	. . .
Securities - Schedule 2 (See Note)	65,973,429.04	63,036,527.74
	$67,552,600.36	$63,620,820.32
Buildings, Land, and Equipment (At Cost)	$ 6,044,814.64	$ 5,807,296.03
Total Assets	$75,048,051.31	$70,838,276.35
LIABILITIES AND FUNDS		
Operating Funds:		
Income taxes, etc., withheld	$ 783.40	$ 286.30
Operating Funds Balance - Exhibit 1	1,058,949.61	1,000,295.07
	$ 1,059,733.01	$ 1,000,581.37
Restricted Grants - Exhibit 2	390,903.30	409,578.63
Endowment, General Reserve, and Special Funds - Exhibit 3	67,552,600.36	63,620,820.32
Buildings, Land, and Equipment Fund - Exhibit 4	6,044,814.64	5,807,296.03
Total Liabilities and Funds	$75,048,051.31	$70,838,276.35

Note: Approximate market value of all securities at June 30, 1962 - $80,867,556

STATEMENT B

SUMMARY STATEMENT OF CHANGES IN FUNDS FOR THE YEAR ENDED JUNE 30, 1962

	Operating Funds (Exhibit 1)	Restricted Grants (Exhibit 2)	Endowment, General Reserve, and Special Funds (Exhibit 3)	Buildings, Land, and Equipment (Exhibit 4)	Total
Balance July 1, 1961	$1,000,295.07	$409,578.63	$63,620,820.32	$5,807,296.03	$70,837,990.05
Additions:					
Investment income	$ 10,267.36	...	$ 3,004,340.32	...	$ 3,014,607.68
Realized capital gain (net)	14,127.78	...	4,104,400.40	...	4,118,528.18
Restricted grants	...	$202,699.89	...	...	202,699.89
Dormitory	7,213.42	...	...	...	7,213.42
Sales of publications	22,774.41	...	...	...	22,774.41
Other income, gifts and bequests	1,362.26	...	15,829.88	...	17,192.14
Expenditures capitalized:					
Current year	...	...	...	$ 214,594.66	214,594.66
Prior years	...	...	...	35,200.00	35,200.00
Gift capitalized:					
Prior years	...	...	...	17,585.00	17,585.00
By transfer:					
Budget appropriation - July 1, 1961 to June 30, 1962	2,848,480.00	...	(2,848,480.00)	...	...
American Geophysical Union	2,250.00	(2,250.00)	...	...	...
Embryology Building Program	1,000.00	...	(1,000.00)	...	...
Harkavy Fund - Income	1,000.00	...	(1,000.00)	...	...
Harry Oscar Wood Fund - Income	24,133.95	...	(24,133.95)	...	...
	$2,932,609.18	$200,449.89	$ 4,249,956.65	$ 267,379.66	$ 7,650,395.38
Deductions:					
Expenditures	$2,873,954.64	$219,125.22	$ 318,176.61	...	$ 3,411,256.47
Disposition of equipment	...	...	...	$ 29,861.05	29,861.05
	$2,873,954.64	$219,125.22	$ 318,176.61	$ 29,861.05	$ 3,441,117.52
Net change during the year	$ 58,654.54	($ 18,675.33)	$ 3,931,780.04	$ 237,518.61	$ 4,209,277.86
Balance June 30, 1962	$1,058,949.61	$390,903.30	$67,552,600.36	$6,044,814.64	$75,047,267.91

EXHIBIT 1

CHANGES IN OPERATING FUNDS FOR THE YEAR ENDED JUNE 30, 1962

Balance July 1, 1961		$1,000,295.07
Additions - Statement B:		
Distributions:		
Investment income	$ 10,267.36	
Realized capital gains, net	14,127.78	
Dormitory	7,213.42	
Sales of publications	22,774.41	
Other income	1,362.26	
Transfers:		
General Reserve Fund - Budget Appropriations July 1, 1961 to June 30, 1962.	2,848,480.00	
American Geophysical Union	2,250.00	
Embryology Building Program	1,000.00	
Harkavy Fund - Income	1,000.00	
Harry Oscar Wood Fund - Income	24,133.95	2,932,609.18
Total available for expenditure		$3,932,904.25
Expenditures:		
Salaries	$1,525,418.82	
Equipment	200,018.06	
Laboratory	185,553.32	
Buildings - fuel, lights, etc.	111,221.45	
Shop	12,432.96	
Travel	80,754.62	
Dormitory	10,391.84	
Operating	125,127.96	
Financial Administration - investment and custody fees	63,010.71	
Insurance Premiums	24,131.02	
General Publications	61,177.80	
Other publication expense	25,571.07	
Fellowships	101,646.50	
Awards	24,000.00	
Retirement Plan Contributions	203,633.91	
Pensions	46,791.05	
Hospitalization Plan and Collective Insurance	42,009.81	
Social security taxes	31,063.74	
Total expenditures		2,873,954.64
Balance June 30, 1962		$1,058,949.61

EXHIBIT 2 CHANGES IN RESTRICTED GRANTS FOR THE YEAR ENDED JUNE 30, 1962

	Balance July 1, 1961	Grants Received	Expenditures		Transfers	Balance June 30, 1962
			Salary	Other		
Departmental Research Operations:						
Department of Genetics:						
The National Foundation	...	$ 1,250.00	...	$ 1,085.42	...	$ 164.58
U. S. Public Health Service RG 149	$ 885.70	12,250.00	$ 8,958.36	822.95	...	3,354.39
U. S. Public Health Service C 2158	8,718.13	19,424.75	8,789.00	15,883.86	...	3,470.02
U. S. Public Health Service GPD 12095	...	500.00	...	495.00	...	5.00
Geophysical Laboratory:						
American Geophysical Union	...	6,000.00	3,750.00	...	$2,250.00	...
Arthur L. Day Library - Gift	...	2,750.00	...	322.23	...	2,427.77
National Science Foundation G 17491	3,078.68	...	...	3,078.68	...	...
Department of Terrestrial Magnetism:						
Geological Survey of Finland	...	18,639.14	...	1,136.23	...	17,502.91
National Science Foundation G 5410	27,681.32	...	...	25,704.28	...	1,977.04
National Science Foundation Y/23.1/321	62,602.90	...	...	17,108.32	...	45,494.58
National Science Foundation G 9770	94,407.67	...	...	36,276.80	...	58,130.87
National Science Foundation G 13396	18,240.18	...	...	17,885.59	...	354.59
National Science Foundation G 14593	1,866.41	16,500.00	...	17,405.45	...	960.96
National Science Foundation G 19568	...	58,000.00	...	4,166.30	...	53,833.70
National Science Foundation G 22227	...	40,000.00	...	...	...	40,000.00
Richard B. T. Roberts - Gift	...	700.00	...	700.00	...	...
Department of Embryology:						
U. S. Public Health Service CF 9529	339.50	...	...	339.50	...	...
U. S. Public Health Service FF 280	...	8,186.00	...	5,684.17	...	2,501.83
U. S. Public Health Service BT 744	...	500.00	...	500.00	...	...
U. S. Public Health Service GPD 5309	...	500.00	...	500.00	...	...
Mount Wilson Observatory:						
Anonymous	9,500.00	2,500.00	...	...	...	12,000.00
Research Projects, Fellowships, etc.:						
Carnegie Corporation of New York:						
Natural Science Fellowships	177,825.00	...	...	44,099.94	...	133,725.06
National Physical Laboratory	...	15,000.00	...	...	...	15,000.00
Terrestrial Magnetism:						
Telescope Image Converter	4,433.14	...	...	4,433.14	...	...
Total	$409,578.63	$202,699.89	$21,497.36	$197,627.86	$2,250.00	$390,903.30

EXHIBIT 3

CHANGES IN ENDOWMENT, GENERAL RESERVE, AND SPECIAL FUNDS FOR THE YEAR ENDED JUNE 30, 1962

	Balance July 1, 1961	Investment Income	Realized Capital Gain, net	Other Income, Gifts and Bequests	Appropriations	Transfers	Expenditures	Balance June 30, 1962
Endowment Funds:								
Endowment Fund	$52,851,419.42	...	$3,466,095.09	...	...	...	...	$56,317,514.51
Capital Reserve Fund	4,951,480.20	...	324,821.43	...	...	...	...	5,276,301.63
General Reserve Fund:								
Unappropriated	1,791,490.57	$2,981,035.00	268,909.59	$15,829.88	($3,116,993.00)	...	...	1,940,272.04
Appropriated:								
Budget (1961-1962)	2,848,480.00	...	...	...	(2,848,480.00)	...	...	...
Budget (1962-1963)	...	...	...	...	3,116,993.00	...	...	3,116,993.00
Building Program	348,059.92	...	...	...	...	($ 1,000.00)	$318,176.61	28,883.31
Special Funds:								
Bickel Fund	20,771.54	883.45	1,204.57	...	...	...	...	22,859.56
Colburn Fund	167,592.06	...	11,029.37	...	...	...	...	178,621.43
George E. Hale Relief Fund	7,776.74	317.58	431.32	...	...	...	...	8,525.64
Harkavy Fund	6,883.23	...	396.72	...	...	...	...	7,279.95
Harkavy Fund - Income	2,471.85	358.81	92.51	...	...	(1,000.00)	...	1,923.17
Harriet H. Mayor Relief Fund	9,937.23	...	613.12	...	...	...	...	10,550.35
Special Purpose Funds	84,768.87	3,581.49	4,883.37	...	...	...	...	93,233.73
Teeple Fund	14,844.47	...	975.77	...	...	...	...	15,820.24
van Gelder Fund	1,908.36	...	118.88	...	...	...	...	2,027.24
Woloff Fund	35,373.15	1,503.81	2,050.44	...	...	...	...	38,927.40
Harry Oscar Wood Fund - Bequest	451,936.22	...	21,643.62	...	...	3,558.96	...	477,138.80
Harry Oscar Wood Fund - Income	25,626.49	16,660.18	1,134.60	...	...	(27,692.91)	...	15,728.36
Total	$63,620,820.32	$3,004,340.32	$4,104,400.40	$15,829.88	($2,848,480.00)	($26,133.95)	$318,176.61	$67,552.600.36

EXHIBIT 4

CHANGES IN BUILDINGS, LAND, AND EQUIPMENT FUND FOR THE YEAR ENDED JUNE 30, 1962

	Balance July 1, 1961	Expenditures (Note)	Deductions	Transfers	Balance June 30, 1962	Classification of June 30, 1962 Balance: Buildings and Land	Library	Equipment
Departments of Research:								
Department of Plant Biology Stanford, California	$ 184,667.52	$ 1,562.31	...	...	$ 186,229.83	$ 75,519.58	$ 32,269.89	$ 78,440.36
Department of Genetics Long Island, New York	1,244,026.95	39,342.30	$ 1,373.67	...	1,281,995.58	1,010,148.66	93,389.36	178,457.56
Geophysical Laboratory Washington, D. C.	543,892.30	69,365.83	2,750.00	...	610,508.13	170,383.79	84,012.28	356,112.06
Mount Wilson Observatory Pasadena, California	1,728,555.17	11,296.19	1,852.85	...	1,737,998.51	294,584.03	92,804.49	1,350,609.99
Department of Terrestrial Magnetism Washington, D. C.	1,075,295.71	51,283.27	12,289.25	$4,554	1,118,843.73	401,081.29	51,404.27	666,358.17
Department of Embryology Baltimore, Maryland	128,328.78	66,177.59	11,353.41	...	183,152.96	...	14,778.08	168,374.88
Total Departments of Research	$4,904,766.43	$239,027.49	$29,619.18	$4,554	$5,118,728.74	$1,951,717.35	$368,658.37	$2,798,353.02
Office of Administration Washington, D. C.	902,529.60	28,352.17	241.87	(4,554)	926,085.90	846,029.54	...	80,056.36
Total	$5,807,296.03	$267,379.66	$29,861.05	...	$6,044,814.64	$2,797,746.89	$368,658.37	$2,878,409.38

Note: Current Expenditures for Equipment:

Restricted Grants	$ 14,576.60
Operating Funds	200,018.06
Expenditures Capitalized:	
Prior Years	21,600.00
Current Year	13,600.00
Gift Capitalized - Prior Years	17,585.00
Total	$267,379.66

SCHEDULE 1

BUDGET SUMMARY OF OPERATING FUNDS FOR THE YEAR ENDED JUNE 30, 1962

	Unexpended Appropriations July 1, 1961	Budget Appropriation for the fiscal year ended June 30, 1962	Continuing Appropriations	Allotments	Total Appropriations	Disposition of Total Appropriations		
						Total Expenditures	Unexpended Appropriations	
							Transferred to General Contingent Fund	Reserved for Liabilities and Commitments and General Contingent Fund
Departmental Research Operations:								
Department of Plant Biology. . .	$ 3,589.09	$ 118,000.00	. . .	$ 7,144.44	$ 128,733.53	$ 97,251.34	$ 24,278.30	$ 7,203.89
Department of Genetics	9,444.53	197,300.00	. . .	17,050.00	223,794.53	190,331.18	24,669.83	8,793.52
Dormitory	3,747.67	2,000.00	$ 7,213.42	. . .	12,961.09	9,040.27	. . .	3,920.82
Geophysical Laboratory	16,144.24	333,100.00	. . .	73,303.56	422,547.80	384,620.13	5,075.80	32,851.87
Mount Wilson Observatory	19,401.30	357,900.00	362.26	5,873.24	383,536.80	352,456.10	16,181.99	14,898.71
Department of Terrestrial Magnetism	69,786.42	430,600.00	. . .	41,490.26	541,876.68	464,888.28	19,758.05	57,230.35
Department of Embryology	44,441.94	252,180.00	. . .	37,159.18	333,781.12	316,646.95	5,940.40	11,193.77
Total Departmental Research Operations	$ 166,555.19	$1,691,080.00	$ 7,575.68	$182,020.68	$2,047,231.55	$1,815,234.25	$ 95,904.37	$ 136,092.93
Administration	6,220.21	461,600.00	. . .	69,934.63	537,754.84	519,777.05	5,978.08	11,999.71
General Operations	113,584.72	339,000.00	. . .	(305,146.14)	147,438.58	. . .	. . .	147,438.58
General Publications	69,512.32	10,000.00	22,774.41	. . .	102,286.73	61,556.67	. . .	40,730.06
Research Projects, Fellowships, etc.	133,396.64	21,700.00	1,000.00	106,635.68	262,732.32	153,888.16	13,550.66	95,293.50
Retirement Plan Contributions . . .	15,000.00	212,500.00	. . .	. . .	227,500.00	203,633.91	8,866.09	15,000.00
Retirement Plan - Life Contingency Account	48,399.47	16,000.00	. . .	5,300.41	69,699.88	. . .	. . .	69,699.88
Pension Fund	134,799.61	28,000.00	. . .	19,094.73	181,894.34	46,791.05	. . .	135,103.29
Hospitalization Plan and Collective Insurance	. . .	40,100.00	. . .	1,909.81	42,009.81	42,009.81	. . .	. . .
Social Security Taxes	. . .	28,500.00	. . .	2,709.29	31,209.29	31,063.74	. . .	145.55
Total	$ 687,468.16	$2,848,480.00	$31,350.09	$ 82,459.09	$3,649,757.34	$2,873,954.64	$124,299.20	$ 651,503.50
General Contingent Fund	312,826.91	. . .	. . .	(29,680.00)	283,146.91	. . .	(124,299.20)	407,446.11
Total	$1,000,295.07	$2,848,480.00	$31,350.09	$ 52,779.09	$3,932,904.25	$2,873,954.64	. . .	$1,058,949.61

SCHEDULE 2

SECURITIES, JUNE 30, 1962 AND INCOME RECEIVED DURING THE YEAR

	Book Value	Approximate Market Value	Per Cent of Total Investments: Book Value	Per Cent of Total Investments: Approximate Market Value	Income Received
Bonds:					
United States Government	$ 3,686,131.68	$ 3,720,192	5.55	4.60	$ 110,457.88
Foreign and International Bank	2,641,408.22	2,571,828	3.97	3.18	112,610.02
Public Utility	10,122,191.59	9,674,380	15.23	11.96	381,809.58
Communication	3,907,499.98	3,597,188	5.88	4.45	145,665.40
Railroad	368,218.96	317,610	.55	.39	16,088.97
Railroad Equipment Trust	76,685.33	79,485	.12	.10	3,318.75
Industrial and Miscellaneous	18,174,738.94	18,048,824	27.34	22.32	778,336.78
Total Bonds	$38,976,874.70	$38,009,507	58.64	47.00	$1,548,287.38 (a)
Stocks:					
Preferred	$ 1,619,411.87	$ 1,506,863	2.44	1.87	$ 71,650.00
Common	25,868,007.76	41,351,186	38.92	51.13	1,372,513.64
Total Stocks	$27,487,419.63	$42,858,049	41.36	53.00	$1,444,163.64
Total	$66,464,294.33	$80,867,556	100.00	100.00	$2,992,451.02

(a) After deducting bond premium amortization of $21,521.05

SCHEDULE OF SECURITIES

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
	United States Government Bonds			
$ 100,000	United States of America, Treasury Bills	7-26-62	$ 99,770.00	$ 99,785
160,000	United States of America, Treasury Bills	10-25-62	157,705.60	158,479
1,105,000	United States of America, Ctf. of Ind., 3¼s	1963	1,105,000.00	1,105,691
1,470,000	United States of America, Treasury Notes 3¾s	1964	1,471,370.80	1,480,106
403,000	United States of America, Treasury Notes 4⅞s	1963	402,285.28	412,068
450,000	United States of America, Treasury Notes 5s	1964	450,000.00	464,063
$3,688,000	Total United States Government		$3,686,131.68	$3,720,192
	Foreign and International Bank Bonds			
$ 250,000	Aluminum Co. of Canada, Ltd., S. F. Deb. 3⅞s	1970	$ 251,561.49	$ 247,813
500,000	Aluminum Co. of Canada, Ltd., S. F. Deb. 4½s	1980	508,695.20	500,000
150,000	Australia (Commonwealth of) 4½s	1971	147,750.00	145,500
137,000	Australia (Commonwealth of) 5s	1972	137,000.00	137,514
250,000	British Columbia Power Commission, S. F. Deb. Series "L" 4⅜s	1987	245,000.00	240,000
125,000	Intl. Bank for Reconstruction & Development, 3s	1976	125,000.00	108,438
125,000	Intl. Bank for Reconstruction & Development, 3⅜s	1975	123,125.00	115,938
250,000	Intl. Bank for Reconstruction & Development, 4½s	1977	250,000.00	252,500
150,000	Noranda Mines Ltd., S. F. Deb. 4¾s	1968	151,399.03	133,875
200,000	Shawinigan Water & Power Co., 1st Mtg. & Collat. Tr. S. F. Series "M" 3s	1971	203,240.00	161,500
500,000	Toronto (Municipality of Metropolitan), S. F. Deb. 5s	1979	498,637.50	528,750
$2,637,000	Total Foreign and International Bank		$2,641,408.22	$2,571,828
	Public Utility Bonds			
$ 250,000	California Oregon Power Co., 1st Mtg. 3⅞s	1986	$ 252,622.54	$ 229,688
125,000	Columbia Gas System, Inc., Series "B" 3s	1975	126,745.43	108,750
250,000	Columbia Gas System, Inc., Series "F" 3⅞s	1981	245,937.50	240,313
237,000	Columbus & Southern Ohio Electric Co., 1st Mtg. 3¼s	1970	242,328.42	218,633
300,000	Commonwealth Edison Co., 1st Mtg. Series "R" 3½s	1986	300,612.54	264,375
300,000	Consolidated Edison Co. of N.Y., 1st & Ref. Mtg. Series "L" 3⅝s	1986	303,222.91	266,250
300,000	Consolidated Edison Co. of N.Y., 1st & Ref. Mtg. Series "N" 5s	1987	301,969.20	315,375
300,000	Consolidated Natural Gas Co., Deb. 2¾s	1968	300,231.22	283,125
150,000	Consumers Power Co., 1st Mtg. 4s	1986	151,194.42	143,438
203,000	Consumers Power Co., 1st Mtg. 4¾s	1987	204,090.77	210,866
300,000	Florida Power Corporation, 1st Mtg. 3⅞s	1986	301,796.95	280,875
500,000	Illinois Power Co., 1st Mtg. 3¾s	1986	497,937.50	458,750
200,000	Minnesota Power & Light Co., 1st Mtg. 3⅛s	1975	202,126.87	174,750
250,000	Niagara Mohawk Power Corp., Gen. Mtg. 3⅝s	1986	252,704.50	224,688
400,000	Niagara Mohawk Power Corp., Gen. Mtg. 4⅞s	1987	402,938.35	418,000
100,000	Ohio Power Co., 1st Mtg. 3¼s	1968	101,500.00	96,125
200,000	Pacific Gas & Electric Co., 1st & Ref. Mtg. Series "X" 3⅛s	1984	201,283.28	164,000
300,000	Pacific Gas & Electric Co., 1st & Ref. Mtg. Series "Y" 3⅜s	1987	305,584.79	257,625
250,000	Pacific Gas & Electric Co., 1st & Ref. Mtg. Series "BB" 5s	1989	251,660.34	263,125
87,000	Panhandle Eastern Pipe Line Co., S. F. Deb. 3¼s	1973	87,677.86	80,258
50,000	Philadelphia Electric Co., 1st & Ref. Mtg. 2⅞s	1978	49,687.50	41,813
500,000	Philadelphia Electric Co., 1st & Ref. Mtg. 4⅝s	1987	500,000.00	514,688
207,000	Philadelphia Electric Power Co., 1st Mtg. 2⅝s Guar.	1975	209,236.62	174,915
250,000	Potomac Electric Power Co., Deb. 4⅝s	1982	255,185.60	254,375
200,000	Public Service Co. of Indiana, 1st Mtg. Series "F" 3⅛s	1975	202,220.21	176,250
400,000	Public Service Co. of Indiana, 1st Mtg. Series "L" 4⅞s	1987	400,000.00	413,000
500,000	Public Service Electric & Gas Co., 1st & Ref. Mtg. 4⅞s	1987	503,995.49	522,500
250,000	Southern California Edison Co., 1st & Ref. Mtg. Series "G" 3⅝s	1981	247,765.00	223,750
250,000	Southern California Edison Co., 1st & Ref. Mtg. Series "H" 4¼s	1982	251,484.40	247,813
200,000	Southern California Edison Co., 1st & Ref. Mtg. Series "J" 4⅞s	1982	201,763.11	208,500
191,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 3s	1969	192,856.18	178,585
500,000	Tennessee Gas Transmission Co., 5s	1982	505,000.00	502,188
265,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 5¼s	1977	265,000.00	274,275

SCHEDULE OF SECURITIES—Continued

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
	Public Utility Bonds—Concluded			
$ 500,000	Union Electric Co., 1st Mtg. 3¾s	1986	$ 500,089.75	$ 458,750
265,000	United Gas Corp., 1st Mtg. & Coll. Tr. 2¾s	1967	265,000.00	243,138
235,000	Virginia Electric & Power Co., 1st & Ref. Mtg. Series "M" 4⅛s	1986	238,742.34	228,831
300,000	Washington Water Power Co., 1st Mtg. 4⅞s	1987	300,000.00	312,000
$10,065,000	Total Public Utility		$10,122,191.59	$9,674,380
	Communication Bonds			
$ 150,000	American Telephone & Telegraph Company, Deb. 2¾s	1975	$ 150,956.25	$ 127,875
350,000	American Telephone & Telegraph Company, 3¼s	1984	358,963.68	300,125
800,000	American Telephone & Telegraph Company, Deb. 3⅞s	1990	817,827.48	736,000
500,000	American Telephone & Telegraph Company, Deb. 4⅜s	1985	504,805.20	502,500
400,000	Illinois Bell Telephone Co., 1st Mtg. Series "E" 4¼s	1988	404,471.60	397,000
200,000	Mountain States Telephone & Telegraph Co., Deb. 3⅛s	1978	200,770.00	169,250
100,000	New York Telephone Co., Ref. Mtg. Series "E" 3⅛s	1978	100,739.59	85,375
200,000	Pacific Telephone & Telegraph Co., Deb. 3¼s	1978	202,428.04	174,250
300,000	Pacific Telephone & Telegraph Co., Deb. 4⅜s	1988	305,984.22	300,750
250,000	Southern Bell Telephone & Telegraph Co., Deb. 4s	1983	251,044.30	238,750
300,000	Southern Bell Telephone & Telegraph Co., Deb. 5s	1986	305,509.62	316,500
300,000	Southwestern Bell Telephone Co., Deb. 3⅛s	1983	304,000.00	248,813
$ 3,850,000	Total Communication		$3,907,499.98	$3,597,188
	Railroad Bonds			
$ 100,000	Chesapeake & Ohio Railway Co., Gen. Mtg. 4½s	1992	$ 99,464.29	$ 96,000
267,000	Fort Worth & Denver Railway Company, 1st Mtg. 4⅜s Guar.	1982	268,754.67	221,610
$ 367,000	Total Railroad		$ 368,218.96	$ 317,610
	Railroad Equipment Trust Bonds			
$ 50,000	Chicago, Burlington & Quincy Railroad Co., Eq. Tr. 2¼s Guar.	1963	$ 47,839.05	$ 49,485
30,000	Pennsylvania Railroad Company, Eq. Tr. Series "S" 2⅜s Guar.	1962	28,846.28	30,000
$ 80,000	Total Railroad Equipment Trust		$ 76,685.33	$ 79,485
	Industrial and Miscellaneous Bonds			
$ 250,000	Aluminum Co. of America, S. F. Deb. 4¼s	1982	$ 250,000.00	$ 250,000
234,000	Bristol-Myers Co., Deb. 3s	1968	234,310.66	226,103
550,000	C. I. T. Financial Corp., Deb. 4¾s	1970	536,937.50	570,625
400,000	Commercial Credit Co., Notes 3⅝s	1976	406,626.56	364,000
400,000	Continental Oil Company (Del.), S. F. Deb. 3s	1984	403,707.00	336,000
105,000	Corn Products Co., Sub. Deb. 4⅝s	1983	109,557.13	109,200
500,000	Crown Zellerbach Corp., Prom. Note 4⅛s	1981	500,000.00	476,250
400,000	Federal Farm Loan Consolidated, 4⅜s	1969	394,000.00	406,000
200,000	Federal Farm Loan Consolidated, 4½s	1964	201,714.29	203,375
285,000	Federal Farm Loan Consolidated, 4½s	1970	284,330.96	290,700
1,025,000	Federal National Mortgage Association, 4⅛s	1970	1,015,070.31	1,014,750
465,000	Federal National Mortgage Association, 4⅛s	1971	466,003.98	460,350
400,000	Federal National Mortgage Association, 4⅝s	1970	394,500.00	409,000
500,000	Federal National Mortgage Association, 5⅛s	1972	498,125.00	532,500
500,000	Food Machinery & Chemical Corp., S. F. Deb. 3.80s	1981	500,000.00	465,000
364,000	Four Corners Pipe Line Company, Sec. Note 5s	1982	364,000.00	383,110
500,000	General Electric Credit Corp. (N. Y.) Prom. Note 5s	1975	500,000.00	510,000
200,000	General Motors Acceptance Corp., Deb. 3½s	1972	202,454.62	184,000
480,000	General Motors Acceptance Corp., Deb. 4s	1979	435,037.50	450,000
200,000	General Motors Acceptance Corp., Deb. 5s	1977	195,000.00	210,000
200,000	General Motors Acceptance Corp., Deb. 5s	1981	199,000.00	211,000
150,000	General Portland Cement Co., Conv. Sub. Deb. 5s	1977	153,375.09	163,500
275,000	Goodrich (B. F.) Company, 1st Mtg. 2¾s	1965	275,144.85	268,125

SCHEDULE OF SECURITIES—Continued

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
	Industrial and Miscellaneous Bonds—Concluded			
$ 662,121.59	Instlcorp, Inc., Collat. Tr. Note Series A-16	1991	$ 640,004.46	$ 660,466
553,035.63	Instlcorp, Inc., Collat. Tr. Note Series A-19	1991	534,736.60	550,270
289,007.18	Instlcorp, Inc., Collat. Tr. Note Series A-21	1991	278,891.94	289,007
352,773.34	Instlcorp, Inc., Collat. Tr. Note Series A-23	1991	347,058.58	351,891
516,359.57	Instlcorp, Inc., Collat. Tr. Note Series A-36	1992	493,984.64	496,996
400,000	Intl. Harvester Credit Corp., Deb. 4⅝s	1979	398,000.00	415,000
300,000	Kaiser Aluminum & Chemical Corp., 1st Mtg. 5½s	1987	300,000.00	315,000
236,000	Lorillard (P.) Company, Deb. 3s	1963	236,000.00	233,640
200,000	Montgomery Ward Credit Corp., Deb. 4⅞s	1980	199,000.00	207,000
195,000	National Dairy Products Corp., Deb. 2¾s	1970	196,043.94	176,719
488,000	Phillips Petroleum Company, S. F. Deb. 2¾s	1964	488,488.91	480,680
150,000	Quaker Oats Co., Deb. 2⅝s	1964	148,922.50	145,688
100,000	Riegel Paper Corp., S. F. Deb. 3⅜s	1981	100,000.00	95,000
250,000	Scovill Mfg. Co., Deb. 4¾s	1982	246,250.00	247,500
300,000	Seagram (Joseph E.) & Sons, Incorporated, Deb. 2½s	1966	298,500.00	288,000
525,000	Sears Roebuck Acceptance Corp., Sub. Deb. 4⅝s	1977	511,505.00	525,000
300,000	Sinclair Oil Corporation, Conv. Sub. Deb. 4⅜s	1986	314,788.96	295,875
300,000	Superior Oil Company, The (California), Deb. 3¾s	1981	300,000.00	280,500
215,000	Talcott (James), Inc., Senior Note 5½s	1966-80	212,850.00	224,944
300,000	Texas Corporation, Deb. 3s	1965	302,071.76	294,000
250,000	Tidewater Oil Company, S. F. Deb. 3½s	1986	250,000.00	212,500
905,926.01	Trailer Train Company, 4⅞s	1976	905,926.01	917,250
451,000	Tremarco Corporation, 1st Mtg. Series "E" 5s	1983	451,000.00	466,785
346,000	Union Oil Co. of California, Deb. 2¾s	1970	350,074.87	311,400
400,000	Westinghouse Electric Corp., Deb. 2⅝s	1971	401,745.32	346,000
250,000	Whirlpool Corporation, S. F. Deb. 3½s	1980	250,000.00	215,625
500,000	Woolworth (F. W.) Co., Prom. Note 5s	1982	500,000.00	512,500
$18,318,223.32	Total Industrial and Miscellaneous		$18,174,738.94	$18,048,824
$39,005,223.32	Bonds — Funds Invested		$38,976,874.70	$38,009,507

Number of Shares			
	Preferred Stocks		
1,500	Appalachian Power Co., 4½% Cum. Pref.	$ 159,000.00	$ 142,500
1,500	Bethlehem Steel Corporation, 7% Cum. Pref.	183,637.50	211,875
3,800	Carrier Corporation, 4½% Cum. Pref.	197,931.28	180,500
1,900	Consolidated Edison Co. of N. Y., $5.00 Cum. Pref.	202,815.50	198,550
800	National Distillers and Chemical Corp., 4¼% Cum. Conv. Pref.	80,000.00	69,000
2,000	Niagara Mohawk Power Corp., 3.60% Cum. Pref.	207,990.00	146,000
1,300	Ohio Power Co., 4½% Cum. Pref.	144,630.02	123,663
3,100	United States Steel Corporation, 7% Cum. Pref.	443,407.57	434,775
15,900	Total Preferred Stocks	$1,619,411.87	$1,506,863
	Common Stocks		
5,000	Aetna Casualty & Surety Co.	$ 192,782.20	$ 306,250
28,100	Aluminium Ltd.	791,574.11	540,925
14,350	American Electric Power Co., Inc.	186,637.38	808,981
19,373	American Telephone & Telegraph Company	963,574.35	2,009,949
11,500	Arizona Public Service Co.	432,901.08	296,125
6,200	Armco Steel Corporation	240,383.71	305,350
14,100	Armstrong Cork Company	231,516.80	697,950
10,000	Atchison, Topeka & Santa Fe Railway Co.	166,256.21	228,750
6,000	Campbell Soup Company	362,612.33	534,000
12,000	Caterpillar Tractor Co.	96,913.60	382,500
2,872	Chase Manhattan Bank, N. Y.	81,118.78	198,886

SCHEDULE OF SECURITIES—Concluded

Number of Shares	Description	Book Value	Approximate Market Value
	Common Stocks—Concluded		
4,800	Christiana Securities Co.	$ 356,143.00	$ 787,200
9,800	Coca Cola Company (The)	628,984.09	749,700
18,000	Continental Oil Company, (Del.)	176,753.10	864,000
2,500	Corning Glass Works	59,631.83	290,625
1,500	E. I. du Pont de Nemours & Co.	61,220.33	295,781
11,294	Eastman Kodak Company	134,396.92	1,007,990
9,500	Falconbridge Nickel Mines, Ltd.	550,837.50	424,820
11,474	Farbenfabriken Bayer AG-ADR Par 50 Deutschemark	655,244.34	622,465
7,257	First National City Bank of N. Y.	348,095.50	605,052
12,000	Florida Power & Light Co.	148,863.69	636,000
14,700	Ford Motor Company	849,086.16	1,131,900
3,700	General American Transportation Corp.	153,433.14	201,650
31,100	General Electric Company	647,197.55	1,850,450
28,000	General Motors Corporation	968,006.87	1,354,500
2,700	General Reinsurance Corp.	220,697.46	425,250
16,500	Gillette Company	401,418.90	577,500
21,712	Goodyear Tire & Rubber Company	480,153.74	681,214
18,606	Gulf Oil Corporation	95,259.49	665,165
4,340	Home Insurance Co. of N. Y.	273,523.65	209,134
14,000	Household Finance Corp.	659,231.85	507,500
22,000	Illinois Power Co.	589,126.02	731,500
4,000	Insurance Co. of North America	42,590.65	297,000
9,500	International Business Machines Corp.	148,783.26	3,222,875
13,800	International Nickel Co. of Canada, Ltd.	379,279.52	791,775
8,500	Kellogg Company	332,482.68	461,125
6,800	Kennecott Copper Corporation	446,112.26	481,950
1,025	Litton Industries, Inc.	122,512.02	94,300
4,600	Marquette Cement Manufacturing Co.	190,140.68	158,700
13,000	Mead Corporation	614,427.23	446,875
5,000	Merck & Co.	93,798.41	325,000
5,800	National Cash Register Co.	530,904.80	437,900
10,100	Niagara Mohawk Power Corp.	458,553.32	405,263
2,100	Norfolk & Western Railway Company	217,512.31	183,750
8,600	North American Aviation, Inc.	559,647.34	491,275
9,000	Northwest Bancorporation	231,895.01	333,000
17,000	Ohio Edison Co.	587,855.31	675,750
1,800	Otis Elevator Company	97,869.13	93,150
8,000	Panhandle Eastern Pipe Line Co.	431,553.54	417,000
5,000	Philip Morris Incorporated	493,240.88	364,375
27,000	Philips' Incandescent Lamp Works, Ltd. (N. V. Philips' Gloeilampenfabrieken), Par 25 Florin	784,874.79	1,213,313
10,098	Pittsburgh Plate Glass Co.	713,845.30	489,753
5,500	Republic Natural Gas Co.	. . .	13,406
14,100	Revere Copper & Brass, Inc.	665,214.01	481,163
32,400	Royal Dutch Petroleum Co.	1,190,625.97	1,162,350
12,000	Scott Paper Company	53,041.98	346,500
10,200	Shell Oil Company	170,667.87	321,300
18,500	Standard Oil Co. (New Jersey)	392,518.12	925,000
15,500	Stevens (J. P.) & Co.	476,576.67	476,625
29,704	Texaco, Inc.	297,924.93	1,444,357
500	Texas Instruments Inc.	63,483.24	31,063
7,600	Texas Utilities Co.	163,042.97	304,000
6,000	Travelers Insurance Co.	452,662.70	789,000
30,750	Unilever N. V., Par 20 Florin	1,162,805.47	1,149,281
14,200	U. S. Plywood Corp.	697,928.16	610,600
7,000	United States Steel Corporation	160,003.06	308,875
13,800	Virginia Electric & Power Co.	240,058.49	674,475
783,455	Total Common Stocks	$25,868,007.76	$41,351,186
	Common and Preferred Stocks - Funds Invested	$27,487,419.63	$42,858,049
	Aggregate Investments (Bonds and Stocks)	$66,464,294.33	$80,867,556

SUMMARY OF SECURITY TRANSACTIONS JULY 1, 1961 TO JUNE 30, 1962

Cash awaiting investment - July 1, 1961				$ 318,121.47

Sales and Redemptions

	Gain	Loss	Book Value	
Bonds	$ 12,349.41	...	$ 5,517,353.28	
Common Stocks	4,089,270.39	...	4,683,491.66	
Sale of Stock Rights	16,908.38	...	...	
	$4,118,528.18	...	$10,200,844.94	
Net Gain - Statement B	...	$4,118,528.18	4,118,528.18	
	$4,118,528.18	$4,118,528.18		
Total Sales and Redemptions				$14,319,373.12
Income applied to amortization of bond premium				21,521.05
Gifts and bequests				700.00
				$14,659,715.64
Cash transferred from investment				1,355,159.51
Total				$13,304,556.13

Acquisitions

Bonds	$ 6,121,410.73	
Common Stocks	7,086,220.30	
Total Acquisitions		$13,207,631.03
Cash awaiting investment - June 30, 1962		$ 96,925.10

Abstract of Minutes

of the Sixty-Fourth Meeting of the Board of Trustees

The annual meeting of the Board of Trustees was held in the new laboratory building of the Department of Embryology, Baltimore, Maryland, on Friday, May 11, 1962. Mr. Henry, Chairman of the Board, presided.

The following Trustees were in attendance: Amory H. Bradford, Omar N. Bradley, Vannevar Bush, Caryl P. Haskins, Barklie McKee Henry, Alfred L. Loomis, Keith S. McHugh, Henry S. Morgan, William I. Myers, Garrison Norton (Secretary pro tem), Richard S. Perkins, Elihu Root, Jr., Charles P. Taft, James N. White, and Robert E. Wilson.

The minutes of the Sixty-Third Meeting were approved.

With unanimous consent, Crawford H. Greenewalt and Juan T. Trippe were reelected members of the Board of Trustees.

The Chairman notified the Trustees of the death of Robert Woods Bliss. Mr. Root spoke of the Trustees' high esteem for Mr. Bliss and of his many contributions to the Institution. Mr. Root proposed the following resolutions, which the Trustees adopted unanimously:

> *Be It Resolved,* That the Trustees of the Carnegie Institution of Washington desire to record their deep sense of loss at the death of their distinguished fellow member, Robert Woods Bliss.
>
> *And Be It Further Resolved,* That these resolutions be entered on the minutes of the Institution and a copy be sent to Mrs. Bliss.

The annual report of the President was accepted.

The reports of the Executive Committee, the Finance Committee, the Retirement Committee, the Auditor, and the Auditing Committee were accepted.

To provide for operation of the Institution for the fiscal year beginning July 1, 1962, and upon recommendation of the Executive Committee, the sum of $3,116,993 was appropriated from the General Reserve Fund.

Carl J. Gilbert and William W. Rubey were elected members of the Board of Trustees.

Garrison Norton was elected Secretary of the Board of Trustees to fill the unexpired term of the late Robert Woods Bliss.

Vacancies in standing committees, including one resulting from the resignation of Henry R. Shepley as a member of the Executive Committee, were filled as follows: Amory H. Bradford and Robert E. Wilson were elected members of the Executive Committee for two-year terms, and Robert A. Lovett and James N. White were reelected for three-year terms. The following were reelected for three-year terms: Richard S. Perkins, Elihu Root, Jr., and James N. White as members of the Finance Committee; Omar N. Bradley and Henry S. Morgan as members of the Retirement Committee; and Charles P. Taft as member of the Nominating Committee. The following were elected or reelected for one-year terms: Henry S. Morgan as Chairman of the Executive Committee, James N. White as Chairman of the Finance Committee, Keith S. McHugh as Chairman of the Auditing Committee, Omar N. Bradley as Chairman of the Retirement Committee, Amory H. Bradford as Chairman of the Nominating Committee, and Richard S. Perkins as member of the Nominating Committee.

Articles of Incorporation

Public No. 260. An Act to incorporate the Carnegie Institution of Washington

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That the persons following, being persons who are now trustees of the Carnegie Institution, namely, Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, Samuel P. Langley, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, Ethan A. Hitchcock, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, their associates and successors, duly chosen, are hereby incorporated and declared to be a body corporate by the name of the Carnegie Institution of Washington and by that name shall be known and have perpetual succession, with the powers, limitations, and restrictions herein contained.

Sec. 2. That the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind; and in particular—

(*a*) To conduct, endow, and assist investigation in any department of science, literature, or art, and to this end to cooperate with governments, universities, colleges, technical schools, learned societies, and individuals.

(*b*) To appoint committees of experts to direct special lines of research.

(*c*) To publish and distribute documents.

(*d*) To conduct lectures, hold meetings, and acquire and maintain a library.

(*e*) To purchase such property, real or personal, and construct such building or buildings as may be necessary to carry on the work of the corporation.

(*f*) In general, to do and perform all things necessary to promote the objects of the institution, with full power, however, to the trustees hereinafter appointed and their successors from time to time to modify the conditions and regulations under which the work shall be carried on, so as to secure the application of the funds in the manner best adapted to the conditions of the time, provided that the objects of the corporation shall at all times be among the foregoing or kindred thereto.

Sec. 3. That the direction and management of the affairs of the corporation and the control and disposal of its property and funds shall be vested in a board of trustees, twenty-two in number, to be composed of the following individuals: Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, *Samuel P. Langley,* William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, *Ethan A. Hitchcock,* Elihu Root, John C. Spooner,

Andrew D. White, Charles D. Walcott, Carroll D. Wright, who shall constitute the first board of trustees. The board of trustees shall have power from time to time to increase its membership to not more than twenty-seven members. Vacancies occasioned by death, resignation, or otherwise shall be filled by the remaining trustees in such manner as the by-laws shall prescribe; and the persons so elected shall thereupon become trustees and also members of the said corporation. The principal place of business of the said corporation shall be the city of Washington, in the District of Columbia.

Sec. 4. That such board of trustees shall be entitled to take, hold, and administer the securities, funds, and property so transferred by said Andrew Carnegie to the trustees of the Carnegie Institution and such other funds or property as may at any time be given, devised, or bequeathed to them, or to such corporation, for the purposes of the trust; and with full power from time to time to adopt a common seal, to appoint such officers, members of the board of trustees or otherwise, and such employees as may be deemed necessary in carrying on the business of the corporation, at such salaries or with such remuneration as they may deem proper; and with full power to adopt by-laws from time to time and such rules or regulations as may be necessary to secure the safe and convenient transaction of the business of the corporation; and with full power and discretion to deal with and expend the income of the corporation in such manner as in their judgment will best promote the objects herein set forth and in general to have and use all powers and authority necessary to promote such objects and carry out the purposes of the donor. The said trustees shall have further power from time to time to hold as investments the securities hereinabove referred to so transferred by Andrew Carnegie, and any property which has been or may be transferred to them or such corporation by Andrew Carnegie or by any other person, persons, or corporation, and to invest any sums or amounts from time to time in such securities and such form and manner as are permitted to trustees or to charitable or literary corporations for investment, according to the laws of the States of New York, Pennsylvania, or Massachusetts, or in such securities as are authorized for investment by the said deed of trust so executed by Andrew Carnegie, or by any deed of gift or last will and testament to be hereafter made or executed.

Sec. 5. That the said corporation may take and hold any additional donations, grants, devises, or bequests which may be made in further support of the purposes of the said corporation, and may include in the expenses thereof the personal expenses which the trustees may incur in attending meetings or otherwise in carrying out the business of the trust, but the services of the trustees as such shall be gratuitous.

Sec. 6. That as soon as may be possible after the passage of this Act a meeting of the trustees hereinbefore named shall be called by Daniel C. Gilman, John S. Billings, Charles D. Walcott, S. Weir Mitchell, John Hay, Elihu Root, and Carroll D. Wright, or any four of them, at the city of Washington, in the District of Columbia, by notice served in person or by mail addressed to each trustee at his place of residence; and the said trustees, or a majority thereof, being assembled, shall organize and proceed to adopt by-laws, to elect officers and appoint committees, and generally to organize the said corporation; and said trustees herein named, on behalf of the corporation hereby incorporated, shall thereupon receive, take over, and enter into possession, custody, and management of all property, real or personal, of the corporation heretofore known as the Carnegie Institution, incorporated, as hereinbefore set forth under "An Act to establish a Code of Law for the District of Columbia, January fourth, nineteen hundred and two," and to all its rights, contracts, claims, and property of any kind or nature; and the several officers of such corporation, or any other person having charge of any of the securities, funds, real or personal, books, or property thereof, shall, on demand, deliver the same to the said trustees appointed by this Act or to the persons appointed by them to receive the same; and the trustees of the existing corporation and the trustees herein named shall and may take such other steps as shall be necessary to carry out the purposes of this Act.

Sec. 7. That the rights of the creditors of the said existing corporation known as the Carnegie Institution shall not in any manner be impaired by the passage of this Act, or

the transfer of the property hereinbefore mentioned, nor shall any liability or obligation for the payment of any sums due or to become due, or any claim or demand, in any manner or for any cause existing against the said existing corporation, be released or impaired; but such corporation hereby incorporated is declared to succeed to the obligations and liabilities and to be held liable to pay and discharge all of the debts, liabilities, and contracts of the said corporation so existing to the same effect as if such new corporation had itself incurred the obligation or liability to pay such debt or damages, and no such action or proceeding before any court or tribunal shall be deemed to have abated or been discontinued by reason of the passage of this Act.

Sec. 8. That Congress may from time to time alter, repeal, or modify this Act of incorporation, but no contract or individual right made or acquired shall thereby be divested or impaired.

Sec. 9. That this Act shall take effect immediately.

Approved, April 28, 1904

By-Laws of the Institution

Adopted December 13, 1904. Amended December 13, 1910, December 13, 1912, December 10, 1937, December 15, 1939, December 13, 1940, December 18, 1942, December 12, 1947, December 10, 1954, October 24, 1957, May 8, 1959, and May 13, 1960.

ARTICLE I

The Trustees

1. The Board of Trustees shall consist of twenty-four members with power to increase its membership to not more than twenty-seven members. The Trustees shall hold office continuously and not for a stated term.

2. In case any Trustee shall fail to attend three successive annual meetings of the Board he shall thereupon cease to be a Trustee.

3. No Trustee shall receive any compensation for his services as such.

4. All vacancies in the Board of Trustees shall be filled by the Trustees by ballot at an annual meeting, but no person shall be declared elected unless he receives the votes of two-thirds of the Trustees present.

ARTICLE II

Officers of the Board

1. The officers of the Board shall be a Chairman of the Board, a Vice-Chairman, and a Secretary, who shall be elected by the Trustees, from the members of the Board, by ballot to serve for a term of three years. All vacancies shall be filled by the Board for the unexpired term; provided, however, that the Executive Committee shall have power to fill a vacancy in the office of Secretary to serve until the next meeting of the Board of Trustees.

2. The Chairman shall preside at all meetings and shall have the usual powers of a presiding officer.

3. The Vice-Chairman, in the absence or disability of the Chairman, shall perform the duties of the Chairman.

4. The Secretary shall issue notices of meetings of the Board, record its transactions, and conduct that part of the correspondence relating to the Board and to his duties.

ARTICLE III

Executive Administration

The President

1. There shall be a President who shall be elected by ballot by, and hold office during the pleasure of, the Board, who shall be the chief executive officer of the Institution. The President, subject to the control of the Board and the Executive Committee, shall have general charge of all matters of administration and supervision of all arrangements for

research and other work undertaken by the Institution or with its funds. He shall prepare and submit to the Board of Trustees and to the Executive Committee plans and suggestions for the work of the Institution, shall conduct its general correspondence and the correspondence with applicants for grants and with the special advisers of the Committee, and shall present his recommendations in each case to the Executive Committee for decision. All proposals and requests for grants shall be referred to the President for consideration and report. He shall have power to remove, appoint, and, within the scope of funds made available by the Trustees, provide for compensation of subordinate employees and to fix the compensation of such employees within the limits of a maximum rate of compensation to be established from time to time by the Executive Committee. He shall be *ex officio* a member of the Executive Committee.

2. He shall be the legal custodian of the seal and of all property of the Institution whose custody is not otherwise provided for. He shall sign and execute on behalf of the corporation all contracts and instruments necessary in authorized administrative and research matters and affix the corporate seal thereto when necessary, and may delegate the performance of such acts and other administrative duties in his absence to the Executive Officer. He may execute all other contracts, deeds, and instruments on behalf of the corporation and affix the seal thereto when expressly authorized by the Board of Trustees or Executive Committee. He may, within the limits of his own authorization, delegate to the Executive Officer authority to act as custodian of and affix the corporate seal. He shall be responsible for the expenditure and disbursement of all funds of the Institution in accordance with the directions of the Board and of the Executive Committee, and shall keep accurate accounts of all receipts and disbursements. Following approval by the Executive Committee he shall transmit to the Board of Trustees before its annual meeting a written report of the operations and business of the Institution for the preceding fiscal year with his recommendations for work and appropriations for the succeeding fiscal year.

3. He shall attend all meetings of the Board of Trustees.

4. There shall be an officer designated Executive Officer who shall be appointed by and hold office at the pleasure of the President, subject to the approval of the Executive Committee. His duties shall be to assist and act for the President as the latter may duly authorize and direct.

5. The President shall retire from office at the end of the fiscal year in which he becomes sixty-five years of age.

ARTICLE IV

Meetings

1. The annual meeting of the Board of Trustees shall be held in the City of Washington, in the District of Columbia, in May of each year on a date set by order of the Executive Committee, unless the date and place of meeting are otherwise set by order of the Executive Committee.

2. Special meetings of the Board may be called by the Executive Committee by notice served personally upon, or mailed to the usual address of, each Trustee twenty days prior to the meeting.

3. Special meetings shall, moreover, be called in the same manner by the Chairman upon the written request of seven members of the Board.

ARTICLE V

Committees

1. There shall be the following standing Committees, *viz.* an Executive Committee, a Finance Committee, an Auditing Committee, a Nominating Committee, and a Retirement Committee.

2. All vacancies occurring in the Executive Committee, the Finance Committee, the Auditing Committee, the Nominating Committee, and the Retirement Committee shall be

filled by the Trustees at the next regular meeting. In case of vacancy in the Finance Committee, the Auditing Committee, the Nominating Committee, or the Retirement Committee, upon request of the remaining members of such committee, the Executive Committee may fill such vacancy by appointment until the next meeting of the Board of Trustees.

3. The terms of all officers and of all members of committees, as provided for herein, shall continue until their successors are elected or appointed.

Executive Committee

4. The Executive Committee shall consist of the Chairman, Vice-Chairman, and Secretary of the Board of Trustees and the President of the Institution *ex officio* and, in addition, five trustees to be elected by the Board by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term.

5. The Executive Committee shall, when the Board is not in session and has not given specific directions, have general control of the administration of the affairs of the corporation and general supervision of all arrangements for administration, research, and other matters undertaken or promoted by the Institution. It shall also submit to the Board of Trustees a printed or typewritten report of each of its meetings, and at the annual meeting shall submit to the Board a report for publication.

6. The Executive Committee shall have power to authorize the purchase, sale, exchange, or transfer of real estate.

Finance Committee

7. The Finance Committee shall consist of not less than five and not more than six members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election.

8. The Finance Committee shall have custody of the securities of the corporation and general charge of its investments and invested funds, including its investments and invested funds as trustee of any retirement plan for the Institution's staff members and employees, and shall care for and dispose of the same subject to the directions of the Board of Trustees. It shall have power to authorize the purchase, sale, exchange, or transfer of securities and to delegate this power. It shall consider and recommend to the Board from time to time such measures as in its opinion will promote the financial interests of the Institution and of the trust fund under any retirement plan for the Institution's staff members and employees, and shall make a report at each meeting of the Board.

Auditing Committee

9. The Auditing Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years.

10. Before each annual meeting of the Board of Trustees, the Auditing Committee shall cause the accounts of the Institution for the preceding fiscal year to be audited by public accountants. The accountants shall report to the Committee, and the Committee shall present said report at the ensuing annual meeting of the Board with such recommendations as the Committee may deem appropriate.

Nominating Committee

11. The Nominating Committee shall consist of the Chairman of the Board of Trustees *ex officio* and, in addition, three trustees to be elected by the Board by ballot for a term of three years, who shall not be eligible for re-election until after the lapse of one year. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Nominating Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

12. Sixty days prior to an annual meeting of the Board the Nominating Committee shall

notify the Trustees by mail of the vacancies to be filled in membership of the Board. Each Trustee may submit nominations for such vacancies. Nominations so submitted shall be considered by the Nominating Committee, and ten days prior to the annual meeting the Nominating Committee shall submit to members of the Board by mail a list of the persons so nominated, with its recommendations for filling existing vacancies on the Board and its Standing Committees. No other nominations shall be received by the Board at the annual meeting except with the unanimous consent of the Trustees present.

Retirement Committee

13. The Retirement Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election, and the Chairman of the Finance Committee *ex officio*. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term.

14. The Retirement Committee shall, subject to the directions of the Board of Trustees, be responsible for the maintenance of a retirement plan for staff members and employees of the Institution and act for the Institution in its capacity as trustee under any such plan, except that any matter relating to investments under any such plan shall be the responsibility of the Finance Committee subject to the directions of the Board of Trustees. The Committee shall submit a report to the Board at the annual meeting of the Board.

ARTICLE VI

Financial Administration

1. No expenditure shall be authorized or made except in pursuance of a previous appropriation by the Board of Trustees, or as provided in Article V, paragraph 8, hereof.

2. The fiscal year of the Institution shall commence on the first day of July in each year.

3. The Executive Committee shall submit to the annual meeting of the Board a full statement of the finances and work of the Institution for the preceding fiscal year and a detailed estimate of the expenditures of the succeeding fiscal year.

4. The Board of Trustees, at the annual meeting in each year, shall make general appropriations for the ensuing fiscal year; but nothing contained herein shall prevent the Board of Trustees from making special appropriations at any meeting.

5. The Executive Committee shall have general charge and control of all appropriations made by the Board. Following the annual meeting, the Executive Committee may allocate these appropriations for the succeeding fiscal year. The Committee shall have full authority to reallocate available funds, as needed, and to transfer balances.

6. The securities of the Institution and evidences of property, and funds invested and to be invested, shall be deposited in such safe depository or in the custody of such trust company and under such safeguards as the Finance Committee shall designate, subject to directions of the Board of Trustees. Income of the Institution available for expenditure shall be deposited in such banks or depositories as may from time to time be designated by the Executive Committee.

7. Any trust company entrusted with the custody of securities by the Finance Committee may, by resolution of the Board of Trustees, be made Fiscal Agent of the Institution, upon an agreed compensation, for the transaction of the business coming within the authority of the Finance Committee.

ARTICLE VII

Amendment of By-Laws

1. These by-laws may be amended at any annual or special meeting of the Board of Trustees by a two-thirds vote of the members present, provided written notice of the proposed amendment shall have been served personally upon, or mailed to the usual address of, each member of the Board twenty days prior to the meeting.

Index of Names

Numbers in italic type refer to pages in the Report of the President.