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CHEMISTRY JOINS FORCES WITH BIOLOGY

More than four centuries have passed since Paracelsus of Hohenheim gave scientific medicine its charter in his celebrated hypothesis:

> The human body is a conglomeration of chymical matters; when these are deranged illness results, and naught but chymical medicines may cure the same.

It has taken man a long time to learn even a small part of these "chymical matters," for as recently as 1849 the molecular weight of water was so uncertainly known that this principal ingredient of the body's conglomeration was still being written as HO by many chemists. Indeed, the idea that each atom has a definite combining power was yet to be accepted. Today, what a change! Biochemistry is now the principal battleground of science's attack on disease. The wealth of physiologically useful chemicals whose identification has come out of these studies - such compounds as the vitamins, the hormones, and the antibiotics, to name but three groups - provides powerful evidence in support of the Paracelsian doctrine and has spurred research in hundreds of universities, medical schools, and institutes.

Notable among the centers of biochemical interest is the California Institute of Technology at Pasadena. Here there is no conscious seeking for new vitamins, new hormones, new antibiotics, or any other specific nutritional or therapeutic agent. But there is being conducted a systematic search for the ways in which the body's molecules behave. And because the living process is always associated with huge molecules comprising hundreds, thousands, and even tens of thousands of atoms in a single structure, the program at the

California Institute is being focussed primarily on these giant molecules. Their attractions and repulsions, their combinations and modifications, their breakdown into smaller units and the joining of these into new patterns of molecular architecture - it is such goings-on that the Pasadena scientists are prying into with all the techniques that chemistry can bring to reinforce those of biology. Their inquiry is directed at the most fundamental of all biological processes: reproduction, nutrition, growth, and disease, each studied at the molecular level. If man can learn how these processes operate at the molecular level, perhaps he can better control or alleviate their disorders at the body level. Such, at least, is one visitor's understanding of the purpose of the program, after spending a week in the laboratories, talking with experimenters, watching the experiments, and breathing the atmosphere of California Institute research.

The Attack from Two Sides

Biochemistry has two avenues of approach. One may enter it from either the biological side or the chemical, and usually the main strength of a research program comes from one or the other of these two directions, seldom from both. A remarkable aspect of the dual project at Pasadena is its balance. This is not a case of a biological laboratory adding a chemical department to its facilities, nor yet that of a chemical laboratory taking an interest in biological problems. It is, rather, a joining of forces between two coordinate divisions, each of which is a leader in its field.

The Division of Chemistry at the California Institute was founded by Arthur A. Noyes. He had previously served as acting president of the Massachusetts Institute of Technology and was the director of its Research

Laboratory of Physical Chemistry when he came to Pasadena in 1913. At first Dr. Noyes served the California Institute on a half-time basis, devoting the other half of the year to the Massachusetts Institute. But in 1919 he decided to give himself wholly to the work in California and soon made it foremost in teaching and research. He was a physical chemist; his emphasis was on the inorganic aspects of the science, and aspiring chemists from all over America came to Pasadena to study the fundamentals under the master.

Among these students was Linus Pauling, a recent graduate of the Oregon State Agricultural College. Perhaps Noyes saw in him the man he wanted to train as his successor. At all events, the young Oregonian became a favorite pupil, spent three years of advanced study under Noyes, and was so imbued with the physical aspects of chemistry that he seriously considered specializing in atomic physics. A National Research Fellowship enabled Pauling to spend a year in Munich with one of the world's leading theoretical physicists, Arnold Sommerfeld, and these studies were continued the following year with Niels Bohr at Copenhagen and Erwin Schroedinger at Zurich. But the problems that made the strongest appeal to him were in chemistry; so Pauling remained a chemist, meanwhile continuing his investigation of the forces which operate between atoms and molecules, a study which resulted in his great book, "The Nature of the Chemical Bond." California Institute made him a full professor in 1931, when he was only 30 years of age, and following Dr. Noyes death in 1936 Pauling was appointed to succeed him as chairman of the division and director of the chemical laboratories.

"I was a physical chemist," explained Dr. Pauling, "with this dominating interest in the forces which cause atoms to join into molecules and molecules to react with one another. The forces are electrical, of course,

and depend on the number of protons and electrons present and the order of their arrangement in the structures. This is essentially a physical subject; or, rather, it belongs to that borderland where chemistry and physics merge. In these studies I naturally selected the simpler molecular structures to work with, such as the metals and inorganic compounds; but in the course of the investigation I also tested an organic substance whose molecule is large and complicated: the hemoglobin which gives the blood cells their red color. I found that in arterial blood the hemoglobin was repelled by a magnet, but in venous blood it was attracted. This led to a study of the chemical bond between the hemoglobin and the oxygen which it picks up in the lungs. I wanted to consult someone who had specialized on hemoglobin and found the authority in A. E. Mirsky of the Rockefeller Institute for Medical Research. Dr. Mirsky came to the California Institute for a year, and we collaborated on a study which resulted in a joint paper."

This paper attracted the attention of Karl Landsteiner, the discoverer of blood types, and Dr. Landsteiner asked Dr. Pauling if his theory of the chemical bond could throw light on a certain antibody reaction. Landsteiner's request introduced Pauling to the highly complicated specialty of immunology; the two men became close friends and frequent conferees on the subject, with Landsteiner supplying information and hypotheses from the biological angle and Pauling from the chemical. "From that time on I gave a great deal of thought to the chemical aspects of immunology," said Dr. Pauling, "trying to understand, in terms of the chemical bond, how an antibody neutralizes a virus or other antigen." By 1939 he had arrived at a chemical picture of the reaction and reported his results to the American Chemical Society as "A Theory of the Structure and Process of Formation of Antibodies."

Thus, under Dr. Pauling, there was a transition. In the course of a few years the division added to its program the investigation of hemoglobin, antibodies, and other molecular giants which originate only in living systems, while still continuing the basic work in the chemistry of inorganic and simpler organic substances. The Rockefeller Foundation had contributed to this development through financial grants, beginning in 1932. With additions in 1933 and 1934, it had made appropriations to the California Institute for "research in chemistry under Dr. Linus Pauling." The shift in program was reflected in 1938 when the first of five annual appropriations was voted "for the development of chemistry in relation to biological problems." Other grants were earmarked for "researches on the structure of antibodies and the nature of immunological action."

Meanwhile, a transition was also taking place in the Institute's Division of Biology. This division had been organized in 1928 by Thomas Hunt Morgan, who had left the chair of experimental zoology at Columbia University to pioneer this new planting in California. Like Dr. Noyes in physical chemistry, Dr. Morgan was already world-famous in genetics; and his coming to Pasadena brought several strong additions to the faculty, most of them geneticists, and attracted from all parts of the country students who wished to specialize in this science. The Foundation made two grants to support Dr. Morgan's researches.

Genetics lends itself to mathematization more easily than most biological sciences, and perhaps it is rightly called the most "physical" of the branches of biology. Certainly Morgan had a strong urge toward collaboration between biology, on the one hand, and chemistry, physics, and mathematics, on the other. In the first announcement of this division he said: "A closer

relation of these sciences with biology is imperative," and during his regime interrelations with the physical sciences were cultivated. But the main effort of his division was in genetics, and particularly in classical genetics, a field in which Morgan was the acknowledged master. Classical genetics has to do with the morphology of the mechanism of heredity, the position and order of the genes in the chromosomes, and their identification as dominant or recessive in the control of inherited characteristics.

After Dr. Morgan's retirement in 1941, the division was administered for several years by a staff committee headed by Alfred H. Sturtevant. The serological aspects of genetics interested Dr. Sturtevant, and during this period there was increasing collaboration between the two divisions. The Foundation made several grants for joint researches by Drs. Sturtevant and Pauling. But direction of the division by a committee was only a temporary expedient while the Institute was seeking a successor to Dr. Morgan, and toward the end of 1945 he was found in the person of Stanford University's professor of genetics, George W. Beadle.

Beadle's history closely parallels that of Pauling. Both men were National Research Fellows; and as Pauling came to the California Institute to study under Noyes, so Beadle came to study under Morgan. It is also significant that at the time when Pauling was turning his attention more and more to the biological molecules, Beadle was becoming enamoured of chemistry as the handmaiden of genetics. During his ten years at Stanford he devoted most of his research effort to experiments with the bread mold, Neurospora, and was able to demonstrate in this lowly fungus that the processes of nutrition (which are purely chemical) are directed by the genes. Perhaps it is not undue praise to say that Dr. Beadle's work with the mold did more

than any other research to establish the chemical nature of genic action. Classical genetics now has a lively daughter known as chemical genetics, a prolific contributor of new findings to science.

This, then, was the situation in 1946. With chemical research in charge of a biologically-minded chemist, and with this chemically-minded geneticist placed in charge of biological research, the California Institute offered an unusual opportunity. The Divisions of Biology and Chemistry immediately prepared a prospectus outlining "a joint program of research on the fundamental problems of biology and medicine." The program would occupy fifteen years and require a budget of $400,000 a year; and application was made to foundations for support. It was estimated that about five years would be required to bring the program to its full operating capacity, and as interim grants The Rockefeller Foundation appropriated $50,000 in 1946 and an equal amount in 1947, followed this year by a long-term appropriation of $700,000 to be paid in annual installments of $100,000. Thus, $800,000 has been committed within the last three years. The earlier contributions, beginning with the first grant on behalf of Dr. Pauling's work in 1932, have totaled $440,865, making a grand total of $1,240,865 given to California Institute for research in chemistry and biology.

In addition, the joint program of research on the fundamental problems of biology and medicine has attracted support from other funds: $60,000 a year from the National Foundation for Infantile Paralysis, and lesser grants from the Nutrition Foundation, the Hermann Frasch Foundation, and others. As rapidly as competent scientists can be found and trained for the new posts called for by the program, the work is being expanded to the full scope envisioned by the prospectus.

Research Manpower

Of the two essentials to successful research - manpower and equipment - the human element of course is the more important. Wickliffe Rose used to say that his main function as an officer of the Rockefeller Boards was to discover men of superior brains and then back them up through a period sufficient to demonstrate their abilities. What makes the situation at the California Institute so challenging is the presence there of the two staffs of scientists with their already integrated teamwork of biology and chemistry. In 1946, when the joint program was projected, the staff in biology, including all workers from professors to research fellows and assistants, comprised 32 persons; and the corresponding groups in chemistry totaled 86. At present biology is employing the services of 79, and chemistry 97, a grand total of 176 for the two divisions, or an increase of 49 per cent over the status of three years ago. Not all of these staff additions were financed from the funds earmarked for the joint program; some of them represent new fellowships that have become available in the last year or two or chairs recently established by the Institute. But all are engaged in research, and the joint program is benefiting enormously from this increase.

Among the recent staff additions are John G. Kirkwood in chemistry and Max Delbrück in biology. Dr. Kirkwood is in the distinguished line of physical chemists and had held research and teaching positions at Massachusetts Institute of Technology, University of Chicago, and Cornell. He was Todd professor of chemistry at Cornell in 1948 when called to the newly-established Arthur A. Noyes professorship of physical chemistry at the California Institute. Like Dr. Pauling, he has a predilection for the giant molecules, and recently developed a new type of electrophoresis apparatus

with which to study their properties. Tests made at Pasadena within the last few months show that the Kirkwood apparatus will separate the proteins of blood plasma to a finer degree than any other device heretofore used.

Dr. Delbrück is a physicist turned biologist. His primary training was in Germany in theoretical physics, but he became interested in bacteriology, and came to the United States as a Rockefeller Fellow in biology. He has made many contributions to our knowledge of bacteriophages, the invisible viruses which prey upon bacteria. Although these viruses multiply with great rapidity when they penetrate a bacterium, and in some of the experiments seem to do this by a kind of sexual reproduction, in other respects they appear as inert as any chemical. Indeed, each virus seems to be made up of but a few giant molecules. The viruses thus occupy a borderland between the living and the non-living, between biology and chemistry, and study of them constitutes an important part of the joint program. Dr. Delbrück joined the California Institute faculty in 1947, coming from Vanderbilt University.

Other members of the combined staffs could be cited with equally interesting detail. There is Laszlo Zechmeister, formerly of the University of Pecs, Hungary, who came to the California Institute as professor of organic chemistry in 1940. Dr. Zechmeister is an authority in chromatography, an amazing technique for separating carotenoids and other organic pigments out of mixtures - and his specialty is contributing directly to the joint research program. Another worker is Dan H. Campbell, an immunochemist, brought here in 1942 with the assistance of a grant from the Foundation. Dr. Campbell has been collaborating with Dr. Pauling in an effort to synthesize antibodies by direct chemical means - a daring project which if successful may revolutionize the control of infectious disease.

Research Equipment

The laboratories and other campus buildings of the California Institute are in the Mediterranean style of architecture, low structures arranged in pleasing quadrangles, with connecting colonnades. Chemistry is housed in the Gates and Crellin Laboratories, which in effect are one large building in the form of a U. Biology has its headquarters in an adjoining edifice known as the William G. Kerckhoff Laboratories. The juxtaposition of the two buildings has proved to be of great convenience to the growing collaboration between the chemists and biologists, and symbolizes the partnership in research.

In addition to its main facilities in Kerckhoff, biology has several smaller establishments off the campus. Across the street are three laboratories of plant physiology, one of them just being completed at a cost of $400,000, a gift from the Earhart Foundation of Detroit. Half a mile away is another center of botanical research, the Orlando Road Greenhouses; and five miles distant is Arcadia Farm, ten acres devoted to the study of genetics in corn. Fifty miles southeast, at Corona del Mar on the Pacific shore, is the marine laboratory. This is both a center for research and a means of supplying marine animals for experiments in biology and chemistry on the campus. Last summer still another unit was completed, a large animal house which adjoins a section of Kerckhoff on the north. This animal house was built entirely underground, cost $150,000, and embodies all current conveniences for breeding, housing, and caring for experimental animals. It is indispensable to the joint biology-chemistry program.

With this combination of laboratories and auxiliary facilities, the division of biology is really superbly equipped. There is plenty of working

space, but of course as the research program expands to its full scope this will become crowded and additional quarters will be needed. The laboratories of the division of chemistry, with its larger staff, are already seriously crowded; and plans have been drawn for a new building to be erected on the campus in the vacant area north of Kerckhoff and west of Crellin and Gates. This new laboratory, designed in the same architectural style as the older buildings, will be used for the joint chemistry-biology program. It is planned to connect with both Kerckhoff and Crellin and will increase the research quarters of the two divisions by 75 per cent. The estimated cost of the proposed building is $2,000,000, and there are good prospects that a sufficiently large fraction of the necessary funds will be obtained to permit construction to begin this coming year.

Research Objectives

"We are seeking to uncover basic phenomena and learn the principles which govern them, rather than to solve specific practical problems," explained Dr. Beadle. "The action of genes in controlling inheritance, the action of antibodies in neutralizing the destructive effects of bacteria, viruses, and other antigens, the action of hormones in promoting or retarding growth - these are examples of basic phenomena, processes that are fundamental to life. If we understood the basic processes, if we knew precisely what is happening in each in terms of molecular action, we believe the solution of the practical problems would follow almost inevitably."

And so the biology-chemistry team at the California Institute is interested in genes, antibodies, viruses, hormones, biological pigments, and related structures. How does each behave biologically, and how can this behavior be accounted for chemically?

Chemical behavior is related directly to the molecular structure of the reacting substances; therefore one of the principal objectives of the joint research program is chemical analysis. What are the building blocks that enter into the construction of genes and the other entities? How are these building blocks put together, in what order of arrangement, and what are the resulting size and shape of the structure?

"Science is still far from completely analyzing these biological agents," said Dr. Beadle, "but the investigations tend to show that the molecular form known as protein is the key structure. Apparently most of the bodies that we are studying in our program are either simple proteins or conjugated proteins."

Simple proteins are "simple" only by contrast with the vaster architecture of the conjugated molecules. Actually, a simple protein will consist of hundreds, sometimes thousands, of atoms. When placed beside familiar inorganic molecules, such as those of water, sulfuric acid, ammonia, and table salt, even the smallest protein molecule is like a whale among minnows. But a protein is simple in this respect: when it is broken down it does not separate into its hundreds or thousands of individual atoms, but divides into characteristic groups of atoms which the chemists know as amino acids. It is as though when a house was demolished, it broke up into basement, rooms, and attic, rather than into individual bricks and boards. Twenty-three different amino acids have been found in proteins, and the possible combinations that may be formed from these twenty-three building blocks run into countless millions. It is no wonder that proteins occur in the wide variety which makes one man's meat another man's poison. But a number of the most familiar and wholesome substances of the body's equipment

are simple proteins: pepsin and many of the other digestive enzymes, insulin and many of the hormones, albumin, fibrinogen, and many other components of the blood plasma.

The conjugated proteins represent a further step in structure. After a simple protein molecule has been built by the joining together of molecules of different amino acids, it may hook on to a pigment and form a conjugated protein such as the hemoglobin of the blood. Or, it may attach itself to a complicated chain of sugar molecules known as a polysaccharide and form a conjugated protein of another type, such as the mucin of saliva. Another possibility is the joining of a protein with a vitamin - the enzyme carboxylase is of this type. Finally, proteins may be linked with nucleic acids to form nucleoproteins - and here we reach the ultimate of giantism among molecules. For if a simple protein is pictured as a whale among the minnows, a nucleoprotein may be likened to a leviathan with form so tremendous that it might swallow the whale. Nucleic acid alone is a large structure - some of its molecules contain 160,000 atoms - and when units of this size combine with units the size of proteins, the combination is truly enormous. Some of the viruses which Wendell M. Stanley isolated in his studies at the Rockefeller Institute were identified as nucleoproteins and weighed up to 8,000,000 times the weight of hydrogen. Such structures comprise nearly a million atoms.

It is believed that both viruses and genes are nucleoproteins, while the antibodies are thought to be simple proteins consisting of chains of amino-acid residues folded together in a certain way. According to Dr. Pauling's theory, these folded chains of interlinked amino-acid residues (polypeptides, they are called) are afloat in the bloodstream; and whenever

they encounter the bacterium, virus, or other odd body against which they serve to protect the organism, the mutual attractions between the two cause the chain to approach and attach itself to the intruder. The action of the chemical bond thus brings the antibody to overlay an area of the surface of the foreign body with a shield or encrustation which blocks the latter's activity. This explanation is necessarily brief and oversimplified, but perhaps it suggests the action by which an antibody neutralizes a microbe.

"The genes, we believe, exercise an overruling control on all these activities," said Dr. Beadle. "They do this, we think, by serving as the master patterns for the many proteins which function in the processes of life. Thus, there is probably a gene which serves as the template for the body's manufacture of insulin, another which provides the mold for pepsin, and so for albumin, fibrinogen, the polypeptide chain that forms antibodies, and all the rest. There are several thousand genes distributed among the 48 chromosomes of the human body cell, a number sufficient to provide templates for the thousands of big molecules required for health. Diabetes, on this theory, is a consequence of a missing or defective gene, leaving its victim unable to manufacture insulin. Similarly, the bleeders or hemophiliacs lack the normal gene for manufacturing a gamma globulin which is an essential component of the blood-clotting equipment. Our experiments with the bread mold, Neurospora, have demonstrated this genic control of the biochemical processes in numerous instances. We found, for example, that after exposure to ultraviolet radiation, the Neurospora lost its ability to make certain vitamins. The genes which controlled this manufacture had been destroyed, and thereafter the Neurospora languished unless these vitamins were supplied in its food. Similarly, Sterling Emerson of our laboratory found that a minute

change in its genes caused the Neurospora to accept as food a compound that before the change had acted as a poison. Indeed, after mutation, the Neurospora would not grow unless fed a sulfonamide which previously had blocked growth and caused death.[8]

The strongest impression that one brings back from a visit to the Caltech team is the magnitude of the task of analyzing these invisible molecules. As a step toward understanding the proteins, the group is working first on the amino acids, trying to map precisely the structure of these protein building-blocks. Robert B. Corey of the chemistry staff spent a year and a half analyzing the configuration of glycine, the simplest of the amino acids. He bombarded it with x-rays, and measured the angles at which the rays bounded off the molecule. In this way he not only determined the position of each carbon atom, each oxygen, and each hydrogen in the glycine, but actually measured the distances between the atoms. After completing this job, Dr. Corey went on to alanine, which is larger and more complicated. The experience he had gained on glycine stood him in good stead, and he required only a year to work out the exact pattern of alanine. He has now taken up a still more complicated amino acid, threonine, and this winter is deep in a study of it. In this way, the group plans to move from the amino acids to more complicated structures, with the hope that eventually they may be able to dissect some of the proteins, perhaps even nucleoproteins, into their integral parts.

FOUR BOOKS IN THE NATURAL SCIENCES

Among the books in the natural sciences which were published during 1948, four may be listed as of special interest because of the relationship of the authors and their subjects to projects fostered by the Foundation.

Mathematical Biophysics, by Nicholas Rashevsky. The University of Chicago Press, Chicago, 1948, pp. 669, $6.

This is a revision and extension of a briefer book published in 1938 with the assistance of a grant from the Foundation. Dr. Rashevsky is professor of mathematical biophysics in the University of Chicago. His special field of research has been the attempt to interpret biological processes such as cell division, growth, nutrition, and communication in terms of physical phenomena. His treatment includes a physico-mathematical theory of the operation of the central nervous system.

Cybernetics, by Norbert Wiener. The Technology Press, John Wiley and Sons, New York, 1948, pp. 194, $3.

Another book which undertakes to interpret biological processes in terms of physical phenomena is this new work by Dr. Wiener, professor of mathematics in Massachusetts Institute of Technology. Professor Wiener dedicates the book to Arturo Rosenblueth, physiologist of the Mexican National Institute of Cardiology, and there is a strict appropriateness in this dedication, for Cybernetics treats of a problem on which the mathematician and the physiologist have been collaborating for a number of years, and since early 1946 with the help of a grant from the Foundation. The problem is that of nervous control, how the brain communicates with the muscles and directs their action. As the book discloses, the brain does this in ways quite parallel to those used by dial telephones, thermostat regulators, electronic calculators, and other automatic machines. "In their more elaborate forms," says Professor Wiener, "modern computing machines are capable of memory, association, choice, and many other brain functions. Indeed, the experts have gone so far in the elaboration of such machines that we can say the human brain behaves very much like the machines. The construction of more complex mechanisms actually is bringing us closer to an understanding of how the brain itself operates." The word

cybernetics was coined from the Greek "kybernetes," meaning steersman, and Wiener uses it as the name for the new science "communication and control in the animal and the machine." His book is heavily loaded with mathematical equations and other technicalities, and is hardly a manual for the layman, but a very readable interpretation was written by Wiener for the "Scientific American" and appeared in its November number.

Submicroscopic Morphology of Protoplasm and Its Derivatives, by Albert Frey-Wyssling. Elsevier Publishing Company, Amsterdam, 2nd edition, 1948, pp. 263, $6.

Professor Frey-Wyssling was a fellow of the International Education Board in 1925-26, and in recent years his work at the Eidgenössische Technische Hochschule in Zurich has received support from the Foundation. He and a small group of associates in Zurich are specialists in the study of structures beyond the reach of the optical microscope - such as the minute morphology of chromosomes, slime molds, cellulose, and silk. The present work is a new edition, in an English translation, of a work that has long been standard in this difficult field.

Radioactive Indicators, Their Applications in Biochemistry, Animal Physiology, and Pathology, by George Hevesy. Interscience Publishers, Inc., London, 1948, pp. 556, $10.

Professor Hevesy was among the first, perhaps the very first, to use radioactive elements as tracers in biochemical research. As early as 1923 he published an article under the title, "The Absorption and Translocation of Lead by Plants: A Contribution to the Application of the Method of Radioactive Indicators in the Investigation of the Change of Substance in Plants." The experiments which produced this paper were pioneer studies, made at a time when the only available radioactive elements were those of natural occurrence. Hevesy used thorium B, an isotope of lead with a radioactive half-life of 10.6 hours, to show that lead was heavily concentrated in the root tips of the bean plant and that it was eventually transported to other locations throughout the tissues of the plant. In the ensuing quarter century Hevesy's research has reached into many areas of the rapidly growing science of radiobiology, enabling him to write this authoritative text-book. With the discovery (by the Joliot-Curies in 1934) that ordinary elements like carbon, iodine, iron, and phosphorus can be made radioactive by bombardment, the usefulness of the tracer technique was greatly enhanced. The method is now in wide use in many fields of biology, including the investigation of fundamental life processes, such as photosynthesis in plants and nutrition in animals, as well as in the study of pathological conditions, drug action, and other medical problems. Someone has said that its

introduction constitutes as significant an advance in the development of biology as the introduction of the microscope more than two and a half centuries ago. Beginning in 1930 when he was at the University of Freiburg, continuing at Copenhagen where he was a refugee from Hitler in 1934, and later at Stockholm where he went when the Nazis overran Denmark, Professor Hevesy's studies have received the support of grants from the Foundation. In 1946 he spent six months in the United States as a visiting professor on a Foundation grant.

RADIOACTIVITY IN PUBLIC HEALTH RESEARCH

The tracer technique which Hevesy discusses in his book on Radioactive Indicators is being applied by the International Health Division of the Foundation in the study of three kinds of disease-transmitting agents: (1) viruses, (2) malaria parasites, and (3) mosquitoes.

In Virus Research

The incubating hen's egg with its growing embryo has become a universal medium for the cultivation of viruses. It takes about 21 days for an egg to hatch, and in the course of this process various systems develop to serve the growing embryo. The most conspicuous are the amniotic sac with its fluid in which the embryo lies, the larger allantoic sac which overhangs and partly envelopes the amniotic, the yolk sac with its rich store of nutrients, and the interconnecting system of blood vessels. Virus injected into any of these systems eventually reaches the embryo, but the selection of the route and technique of inoculation depends on the nature of the virus that is to be cultivated. Usually the virus is introduced into one of the extra-embryonic sacs by means of a needle inserted through a window cut in the shell of the egg. The window is then closed, the egg returned to incubation, and in two to three days the embryo is teaming with the virus.

Most but not all of the viruses which are known to infect animals have been cultivated in egg embryos. Their growth may be recognized in any one of several ways: by the death of the embryo; by the production of characteristic pathological changes in the embryo; by the agglutination of red blood cells, a phenomenon limited to influenza and a few other viruses;

and by the inoculation of material from the embryo into a susceptible animal. If, however, a virus does not kill or produce recognizable changes in the embryo, or fails to cause agglutination of red blood cells, or if no susceptible animal is readily available, the growth of the virus within the egg may escape notice. The problem is to find other means of identifying the presence of unknown viruses - and it was with this in mind that Richard M. Taylor and his associates of the International Health Division Laboratories decided to experiment with radioactive phosphorus.

Phosphates are among the mineral compounds necessary to metabolism, and therefore it was felt that the introduction of such substances into the developing egg would have no adverse effect on the embryo. By using a radioactive isotope of phosphorus, p32, which has a half-life of 14.2 days, the compound was provided with a tag which betrayed its presence. The idea was to inject the phosphate into various parts of the developing egg and see if its transport to other parts was affected by the presence of virus.

For the first series of experiments only healthy eggs were used, the object being to get the normal result unaffected by the virus. Dr. Taylor and his co-workers injected the radioactive phosphate into the allantoic sac, and then at intervals of two, three, and four days took sampling of the allantoic fluid, amniotic fluid, yolk-sac fluid, blood, and embryonic tissue, and tested each for radioactivity to see where and to what degree the phosphorus had dispersed. They found that most of it remained within the allantoic sac, though small quantities were detected in the blood stream and in the embryonic tissue. Taking other healthy eggs, they next thrust the needle into the amniotic sac of each and deposited the phosphate there. Tests for radioactivity showed that the phosphorus rapidly passed to the other systems,

and by the third day precious little remained in the amniotic fluid. Similar experiments tested the effect of introducing the phosphate into the yolk sac - it passed into the embryo, the blood, to some degree into the allantoic fluid, but none went into the amniotic sac. Similarly, when the compound was injected directly into a blood vessel, the larger amount remained in the blood, some entered the allantoic sac - but again, none passed into the amniotic. The experiments thus indicated that the allantoic sac tends to retain the phosphorus and will also receive it when introduced by other routes. Contrarily, the amniotic sac neither retains nor receives the element.

The question was whether or not the presence of virus would make any difference in this pattern of phosphorus transport. To test it, the experiments which had been made with healthy eggs were repeated with virus-infected eggs. It was found that if the egg was infected with influenza virus, and the phosphate was introduced into the allantoic sac, the radio-activity no longer remained concentrated there, but rapidly diffused to other parts, particularly into the blood stream.

The testing technique is as follows. Samples are taken of the various fluids, a standard quantity of each is placed in proximity to the tube of a Geiger-Müller counter, and the number of radiations given off is counted. Since the radioactive phosphorus atoms discharge beta particles at a rate which is statistically constant, the number of radiations per minute is a direct index to the number of phosphorus atoms present. In one series of experiments, in which the phosphate was injected into the allantoic sac of a healthy egg, specimens were taken of the allantoic fluid and of the blood at intervals of 48, 72, and 96 hours, and equal quantities were tested for radioactivity, with the following results:

	Allantoic fluid	Blood
At the end of 48 hours	1483	
At the end of 72 hours	1332	99
At the end of 96 hours	1308	134

The concentration of the radioactive phosphorus did not change much, and the amount that had passed into the blood stream at the end of the fourth day was about a tenth of that retained in the allantoic sac. But when this experiment was repeated with the virus of influenza injected into the allantoic sac along with the radioactive phosphorus, the results as picked up by the Geiger-Müller counter were strikingly different.

	Allantoic fluid	Blood
At the end of 48 hours	2039	
At the end of 72 hours	831	307
At the end of 96 hours	300	460

Obviously the presence of the virus had a profound effect on the permeability of the membrane enclosing the sac; for four days after its receipt of the injections the allantoic sac has lost more phosphorus to the blood stream than it had been able to retain, and corresponding or even larger amounts had passed into other fluids and into the embryo itself.

As far as the experiments have gone, influenza has a more potent effect on the dispersion of the phosphorus than any other virus. Mumps virus shows an influence, but to a lesser degree, whereas yellow-fever virus has no apparent effect at all. Dr. Taylor and his associates are working at present with throat washings from various undetermined respiratory infections. It would be a great boon if this tracer technique should point the way to a simple test for the identification of specific viruses, or to a sure means of separating viruses out of a mixture.

In Malaria Research

The plasmodium of malaria is a parasite of the red blood cell. It enters a red cell and, after a period of time which varies with the parasite type, the cell bursts and out come a number of new plasmodia. Immediately each of the new brood attacks a fresh red cell, bores into it, and begins a new cycle of growth and multiplication. Without red cells the plasmodium dies, and therefore it is dependent on the cell for certain metabolic functions. But presumably there are other functions which it performs itself, and one of the key problems is to identify the two sets. If we knew what the malaria parasite does for itself and what it must depend on the red cell to do for it, the biochemists and pharmacologists would be better prepared for their effort to develop new antimalarial drugs. To be effective, the drug must block some essential step in the parasite's metabolism - and a preliminary task is to identify that vulnerable function.

Several approaches to the enigma of the plasmodium are being explored in the program of malaria research conducted by Max Theiler in the International Health Division Laboratories. One is of special interest in this connection because it is using radioactive phosphorus. Delphine H. Clarke was intrusted with this research, and before beginning it she spent several weeks at the Radiation Laboratory of the University of California studying tracer technique.

Most investigations of plasmodia have been in animals that were ill of malaria. But for study of its function Dr. Clarke wanted to isolate the parasite as much as possible. She made a rich broth of nutrients, including an extract of ruptured red cells and a phosphate containing radioactive phosphorus. When this nutrient solution was blended in a test tube, she placed

in it a number of living red cells which were infested with the parasites. And then for 24 hours she watched what happened. At the end of that time, the red cells were removed, and the plasmodia measured under the microscope. They were larger: there was no doubt they had grown. Then Dr. Clarke fractionated the plasmodia, grinding them up, and by means of chemical agents separated out the fatty compounds, the nucleo-proteins, and other components. Each of these fractions in turn was tested under the Geiger-Müller counter, and the lively responses of the indicator needle showed radioactivity from each. It was clear that the tagged phosphorus had passed from the solution into the parasite and had entered into the construction of nucleoprotein, fat, and other compounds. But which was responsible for the synthesis - the plasmodium or the cell in which it lived?

Nucleoproteins, those supergigantic molecules which Drs. Beadle and Pauling are studying in the joint biology-chemistry program at Caltech, are believed to be the fraction which indicates growth. So Dr. Clarke directed a series of experiments at the investigation of nucleoprotein synthesis. She worked with animals first, selecting chickens because they are susceptible to malaria. She injected phosphates containing p32 into the blood of healthy chickens, and after 24 hours took blood specimens, fractionated the red cells, and by counts of the radioactivity determined the amount of nucleoprotein that had been synthesized. Then she took malarious chickens, injected them with the phosphate, and after the usual period of time fractionated red cells taken from the infected birds. These cells contained ten times as much nucleoprotein as the healthy cells. The studies were repeated in the test tube, again with healthy red cells and then with malaria-infected cells, and the result was the same: the cells containing the parasites synthesized ten

times as much new nucleoprotein as the healthy cells produced. From these results Dr. Clarke concluded that the synthesis of nucleoprotein can assuredly be used as a measure of the rate of parasite growth.

The experiments indicate that the synthesis of new nucleoproteins (and thus the growth of the parasite) is dependent upon the presence of an abundance of normal red cells or of an extract of such cells. Therefore, although it seems that the formation of nucleoproteins is a function of the parasite itself, nevertheless the normal cells must supply certain materials which enable this synthesis to take place. It is these as yet unknown materials which are now being studied and whose identification would seem to answer the question why the parasite must live within a cell. This in turn may show what kind of drugs we must devise to stop the growth of the parasite and lead to its complete destruction.

In Mosquito Research

Because they serve as vectors of malaria and yellow fever, the study of mosquitoes is as pertinent to the control of these diseases as the study of the plasmodium and virus. For many years the International Health Division has been interested in the flying abilities of the insects. It was important in the establishment of control measures to know how far from an infected village or plantation they could travel. To determine this, mosquitoes were marked by dusting with bronze powder, fluorescent material, or spraying with dyes, and the marked mosquitoes were then released from a central station. When recaptured, it was possible to identify them and thus determine their flight range and how long they had endured the buffeting of inclement weather.

But each of these methods of marking subjected the mosquitoes to handling. Many were injured in the process, and one could not be sure how the survivors were affected physiologically, perhaps weakened in endurance. There was need of a less-violent marking method, and it was found and effectively demonstrated last year in the use of the radioactive tracer technique.

The demonstration was made at the West African Yellow Fever Laboratory near Lagos, Nigeria, by John C. Bugher and associates of the International Health Division. Dr. Bugher explored the possibilities of the method first in 1946, when he was in the United States, working with Dr. Whitman. They showed at that time that if a phosphate containing radioactive phosphorus were dissolved in the water in which larvae were hatching, the phosphorus would be assimilated by the insect and could be identified in the mature mosquito. When Dr. Bugher returned to Africa he took with him a Geiger-Müller counter and other needed apparatus, and soon had an experiment going with radioactive marking. In addition to the phosphorus, he used a radioactive isotope of strontium, Sr^{89}. The method is essentially the same with both, but the strontium has a half-life of 55 days, therefore lasting longer as a marker.

"What we did in Lagos," said Dr. Bugher, "was to put mosquito eggs in a tray of water, and after the eggs had hatched we added the radioactive material - either strontium chloride or disodium phosphate. We did not handle the adults; simply let nature take its course. In one experiment we marked 5,000 mosquitoes in this way, in two others 10,000 each, and in the final test 250,000. Our release point was in the compound of the Yellow Fever Laboratory, and catching stations were set up at intervals of a few hundred feet in all directions. Each catching station consisted of a man,

hired for the purpose, who took off his shirt and sat or stood in the appointed place. Whenever a mosquito landed on him, he captured it in a tube and waited for the next arrival. At the end of the night's vigil each man brought his catch to the laboratory, where the mosquitoes were identified and subjected to the counter for recognition of the radioactive ones. We found that some, flying in the direction of the prevailing wind, traveled as far as 4,000 feet. Against the wind few made any great headway. Some of the mosquitoes were picked up 30 days after their release, but these were rare; most of those recovered were found within the first fifteen days."

The familiar yellow-fever vector Aedes aegypti, which is easy to colonize in the laboratory, was used in the flight-range studies. Dr. Bugher thought it would be helpful to see if the tracer technique could be applied to the forest mosquitoes which breed in the tree holes, such as Aedes africanus, the vector of jungle yellow fever in Central Africa. Accordingly, the strontium compound was added to the water of the tree hole, and later the adult mosquitoes resulting from larvae in this water were easily recognized by the Geiger-Müller counter. By treating a considerable number of tree holes in a restricted area, flight-range and longevity studies may be made of all the tree-hole breeders, with the added advantage that the observations are made under natural conditions.

In the course of identifying the mosquitoes, it was noticed that the females were twice as radioactive as the males. This led Dr. Bugher to test various parts of the insect's body, to see if the radioactive elements were more concentrated in some organs than in others. He found that the larger size of the females accounted in part for their greater absorption of phosphorus, but even more important was their larger mass of malpighian tubes.

This is an abdominal organ which functions somewhat as kidney, and it has a high capacity for absorbing the radioactive material.

When the dissections of different species were compared, some striking contrasts showed up. "In Aedes aegypti we found that about 25 per cent of the radioactive strontium went into the legs and about 30 per cent into the malpighian tubes, whereas in the malaria mosquito Anopheles gambia only 4 per cent was found in the legs and 70 per cent in the malpighian tubes. These contrasts can only mean that there are marked physiological differences between the two species," said Dr. Bugher.

"It is also significant," he continued, "that the newly-emerged mosquito, just changed from pupa to winged insect, has very little of the strontium in the legs, but 24 hours later the legs have acquired their full quota. This points to the fact that the series of profound changes which go on in the pupa do not produce a complete adult. Internal changes are still happening during the 'drying out' process."

Thus, the application of the radioactive tracer technique to mosquito marking, for the purpose of determining flight range, has opened the door to a new way of investigating mosquito physiology - a subject that Dr. Bugher hopes to pursue more fully.