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THE CONTRIBUTIONS OF THE WOODS HOLE OCEANOGRAPHIC INSTITUTION

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Director, Woods Hole Oceanographic Institution

The dislocations of the war years have finally subsided at this Laboratory and it is hoped that the research program has become stabilized for a few years at least. The great majority of investigations having direct practical applications have been terminated so that, of a total budget of about $750,000, which is now virtually assured through 1950, only about one-fifth comes under the heading of applied research or development. Thus, what we consider basic oceanography has grown at Woods Hole from a pre-war budget of about $110,000 to a post-war budget of about $600,000.

This is not an exceptional increase. For the country as a whole, it has been estimated that the oceanographic budget has increased more nearly by a factor of ten and that it will double again during the next ten years. This is only to say that interest in the physics, chemistry, geology and biology of the oceans is growing rapidly. The Federal Government is especially aware of the need for increased knowledge of

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THE MARINE BIOLOGICAL LABORATORY IN 1949

DR. CHARLES PACKARD
Director, Marine Biological Laboratory

The 62nd session of the Marine Biological Laboratory formally opened with the first Friday evening lecture by Dr. Gilbert Tyler. But long before this time investigators had been arriving, some in May and many more before the middle of June. Others will continue to come until late August. By the middle of September most of them will have left, but the workers in the Institute of Muscle Research, under the direction of Dr. Szent-Gyorgyi will remain throughout the year. The laboratory welcomes these newcomers, and wishes that more investigators would find it possible to carry on their research here after the summer season.

As usual, more people applied for places in the laboratories and in the courses than we could accommodate. A few more investigators could have been accepted if there were more rooms available in our residences and in the village houses. The housing situation will not materially improve until a number of investigators build their own homes in the Devil's Lane Tract or elsewhere. There is no need to call attention to

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MEMBERS AND GUESTS OF THE SOCIETY OF GENERAL PHYSIOLOGISTS AT WOODS HOLE

Back row—D. Nachmanson, John Buck, M. M. Brooks, Frank A. Brown, A. M. Chase, Dean Burk, Robert Emerson, C. L. Prosser, Charlotte Haywood, L. J. Milne, Witherow, Frank Skogg, K. V. Thimann, H. Gafron. Middle row—Karl Wilbur, S. V. Perry, L. V. Heilbrunn, W. J. V. Osterhaut, D. R. Goddard, H. F. Blum, M. H. Jacobs, Ethel B. Harvey, Unidentified, Bateman, Kenneth Fisher, Hudson Hoagland. Front row—George Scott, Albert Tyler, Leonard Clark, A. K. Parpart, A. C. Redfield, H. K. Hartline, F. J. Sichel, C. Williams, L. F. Nims, Douglas Marsland, Sterling Hendricks.

the crowded condition of the Mess.

Sixty years ago the laboratory acquired a small steam launch at a cost of $1,350. Later the classes were carried to the collecting areas in the *Vigilant*, a sailing vessel which was towed by the launch. Now we have a fleet of five power boats —*Dolphin, Limulus, Nereis, Sagitta* and *Tern*. The first two are new; the others have been in our service for many years. The *Dolphin*, purchased last year, and used by the classes and the collecting crew is now provided with a Diesel motor. Thus the danger of explosion or fire, has been reduced to a minimum.

This summer there are five labor fellows and five Atomic Energy Commission fellows in residence. The former are investigators who already have their doctorate and are competent to carry on independent research in the fields of biophysics, biochemistry or biological chemistry. The latter are in general a younger group. Each has a definite problem involving the use of radioactive isotopes. One purpose of these fellowships is to train investigators in the new and exacting techniques required in this type of research.

On the third floor of the Brick Building is a "hot laboratory" where transfers of highly active material can be made. Under Dr. Failla's direction it has been equipped with the safeguards necessary to protect the workers from radiations. Funds for the equipment were furnished by the American Cancer Society.

The renovation of the Old Main Building, begun this Spring, is the first major change to be made in the oldest building of the Marine Biological Laboratory. It was in 1888 that the south wing, now used by the Embryology class and some investigators, was erected. The next year the middle portion was added to serve as a lecture room and a library. Then in 1892 the north wing was built. Many notable biologists have occupied the little rooms on the first and second floors. Among these were Whitman, the first director; Loeb, physiologist; F. R. Lillie, embryologist; T. H. Morgan, geneticist; E. B. Wilson, cytologist; and G. N. Calkins, protozoologist. Some of the early pioneers—Conklin, R. S. Lillie, Mathews and Osterhout, have continued to return to the M.B.L., their places of summer work for more than fifty years.

The renovation of the building was made possible through the generosity of the Rockefeller Foundation, which has on previous occasions contributed to this laboratory. The basement, originally almost unexcavated, is now provided with well equipped laboratories used by the Physiology Class and by instructors in other courses. The upper floors will be remodeled next Fall. In the Embryology Laboratory the water table is to be rebuilt and moved toward the back of the room. On the Physiology side, the present stairway will be removed, and a new one will open from the street with stairs both to the basement and to the second floor. The present arrangement of rooms will be much altered. Upstairs the rooms will be enlarged, and some will be provided with salt water tables. It is hoped that insulation of the roof will keep room temperatures within reasonable limits.

With all these changes, the building still retains much of its original character. It will always be called "Old Main" to remind us of our debt to those who first worked in it and laid the foundations of American Biology.

The Contributions of the Woods Hole Oceanographic Institution

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the oceans and it is from this source that most of the money is coming to oceanography. However, it may be significant to point out that our sister laboratory, the Scripps Institution of Oceanography, at present is receiving roughly $400,000 per year from the State of California. Many other coastal states also are making substantial contributions for studies of the ecology of inshore forms, including the State of Massachusetts which has given a contract to this Institution for the study of shellfish.

There are, at present, 264 persons actively engaged in work at the Woods Hole Oceanographic Institution. These can be classified as follows:

Full-time scientific and technical staff	89
Part-time scientific; i.e., summers only	44
Fellowship holders	12
Visiting investigators	13
Secretaries and clerks	19
General maintenance and services	40
Crews of vessels	37
Administration	10

The total summer increase, including fellowship holders and visiting investigators, is 68 which is roughly the pre-war figure.

Of what does this boom in oceanography consist? It will only be possible here to describe very briefly some of the main lines of investigation in which especially rapid progress is being made.

The most active group in oceanography today are those interested in the geology and geophysics of the ocean basins. The recording echo sounder when combined with new, radio-navigational techniques makes it practical to examine in detail the topography of the ocean bottom. During the last few years, the *Atlantis* has accumulated about 70,000 miles of bottom records

and gradually it is being revealed that the bottom of the ocean is as varied and complex as the surface of the land. Recent soundings in the neighborhood of Bermuda show that the island is situated on a ridge of low hills, trending northeast-southwest. Several submerged sea mounts, nearly as high, have been located in the general area. The Hudson River Canyon has recently been traced nearly halfway to Bermuda and at present our newest research vessel *Caryn* is tracing the continuation of this remarkable feature towards Bermuda.

It is evident that the course and character of the ocean currents may be very much influenced in passing through regions of pronounced bottom topography. The *Atlantis* left two weeks ago to study these relationships in the area east of the Grand Banks.

Another development in submarine geology has come about as the result of great improvements in coring tubes. The piston-type coring tube, first used by Swedish oceanographers, now permits cores thirty to fifty feet in length to be obtained from the deepest waters. Since the rate of sedimentation in deep water is relatively slow, there is great hope that much of the recent history of the earth can be worked out rather quickly through studies of such cores. They form an undisturbed record of the changes in depth and climate extending over a period of several million years.

The seismic techniques developed in oil geology have been adapted for use at sea and are allowing the examination of the rock structures underlying the ocean down to ten or fifteen miles below the bottom of the sea. Of special interest is the location of the edge of the granite on which the continents are built and to learn about the character of this edge.

Turning to subjects more usually identified with oceanography, the study of the heat and water vapor exchange between the sea surface and the atmosphere is receiving special emphasis. During the past twenty years it has been the fashion more or less to neglect heating and cooling as a cause of the general circulation, both in the atmosphere and in the hydrosphere. Recent studies of the Director of the Royal Netherlands Meteorological Institute are indicating that even on a day to day basis sea surface temperatures can exert a major effect on the development of the weather. The role of salt nuclei picked up by the air passing over the ocean also is proving to be a fascinating and important study connecting meteorology and oceanography.

It will, of course, be a long time before the distribution of temperature, salinity, oxygen and nutrient chemicals can be described in satisfactory detail. The broad seasonal and geographical aspects of physical oceanography is a study requiring patience and a certain amount of sustained organization. If the collection of data was left entirely to the interests of individual investigators, it would proceed much more slowly than is desirable. In this sense, an oceanographic laboratory has somewhat the role of an astronomical observatory. Vast quantities of routine data must be collected and digested before even the basic problems can be clearly defined. Improved instrumentation, both at sea and in the laboratory, is greatly accelerating the descriptive aspects of physical oceanography. It is not enough that the ships take in large quantities of, for example, routine temperature data. The process of correcting, sorting, averaging and digesting must, if possible, also be facilitated through machines of one kind or another. Although the instrumentation of oceanography has developed rapidly, it is clear that there is still much more to be done. The market for oceanographic instruments will probably always remain small. It is for these reasons that instrument design and construction remains an important activity at our laboratory.

It would be nice to be able to report that biological oceanography is going ahead with as much vigor as the physics and geology of the sea, but unfortunately in this case money is a serious limiting factor. Although it is clear that man will soon have to turn more and more to the sea as a source of protein, and this is already the case in several countries bordering rather barren seas, there is little financial support in this country for basic studies of the productivity of the oceans. We know about how many haddock are to be found on George's Bank and about how much sustained yield can be expected from this area, but when it comes to the productivity of the oceans as a whole we know very little. Quantitative studies of the smaller forms have been made, for they cannot easily escape a net, but as we advance up the food chain in the sea, the quantitative and geographical aspects become very vague indeed.

Here again considerable instrumental development will be required. Two possible quantitative tools for marine biology are suggested by recent refinements in underwater acoustics and underwater photography. However, it also seems likely that marked improvements in the effectiveness of nets of various kinds can be made. Once really good sampling techniques have been devised the marine biologist will be face to face with the same problem that the physical oceanographer already has had to deal with. That is, he will soon be swamped with data, unless means are devised in advance to facilitate the analysis phase of a given investigation.

THE WORK OF THE UNITED STATES FISHERIES LABORATORY AT WOODS HOLE

Dr. Paul S. Galtsoff

Director, United States Fisheries Laboratory

During the years following the end of the World War II, the United States Fisheries Laboratory at Woods Hole was gradually rehabilitated and adapted for year-round operation.

Investigators coming regularly to Woods Hole for the last ten years may recall that the buildings and grounds of the Laboratory were seriously damaged by the hurricanes of 1938 and 1942. Although the most serious defects have been repaired, the signs of the ravages caused by the wind and sea and are still noticeable; the sea wall along the southeast side of the small boat basin is still in ruins, and the pool in which the sharks and seals were formerly displayed has not been restored. The Laboratory was able, however, to rebuild the sea wall around the grounds and to rehabilitate the laboratory building and the residence which have been made suitable for all-year occupancy. The sea water tables, chemical benches and other laboratory equipment which were removed when the station was occupied by the U. S. Navy during the war have been completely restored. The water pipes were repaired and the buildings rewired and reconditioned.

The hatching of marine fish (cod, flounder and mackerel), which for many years had been carried out by the old Bureau of Fisheries, has been discontinued and the hatchery equipment adapted for biological research. In 1947, arrangements were made to transfer the headquarters of the section of the North Atlantic Fisheries Investigations from Cambridge, Mass., to the Woods Hole Laboratory. The necessary rearrangements to provide additional office and laboratory space for investigators and docking facilities for the research vessel *Albatross III* were completed that year. A comprehensive program of fishery research in the North Atlantic and its progress will be discussed in a separate article by Dr. William F. Royce, in charge of the project.

Besides the studies carried on by the North Atlantic Section, the Woods Hole fisheries laboratory is engaged in shellfishery research conducted by Dr. Paul S. Galtsoff and serves as a temporary headquarters for the clam investigations carried on by John B. Glud.

Following a well-established old tradition of working together with other scientific institutions at Woods Hole, the Service made a cooperative agreement in 1947 with the Marine Biological Laboratory for an exchange of services and facilities. The plan has proved mutually pleasant and profitable.

Full cooperation with the Woods Hole Oceanographic Institute is likewise a very important factor in carrying on the research program of the Laboratory. Close association with the officers and personnel of both institutions, mutual assistance in case of emergencies and free exchange of ideas, creates a favorable environment which stimulates research work.

The Aquarium of the Laboratory was reopened to the public in 1947. Thanks to the cooperation of the Supply Department of the Marine Biological Laboratory, it was possible to assemble and display to the public from 55 to 65 different species of fish and invertebrates which occur in local waters. As an innovation, part of the former hatchery room on the first floor was set aside for special exhibits showing the various techniques used in marine biology. Of special interest are the exhibits arranged by the Woods Hole Oceanographic Institute, which show oceanographic instruments, automatic recording devices, underwater photography, sounds recorded in the depths of the sea, and various methods employed by modern science in the study of the ocean.

The Aquarium is open to the public every day, including holidays, from 8:00 A.M. to 8:00 P.M. The number of visitors, particularly on Sundays and holidays is surprisingly large, frequently exceeding 1,000 persons a day.

Thus far, the Service has not been able to obtain sufficient appropriation for the complete rehabilitation of buildings and grounds and for the modernizing of scientific equipment and the Aquarium. Every year, however, the work of reconstruction and rehabilitation continues with the limited funds available for this purpose. The investigators of the Laboratory are confident that with this increased scope of scientific activities the Laboratory will become an important center of research and training in fishery biology.

SEROLOGICAL ASPECTS OF FERTILIZATION

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It seems quite appropriate that a talk on fertilizin and related substances should be presented here at Woods Hole since it was here that the subject was first developed by the late Professor Frank R. Lillie, who was director of this laboratory for many years. It was here, too, that most of the early work in this field was done by Jacques Loeb, Otto Glaser, Alvalyn Woodward, C. R. Moore, E. E. Just, Myra Sampson and G. H. A. Clowes. After the highly interesting early work investigations along this line practically ceased from about 1922 until 1939 when Max Hartmann and his colleagues, working at Naples, and we, in Pasadena, undertook a series of investigations which have continued, with some interruption, during the war years. More recently John Runnström and his co-workers in Stockholm have entered this field. In general the results of the latter investigators agree very well with our own while those of Hartmann and his co-workers differ in several points. Recent detailed reviews of the subject have been written by Bielig and Medem (1949) and by Tyler (1948, 1949).

The present summary is based primarily on the work of the author's laboratory. This work has been concerned with four kinds of substances that have been isolated from eggs and sperm of marine animals; namely, fertilizins from eggs, antifertilizins from sperm, antifertilizins from eggs and egg-membranes lysins from sperm. Early in this work it was shown that the fertilizin of eggs of the sea-urchin and other animals is the macromolecular material of the gelatinous coat, and this has been confirmed by Hartmann, Runnström and others. The gelatinous coat slowly dissolves as the eggs stand in sea-water, yielding the so-called egg-water that has the property of agglutinating homologous sperm. The gelatinous coat can be rapidly dissolved in slightly acidified sea-water, without injury to the rest of the egg, and concentrated solutions of fertilizin thereby obtained. Various tests, including the action of purified proteinases, showed the fertilizins of the sea-urchin and the keyhole limpet (as defined by their agglutinating action) to be of protein nature. By relatively simple extractions and precipitation procedures we have been able to prepare sea-urchin fertilizin in electrophoretically and ultracentrifugally homogeneous form. The purified material contains both amino-acids and sugars and may, therefore, be termed a glycoprotein or mucopolysaccharide (depending upon the terminology adopted). It is of highly acidic character, showing little change in electrophoretic mobility between pH 8.6 and 2.0 This is evidently due to the fact, discovered by Vasseur (1947) and confirmed by us, that it contains over 25 per cent sulphate, most probably linked in the manner of a sulphuric ester. Some of the analytical data obtained on purified fertilizin is given in Table I. The values for the amino-acid and reducing sugar content are minimum due to the fact that a fair amount of not readily analyzable humin residue forms upon acid-hydrolysis. Galactose has been identified as the osazone. Paper chromatography has shown the presence of at least seven different amino-acids which are, most probably, aspartic acid, glutamic acid, threonine, lysine, arginine, phenylalanine and isoleucine in addition to tryptophane in the humin residue.

TABLE I

Analysis of electrophoretically homogeneous preparations of fertilizin of *Strongylocentrotus purpuratus*

Nitrogen	5.6-5.8%
Carbon	33.3%
Hydrogen	5.5%
Sulphate	23%
Phosphate	0.06%
Reducing sugar	>25%
Amino acids	>20%
Glucosamine (?)	1.6%
Galactose	pos.
Glucuronic acid	neg.
Pentoses	neg.
Molecular weight (calc. as sphere)	82,000

On the basis of the results of attempts to separate protein and polysaccharide fractions of fertilizin it is concluded that these do not exist as loosely bound distinct entities in the molecule but rather that amino-acid and sugar residues are firmly inter-linked. In this connection it may be noted that other glycoproteins of somewhat similar composition, in particular those exhibiting the human ABO blood-group activity, have likewise proven refractory to attempts to dissociate protein and polysaccharide constituents (see Morgan, 1947).

The antifertilizin from sea-urchin sperm has also been prepared in electrophoretically homogeneous form. It is an acidic protein, isoelectric at pH 3. Investigations of its amino-acid composition are in progress. One of the workers in

Runnström's laboratory (Hultin, 1947) has suggested that it may be a basic protein, but this is refuted by results published recently by Metz (1949) as well as by Runnström's (1942) and our own data concerning its electrophoretic mobility. Evidence from electron microscopy of extracted sperm shows the antifertilizin to be located on the surface of the nuclear region of the head.

The antifertilizin from eggs and the egg-membrane lysin have likewise been shown to be of protein nature. Dr. Max Krauss, of our laboratory, has obtained good evidence showing that the action of the lysin of keyhole limpet sperm is dependent upon the presence of sulfhydryl groups. Electron microscopy of extracted sperm indicates that the acrosome may be the source of the lysin.

Lillie considered the interaction of fertilizin and sperm to be analogous to that of serological agglutination and evidence has since accumulated that the kind of interaction exhibited by the various substances extracted from the eggs and sperm is essentially that of antigen and antibody. The finding of an antifertilizin within the egg along with fertilizin in the coat, means, then, that in one and the same cell there are a pair of substances that are capable of interacting in antigen-antibody manner. This along with consideration of certain information from the literature of immunology has led to the development of a so-called auto-antibody concept of cell structure, growth and differentiation that has been presented recently in some detail (Tyler, 1947). Briefly this view states that the macromolecular substances of which cells are constructed bear the same relationship to one another as do antigen and antibody and that they are formed in essentially the same manner as are antibodies. In addition to various experiments of others that can be interpreted on the basis of the occurrence of such natural auto-antibodies the author has been able to demonstrate the presence of an auto-antivenin in a venomous reptile, the Gila monster. The view also offers interpretation for certain serological anomalies, such as the Wassermann reaction, auto-agglutination phenomena, specific interaction of virus with cell surface, etc., and it offers the possibility of obtaining protective agents against pathogenic organisms by extraction of the organisms themselves.

Experiments relating to the spontaneous reversal of sperm agglutination by fertilizin in the sea-urchin (a phenomenon now known to occur also in the so-called Hirst reaction of hemagglutination by viruses) led to the finding that fertilizin could be converted, by simple treatments, into a non-agglutinating, "univalent" form. Evidence was also accumulated that fertilizin occurs normally in such "univalent" form in many species of animals and C. B. Metz (1945) discovered that specific agglutination of sperm could be obtained with such non-agglutinating fertilizins by the addition of a non-specific adjuvant obtained from hen's egg-white, serum-albumin or other sources. This latter result is paralleled by recent experiments of Wiener (1948) on "univalent" Rh antibodies which likewise can cause specific agglutination in the presence of certain non-specific proteins. Metz's results provide strong support for Lillie's view that the fertilizins are of general distribution throughout the animal kingdom.

As a side-line of some practical, as well as theoretical, interest a series of experiments were undertaken in which immune antibodies against mammalian blood cells, pathogenic bacteria and toxins were converted into the "univalent" form by photo-oxidation and various properties of such antisera examined. The anaphylactic properties of such treated sera were found to be greatly reduced. The evidence also showed that the "univalent" antidiptherial antibodies were capable of neutralizing the toxin but that "univalent" antipneumococcal antibodies were incapable of acting as protective antibodies. With respect to serum-sickness factors there is, then, considerable improvement in the antisera in the former case but not in the latter. It was found, too, that the "univalent" anti-blood cell antibodies were incapable of acting as hemolytic sensitizer, or of fixing complement, and this offers some support of Heidelberger's views concerning the mechanism of complement fixation.

Investigations of the role of fertilizin and antifertilizin in fertilization have shown that, when present on the surface of the respective gametes, they facilitate the process. When present in solution, however, they block fertilization, presumably because the interaction of the sperm with fertilizin, or of the eggs with antifertilizin, is completed before contact is made between the effective surfaces of the gametes. It has not, as yet, been possible to determine with any certainty whether or not fertilizin-antifertilizin interaction is also essential for fertilization since, in the experiments on removal of fertilizin by methods that do not injure the rest of the egg, a minute layer of this substance evidently remains firmly bound to the surface. However, results of experiments employing immune antibodies against antifertilizin favor the view that the interaction is essential for fertilization. The role of the egg-membrane lysin, in species in which this agent has been demonstrated is manifestly to enable the sperm to penetrate the membrane barriers that surround the unfertilized

egg. For the antifertilizin within the egg, Lillie had proposed a role in activation and establishment of the block to polyspermy but evidence concerning this is still lacking. Lillie's demonstration that fertilizin is obtainable from no other tissue than the gametes has been amply confirmed and this serves as a basis for understanding the tissue-specificity of fertilization. An extensive investigation has been made, and is in progress, concerning the problem of species-specificity. In general, the results show that the degree of cross-reaction of fertilizin and antifertilizin of various species is greater than the degree of cross-fertilization. Thus the specificity of fertilizin-antifertilizin interaction is not in itself sufficient to account for that of fertilization. Similar results are obtained with the lytic agent of sperm. Also, the specificity of these substances as antigens in rabbits is not as great as that of fertilization. It appears then that other specific factors must be involved and this is not too surprising since it is quite likely that many other substances besides those discussed here are concerned in various steps in the process of fertilization. On the other hand, it should be noted that where cross-reaction between fertilizin and antifertilizin is lacking fertilization also fails to occur.

A scheme has been proposed (Tyler, 1948) for the manner in which fertilizin-antifertilizin interaction may account for the approach and specific attachment of sperm to egg surface. As noted above Lillie also suggested that activation of the egg might involve these substances. At present the best available hypothesis concerning activation is that proposed by Heilbrunn (1943), which involves a protoplasmic gelation or clotting reaction initiated by a release of calcium. It is of interest to note, then, that the fertilizin-antifertilizin reaction is largely dependent upon the presence of calcium, as Loeb first showed and as Vasseur (1949) has recently demonstrated in some detail and, that fertilizin shows (see Immers, 1949) some heparin-like activity.

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Note: An abstract of a Friday Evening Lecture delivered July 1, 1949, at the Marine Biological Laboratory.

THE MECHANISM OF COLOR CHANGES IN CRUSTACEANS

Dr. Frank A. Brown, Jr.

Professor and Chairman of the Department of Biological Sciences, Northwestern University

The capacity for exhibiting changes in body coloration is widely distributed among higher animals. It is usually found only in species which possess well-developed eyes and central nervous systems and the color changes are ordinarily complexly controlled through these organs. Of all the organisms showing color changes no group shows more striking examples of this capacity than the decapod crustaceans. Within this group the character of the changes spread through a wide range. Some species, as for example the fiddler crab, Uca, has as its major natural response simply a darkening of the body by day and a blanching by night. The sand shrimp, Crago, and the common prawn, Palaemonetes, on the other hand, are able to darken upon dark-colored backgrounds and lighten upon light-colored ones. In addition, both of these latter animals are able to a good extent to become even the color of colored backgrounds upon which they come to rest. This is especially true of Palaemonetes. Still other crustaceans as, for example, the gulf-weed crab, Planes, and the shrimps, Latreutes and Hippolyte, appear able to imitate not only the color but also the pattern of the coloration of the algae upon which they live.

These color changes in the crustaceans are accomplished by means of special effector organs, the chromatophores, which are widely distributed over the surface of the body. These chromatophores are highly branched, multinucleate cells, each containing one (monochromatic), two (dichromatic) or more (polychromatic) pigments. The chromatophores normally function in both of two ways in the production of color changes. One of these two activities is referred to as physiological color change and involves the mechanical movement of the pigment within the chromatophore. Any given pigment may concentrate into a spherical mass in the chromatophore center and thus not contribute to the gross coloration of the animal, or it may disperse out into the chromato-

phore branches and contribute to the coloration. By an appropriate differential activity of the chromatophoral pigments in physiological color changes, a prawn like Palaemonetes, possessing red, yellow, blue, and white pigments can readily assume the color of any background upon which it is placed. The rates at which physiological color changes are accomplished are relatively rapid, the time required for a pigment to pass from a maximally concentrated to a maximally dispersed state, or *vice-versa*, ranging from about five minutes to a few hours depending upon the chromatophore type. As would be expected, the accuracy with which the background color can be matched by physiological color changes alone, depends upon the amounts of the required pigments present within the chromatophores.

The second of the two primary activities of chromatophores is termed morphological color change. This activity involves a differential synthesis or destruction of the pigments within the chromatophore. In Palaemonetes, for example, the red and blue pigments gradually disappear from the chromatophores of specimens kept upon a white background. Animals kept upon a black background show, on the contrary, a gradual gain in the amount of these dark pigments in the chromatophores. These morphological color changes are much slower than the physiological ones and require days or even weeks before the maximum adaptive change is completed. Extraction of the total pigment in the bodies of animals undergoing extensive morphological changes shows clearly that these changes involve chemical alteration in the pigments and not simply a translocation of the pigments within the body.

It was for many years assumed that the chromatophores of crustaceans were directly under the control of nerves. Numerous attempts to demonstrate histologically the presence of nerves passing to the chromatophores all resulted, however, in failure. Furthermore, when all the known nerve supply to a region of the body was transected, there seemed to be no disturbance, whatsoever, of the capacity of that region for color change. Even the accurately detailed tint-adaptation of a shrimp like Palaemonetes occurs in such denervated regions of the body.

About twenty years ago, two investigators, working independently, demonstrated an action of blood-borne principles in the control of crustacean chromatophores. Koller, in Germany, utilizing the technique of blood transfusion, showed that blood from a black donor darkened a pale recipient and that blood from a yellow donor caused a pale recipient to become yellow. Perkins, in this country, observed that occlusion of a blood vessel resulted in an immediate cessation of the color response of that region of the body supplied by that vessel, and that the ability to respond reappeared at once following restoration of the normal blood supply. Perkins demonstrated, in addition, that an aqueous extract of the eyestalks of the shrimp, Palaemonetes, lightened dark specimens into which it was injected, and, conversely, removal of the eyestalks left them permanently darkened. On the basis of these observations Perkins postulated the eyestalks to contain sources of a lightening hormone. At about the same time Koller reported that aqueous extracts of the rostral region of the sand-shrimp, Crago, darkened pale specimens and destruction of the region resulted in their remaining permanently pale. Koller therefore postulated a source of a darkening hormone to lie in the rostral region. These last observations of Koller have not yet been confirmed despite a number of attempts to do so.

It appears clear at the present time that hormones conveyed by the blood constitute the only chromatophore-activating agents for the crustaceans. The great complexity of the endocrine mechanism which must be involved becomes apparent when one recalls the independent activities of the several pigments in single individuals in the course of adaptation to different colored backgrounds. Palaemonetes possesses four kinds of pigments each of which is capable of activity independent of each of the other three. It is clearly evident that a *minimum* of three hormones must be present in this shrimp to account for this complex differential activity of the pigments.

Investigations during the past few years have provided us with considerable information as to sources, numbers and the activities of hormones influencing the various chromatophore types. The two major sources appear to be the sinus glands in the eyestalks and various regions of the central nervous system.

The sinus glands are minute organs which are richly charged with secretory granules; these granules render the gland readily visible in the freshly dissected eyestalk. The glands are complexly innervated from the brain and optic ganglia. Sinus glands may be easily dissected free of the remaining eyestalk tissue, extracted, and tested for activity by injection into other specimens. It seems quite clear at present that sinus glands of crustaceans possess at least three chromatophorotropic principles. Evidence for this conclusion has come from two types of experiments: comparative physiological studies and chemical fractionation. Physiological studies of the actions of extracts of sinus glands of various species of crustaceans as tested by simultaneous assay of their actions upon two widely different

responding chromatophore types, Uca black pigment and Palaemonetes red pigment, have demonstrated that the activities of the extracts with respect to these two types do not vary in any correlated manner. This is what would be expected were the activity upon one chromatophore type the result of one substance and the action upon the other, of another. Compelling the same conclusion are experiments upon chemical fractionation. An alcohol-soluble fraction of sinus glands from a wide variety of species of crustaceans exhibits a strong action upon Palaemonetes red pigment and little upon Uca black; the alcohol-insoluble fraction, on the contrary, has a strong action upon Uca black pigment and relatively little upon the red of Palaemonetes. There seem, therefore, definitely to be at least two principles in all sinus glands studied.

Further comparative studies of the effects of sinus gland extracts lead to the conclusion that still an additional chromatophorotropic principle is present in some. Extracts of the glands of Palaemonetes and other shrimp, but not of Uca or other true crabs, will, upon injection, lighten the telson and uropods of black Crago. It is still unknown as to whether the shrimp sinus glands possess three hormones and the crab, only two, or whether the glands of both groups possess only two, with one of the two differing in properties between the two groups.

The presence of chromatophorotropins within the central nervous system is of general occurrence among the crustaceans. The actions of these principles may supplement or may antagonize those of the sinus glands, depending upon the chromatophore type. The general roles of these substances in color change may perhaps be most lucidly illustrated by a description of a few types of results obtained with the shrimp, Crago. When one removes the eyestalks with their included sinus glands from this sand shrimp, a very characteristic color change ensues. First, the whole shrimp becomes very pale, except for the telson and uropods which become intensely black. After about an hour the shrimp gradually assumes a typical coloration of eyestalkless specimens, a mottled body and a completely pale tail; this coloration is then retained indefinitely. One can reproduce the transitory color change just described, by strong electrical or other stimulation of the cut ends of the optic stalks in the eyestalkless animals. The same transitory response can be obtained by injection of an aqueous extract of the central nervous system of another specimen. Incidentally, this peculiar coloration involving a light body and a dark tail is so often observed in normal individuals of the genus, that one common Pacific coast species bears the specific name, *nigricauda*.

If instead of applying a strong stimulus to the cut ends of the optic stalks, a mild electrical stimulus is used, the response is quite different; the whole animal now undergoes a transitory blackening. It can easily be seen that at least two active principles from somewhere within the central nervous system are here involved. A study of the various parts of the central nervous system reveals that whereas an extract of any major portion of the system will lighten the body of darkened Crago, only extracts of the minute tritocerebral commissure, connecting the two circumoesophageal connectives just posterior to the oesophagus, will both lighten the body and blacken the telson and uropods. If one goes further and now extracts the tritocerebral commissure with alcohol, this fraction, like the other parts of the nervous system, will lighten the body but not darken the tail. The alcohol-insoluble residue, now freed from its body-lightening activity, blackens not only the tail of Crago, but the whole body as well. The central nervous system of Crago, therefore, clearly appears to have two principles, one of whose activities is to lighten the body but not the tail (sinus gland extract lightens both), and the other darkens the whole body. The mild stimulation of the optic stalk therefore caused a selective liberation of only one of the principles; the strong stimulation produced extensive liberation of both.

A histological examination of the tritocerebral commissures has disclosed that the neurilemma of this region shows a greatly thickened area whose cells are filled with secretory granules.

When one examines the nervous systems of other crustaceans for the presence of this Crago-lightening and this Crago-darkening activity, it is found that all of the decapod crustaceans except the true crabs possessed both of these principles though their distributions within the nervous systems differ from genus to genus. True crabs, such as Uca, do not possess the Crago-darkening activity in any part of their nervous system. It can readily be demonstrated, however, that the nervous system of Uca possesses two chromatophorotropins. One of the latter is a white pigment concentrating principle, the other is a black pigment dispersing one, and these two show quite different distributions through the system.

It is seen from the foregoing that sources distributed within the central nervous system and the sinus glands are important in the control of the complex chromatophore systems of crustaceans. There is no reason to believe that these initial demonstrations of a few principles from these sources have provided a complete picture. Undoubtedly, more will be shown to exist. Furthermore, little or nothing is known of the nature

of joint actions of the various principles.

There are three general kinds of responses of crustacean chromatophore systems to environmental factors. The first general type is a response to total illumination. In this response it seems to be rather generally true that the greater the illumination the greater the degree of dispersion of all the pigments, both dark and light. This is apparently the primitive response of the system and is probably quite comparable to the primary or embryonic one of the vertebrate. It can be demonstrated by such a technique as shielding a limited portion of the integument that this response is at least in good measure an "independent-effector" one in the crustaceans. On the basis of this response, crustaceans tend, other factors being equal, to become opaque in bright light and transparent in dim light.

A second general kind of response is one to the albedo, or in other words, a response to the ratio of incident to reflected light striking the eye. This type of response is obviously dependent upon the possession of a complex eye. A good white background diffusely reflects about 1/3 of the incident light and a good black background diffusely reflects about 1/200 of the incident light, hence the ratios in the two instances are 3 and 200 respectively. The albedo responses of the chromatophore system are ones resulting in a mimicking of the shade of the background upon which the animal lies. By this response the dark pigments typically disperse and the white pigment concentrates when the animal is upon a black background. The pigments assume the opposite condition upon a white background. It is clear from what has been said that the albedo response and total illumination response may supplement one another (*e.g.* the white pigment on black and white backgrounds) or oppose one another (*e.g.* dark pigment upon black and white backgrounds). The imitating of the colors of backgrounds similarly is an albedo type of response, but here obviously there is involved a capacity for color perception as well.

The third major type of response of the chromatophore system to environmental factors involves temperature. Elevation of the temperature above the normal range tends to disperse white pigment and to concentrate dark pigment. The result is that at these higher temperatures the body reflects more, and absorbs less, radiant energy. This would appear to serve to some degree as a body-temperature regulating mechanism.

All three of the kinds of responses are normally operating simultaneously upon any given chromatophore system, but the relative influences of the three vary with the species, the chromatophore type, and the magnitude of the intensity factor for each in any given situation. It can be seen that the characteristics of the responses of the system that have just been described are all compatible with all of the four commonly postulated adaptive significances of color change in animals in general. A function of obliterative coloration appears to be fulfilled by the albedo response. A function of protection of the body from injury due to excessive illumination appears subserved by the total illumination response. The temperature response appears consistent with the hypothesis that the system helps protect the body from excessive elevation in body temperature due to sunlight absorption. Both the temperature and total illumination responses appear to be such as to favor the absorption of heat through radiation within the viable temperature range.

The chromatophore systems of crustaceans are in many instances also subject to persistent endogenous rhythms. The operation of such a rhythm is especially conspicuous in the fiddler crab, Uca, which tends to be dark in color by day and pale by night. This diurnal color change persists with remarkable regularity even when the animal is kept in constant darkness and temperature. Under such constant conditions the rhythm has been observed to remain completely in phase with the solar day-night cycle for several weeks. The frequency of the rhythm is independent of temperature through a wide range. Uca may be taken from room temperature and placed in a constant temperatured darkroom at any temperature between 6 and 26° C. and in every instance the rhythm retains very precisely its twenty-four hour frequency. Despite the temperature independence the rhythm appears to be based upon a metabolically operated mechanism. This is indicated by such an observation as the result of chilling animals in a darkroom for several hours at 0 to 3° C. When the animals are rewarmed the rhythm immediately returns as a twenty-four hour cycle, but one that is now permanently out of phase with the earlier rhythm by a length of time approximately equal to the period of chilling.

The temperature-independence of the rhythm through such a wide range of temperatures is most remarkable, and, I believe, a unique situation in biology. It is a phenomenon whose explanation can scarcely be postulated at the present time. It seems obvious, however, that there must be within this poikilothermic crab, Uca, a very precise temperature-compensating mechanism.

Recent work has given us some information as to characteristics of response of the rhythmical mechanism and some interesting suggestions as to the nature of its organization. In constant illumi-

nation the rhythm of color change in Uca gradually becomes weaker and weaker and after four or five days the rhythm is completely lost and the animal remains continuously dark. The rhythm in such inhibited animals will immediately reappear upon placing the animals in constant darkness. The frequency of this restored rhythm is exactly twenty-four hours but the phase is determined by the time the animals are placed in the darkness. If such inhibited animals are placed in darkness at 7 a.m. the rhythm will be approximately 6 hours out of phase with the normal day-night cycle; if, however, they are placed in darkness at 1 p.m., 7 p.m., or 1 a.m., they are completely in phase. These results indicate clearly that the light-inhibited crabs must still possess a twenty-four hour rhythmicity which is expressed here as a cycle of sensitivity change. Furthermore, it is seen that a single light change if administered during a critical period, can abruptly alter the cycle.

The rhythm of color change in Uca can be reversed by illumination at night and darkness by day. This reversal is characterized by a gradual inhibition of the rhythmic color changes over a two- or three-day period and then a gradual increase in amplitude of the rhythm in its new and reversed phase. This rhythm can be restored to its normal phase by returning the crabs to the normal day-night environment. The return is again characterized by an initial period of inhibition and after a few days the resumption of the original rhythm.

The responses of the rhythm to alternating six-hour periods of light and darkness are interesting and instructive ones. If the crabs are illuminated between 7 a.m. and 1 p.m. and between 7 p.m. and 1 a.m. and darkened during the intervening periods, the cycle of color change is abruptly thrown six hours out of phase with the previous one. In this case there is no initial inhibition. The behavior of the rhythm upon placing these animals in constant darkness now depends upon the time of the last period of illumination. If the last illumination period was 7 p.m. to 1 a.m. there is a gradual creeping of the rhythm to its original phase over a four- to five-day period in darkness. In previously reversed animals, there is a comparable gradual return to the reversed rhythm when the last period of illumination was 7 a.m. to 1 p.m. On the contrary, if the last illumination was 7 a.m. to 1 p.m. (or 7 p.m. to 1 a.m. in reversed animals) the return to the earlier phase of the rhythm is immediate.

These last two types of experiments appear to indicate two things: (*1*) the influence of one of the six-hour light periods is fully cancelled by the second, and therefore only a single light period induced the slowly transitory alteration, and (*2*) there are two centers of rhythmicity with only one of them altered here; an unaltered one gradually restores the original phase in this instance.

All of the characteristics of responses of the rhythmical mechanism of Uca appear to be simply explained in terms of such an hypothesis as the following. There are two rhythmical centers operating in the maintenance of the normal rhythm of color changes. One of the centers which might be referred to as Center I possesses a deep-seated twenty-four hour rhythmicity which persists throughout the life of the animal. A second center, Center II, also possesses a rhythmicity but this latter rhythm persists for only a few days in the absence of influence from Center I. Continuing with this hypothesis, light acts in such a manner as to inhibit the rhythmic influence of Center I on Center II. Center II, thus cut off from Center I, gradually loses its rhythm. Center II can not alter the phase of of Center I but center I can gradually alter Center II when the later is out of phase with the former.

The evidence appears to indicate that each of the two centers may have its rhythm abruptly altered independently of the other by light stimuli of specific sorts presented during sensitive periods in its cycle. Those stimuli such as reversal of illumination, and placing animals which had been inhibited, into darkness at 7 a.m. must have induced an alteration in Center I. Transitory alterations such as that induced by a six-hour period of illumination from 7 p.m. to 1 a.m., would be presumed to have effected an alteration in Center II.

Note: Based on a lecture presented at the Marine Biological Laboratory.

LABILE *P* IN NUCLEIC ACIDS

By Abel Lajtha
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There is a striking parallelism between muscle and other organs, for example, kidney and liver. If we let rabbit muscle or any other kind of muscle stand at room temperature, the *ATP* in it is gradually split; parallel with the decreasing *ATP* concentration, the elasticity of muscle fibers decreases, a stiffness gradually develops (rigor mortis), and the solubility of the highly viscous

muscle protein actomyosin decrease.

The post mortem changes in kidney are analogous. If we mix fresh minced kidney with strong salt solutions, a greatly viscous extract is obtained; the sticky solution shows a strong double refraction of flow. If, before extraction of the kidney, we let it stand at room temperature for about half an hour, the viscosity of the solution will be very small, there will be no *DRF*, and it does not appear sticky—showing that only very small amounts of the kidney structure-protein went into solution, if any did at all.

In the muscle the changes are mostly restored by adding physiological amounts of *ATP*. Even large amounts of *ATP* don't restore the lost solubility of structure proteins in kidney.

The analysis shows that compared with the muscle, there is only about one tenth as much *ATP* in the kidney. The question arises whether in kidney the nucleic acid plays the rôle played by *ATP* in muscle. The first question in approaching this problem is whether nucleic acids contain labile *P*.

Nucleic acids were prepared from kidney and liver in three different ways. In one set of experiments emphasis was laid on purity of the product, in another on quantitative yields, and in the third on mildness of the method avoiding all possibility of hydrolysis.

To prepare pure nucleic acids the organs were washed with cold trichloracetic acid, then with lipoid solvents and finally with strong NaCl solution reprecipitated with acids several times and washed with lipoid solvents again several times at pH 2.5. As in the other methods, I followed the purification with pentose and desoxypentose tests and with ultra-violet absorption spectra. With this type of reprecipitation, we get pure nucleic acids very fast, and working at low temperature, we can retain almost all labile *P*.

To get quantitative results, I extracted the organs with hot NaCl solution containing five per cent, Na_2CO_3, extracting three times for about 15 minutes. The analysis of the remainder showed that about 98 per cent of the *P* containing compounds were dissolved. Precipitation was made complete with the combined action of acid and alcohol. This method, however, must be corrected, as experiments with nucleic acids prepared in another way and after being boiled in basic NaCl solution showed that about ten per cent of the labile *P* is split off by 45 minutes of boiling in a salt solution containing five per cent Na_2CO_3.

To work as fast as possible and retain all labile *P* groups, the organ was washed with cold alcohol and then with water and then extracted in many ways. One of the methods used, for example, was just washing it with hot water. All the extracting solutions were then analysed for nucleoprotein and labile *P* afterwards.

The result of these experiments is that the nucleic acids of kidney and liver contain a labile *P* which is hydrolysed in normal acid in ten minutes and which amounts to about 20 per cent of the total P.

This would show that for approximately every tetranucleotide unit there is one labile *P* in the nucleic acids.

NOTE: Based on a paper presented at the Marine Biological Laboratory.

ON THE STRUCTURE OF FIBRIN CLOTS

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The mechanism of the transformation of fibrinogen into fibrin is largely unknown. Several theories were formulated to explain the action of thrombin, but none of them proved to be satisfactory. Such a fundamental question as whether the fibrin molecules are bound together through co-valent bonds, or whether they are held only by weak forces, like the van der Waals forces, or hydrogen bonds is still unanswered.

In the present investigation attempts were made to solve this question by studying the solubility of fibrin clots in urea solutions.

Several investigators of the late nineteenth Century reported the solubility of fibrin clots in concentrated urea solutions.[1, 2] Wöhlisch and his co-workers,[3, 4] however, could not confirm this finding. The problem has considerable importance, because the protein gels and coagula, where the particles are bound by weak secondary forces, are all soluble in urea solutions. Insolubility may thus be an indicator of co-valent bonds between the particles.[5]

Loránd,[6] reinvestigating the problem, found fibrin resulting from the action of thrombin upon pure fibrinogen to be readily soluble in urea solutions. On the other hand, fibrinogen clotted by thrombin in the presence of serum and calcium ions, was insoluble. This finding may explain the contradictory results of different authors.

In our investigations the solubility of pure

fibrin clots was confirmed, and the reversibility of the dissolution demonstrated. These facts seem to justify the conclusion that by the clotting process no co-valent bonds are formed.

When urea is dialysed from a fibrin solution the clot is reconstituted. Similarly, if the fibrin solution is diluted with distilled water, the urea concentration being lowered in this way, the solution suddenly undergoes gelification. The pH of the fibrin solution during the dialysis decides whether a coarse or a fine type gel will be formed.

The dissolving effect of urea depends on its concentration and the pH of the system. It is easier to investigate the interaction of these factors by studying the inverse phenomenon *i.e.* the gelation of a fibrin solution. The fibrin dissolved in urea solution was mixed with buffers of different pH and distilled water in order to have different urea concentrations and pH. The viscosity of the solutions was determined. At 30% urea concentration, over the entire pH range studied, the viscosity of fibrin solutions did not differ from that of fibrinogen in similar conditions. The solutions had Newtonian viscosity and showed no double refraction of flow. At 20% urea concentration the viscosity rose at pH values more alkaline than 7.6 and reached a maximum at pH 8.6; it then decreased again to the original value by further alkalinisation. At still lower urea concentrations the increase of viscosity started at a pH which was the more acidic the lower the urea concentration was and finally led to a gelation of the solution. In the region of rising viscosity the solutions became thixotropic; at the same time a strong double refraction of flow appeared.

Urea affects the electrostatic forces between charged groups by increasing the dielectric constant of the medium. Although this effect may have some importance, it is far from being the cause of the dissolution of fibrin. Dipolar ions, like glycine for example, have a much greater effect on the dielectric constant. In spite of this, a fibrin solution in 15% urea, when diluted with 2/*M* glycine, gelified exactly at the same degree of dilution as when it was diluted with distilled water.

Most probably urea affects the hydrogen bonds of the protein molecules formed between the -NH- and -CO- groups of adjacent polypeptid chains. If this mechanism is really the cause of the dislocation of the fibrin particles, the hydrogen bonds must play a considerable rôle in the building of the gel.

The viscosimetric behavior of fibrin solutions in 30% urea indicates that the particles, in respect to their shape and size, are probably identical with those of fibrinogen. Thrombin does not alter the shape and size of the fibrinogen molecules; it only modifies some of their physicochemical properties.

The solubility of fibrin in urea solutions makes it possible to investigate the electrophoretic mobility of this protein. If the action of thrombin involves some of the ionizing groups of fibrinogen, a study of the differences in electrophoretic mobilities between fibrinogen and fibrin may give some information about the nature of the process of clotting.

The electrophoretic mobility of fibrinogen and fibrin, dissolved in 20% urea, was determined at different pH in the Tiselius apparatus. It was found that the mobility curve of fibrin is always below that of fibrinogen, *i.e.*, in the region more alkaline than the isoelectric point the fibrinogen is the faster, whereas in the region acidic to the isoelectric point fibrin is the more rapid; the isoelectric points of the two proteins are very close together. Fibrinogen is isoelectric at pH 5.5, fibrin at 5.6.

The mobility differences of the two proteins are very small. To exclude the possibility that the observed differences were due to experimental error, control runs were made with a mixture of fibrinogen and fibrin at each pH studied. The two components separate slowly in conformity with the mobility differences obtained in runs with a single component.

The results indicate that the net charge of fibrin in the zone of clotting is lower than that of fibrinogen. The lower charge favors the approach of the fibrin particles and thus their binding. The decrease of the net charge may be the result of an increase in the free NH_2 groups of the protein. If we suppose that the fibrin particles are bound by hydrogen bounds between $-NH_2-$ and -CO- groups, the increase in the number of free NH_2 groups will favor also the gelation process.

The results are in accord with our earlier investigations[7] in which we were able to demonstrate the role of NH_2 groups in the process of clotting.

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NOTE: Based on a report presented at the Marine Biological Laboratory, August 2, 1949.

INVESTIGATIONS ON MUSCLE FIBERS

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The glycerinated muscle preparation described by Dr. A. Szent-Gyorgyi (*Biological Bulletin* **36**, 140, (1949) gives an excellent material for the study of muscle contraction itself, without being influenced by conductivity and transmission. The question is how far the behaviour of this muscle preparation corresponds to that of the actomyosin thread.

The material used consisted of thin bundles of the rabbit psoas washed with 50% glycerol for 2-9 days. The muscle preparation was made to contract by placing it in 0.2% *ATP* plus varying NaCl concentrations at pH 7.0 in the presence of 0.0015 *M* $MgCl_2$. There was no difference between Na or K.

According to the experiments of our laboratory, in the presence of *ATP*, actomyosin can only be either in the contracted or in the dissociated state. At low NaCl concentrations (0.05 — 0.2 *M* NaCl) the actomyosin thread is maximally contracted. As one increases the salt concentration a critical point is reached, at which contraction no longer occurs, instead the actomyosin dissolves and dissociates, as shown by its low viscosity. In the case of the actomyosin thread this critical point falls to 0.2-0.22 *M* NaCl concentration.

Repeating the same experiment with muscle fibers washed with glycerol the results are quite different. The contraction of the fibers is practically independent of salt concentration and maximal contraction is obtained by varying the salt concentration from 0.015 up to 0.5 *M* NaCl, the highest salt concentration used. The degree of contraction amounted to 60-70% of the original length at every salt concentration investigated. The close packing and the prolonged washing in glycerol stabilizes the actomyosin in the fibers so much that *ATP* could not dissociate it even at high salt concentrations. If the actomyosin has existed in the muscle fibers in the associated state for some time it would become very stable and hence would contract in the presence of *ATP* over a wide range of salt concentration.

If we want to start with a muscle where actomyosin is dissociated we have to loosen the structure by high concentration of ions which have a specific dissociating action. This can be done for instance with 0.1 *M* Na-pyrophosphate at pH 7.5, or with 0.1 *M* $NaHCO_3$ in the presence of 0.3 *M* NaCl, or with 0.4 *M* NaOCN at pH 8.8 or with 0.05 *M* Na-tri-phosphate of pH 7.5. Treating the fibers with these solutions there is a pronounced difference in physical appearance. The originally opaque, white, completely inelastic and brittle muscle becomes transparent and slightly elastic. The effect takes place within 1 minute, though usually 4 minutes incubation time was used. (Immersing the fibers into these solutions brings about a slight shortening in the absence of *ATP* too, which does not exceed 10-15% of the original length. This was kindly observed by Dr. A. G. Matoltsy under the microscope; it is most probably due to the dissociation of the rigid actomyosin structure. This shortening was not taken into account in these measurements.) The fibers after treatment with pyrophosphate, or with the solutions described above, behave like fresh actomyosin or perhaps like fresh muscle. The contraction depends on a critical salt concentration between 0.1 and 0.2 *M* NaCl.

Below and above this salt concentration range there is no contraction. At 0.015 *M* NaCl concentration the fibers remain relaxed in spite of the presence of *ATP*. Between 0.1 and 0.2 *M* NaCl the fibers contract maximally. The upper salt limit of contraction varies somewhat and is around 0.25 *M* NaCl. The transition to contraction is usually sharp, though the single points differ only by 0.05 *M* NaCl. *ATP* does not cause the contraction of the fibers treated with pyrophosphate at higher salt concentrations. The contraction of the pyrophosphate treated fibers occurs thus in two steps. The first one is the actomyosin formation, the second the contraction due to the effect of *ATP*.

The dissociating action of pyrophosphate is reversible. If we put the pyrophosphate treated muscle into 0.1 *M* NaCl for about 10 minutes in the absence of *ATP*, it begins again to behave as glycerinated muscle untreated with pyrophosphate. That means the fibers contract again at every salt concentration employed, though over 0.4 *M* NaCl concentration the contraction is not maximal, showing that the actomyosin formed in 0.1 *M* NaCl is not as stable as the actomyosin formed during the prolonged washing in glycerol. The same reversal effect can be obtained by putting the muscle into 50% glycerol after pyrophosphate treatment, though here a longer incubation time (about 30 minutes) is needed.

Starting with the glycerinated muscle we have actomyosin in a very stabilized form, after pyrophosphate treatment we have fibers where the actomyosin is dissociated. One can study separ-

ately the actomyosin formation and the contraction by the aid of these preparations. *E.g.*, $NaHCO_3$ does not inhibit the contraction, even at 0.1 *M* concentration, the glycerinated fibers contract maximally. After pyrophosphate treatment the contraction is inhibited in the presence of 0.03 *M* $NaHCO_3$, showing that $NaHCO_3$ inhibits actomyosin formation.

The results indicate at least two different steps in the contraction of muscle after pyrophosphate treatment. The first is actomyosin formation. That depends on salt concentration. The second is the contraction itself, which does not depend on salts. *ATP* causes contraction only after actomyosin is formed or under conditions, where formation of actomyosin is favored.

Note: Based on a report presented at the Marine Biological Laboratory.

EVIDENCE FOR ACTIVITY OF *DNAse* IN MITOSIS BY USE OF d-USNIC ACID

By Dr. Alfred Marshak

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Along the coast of California there is a good deal of fog during the summer. In the neighborhood of Monterey, the fog comes in almost every night and remains for a good portion of the day. A lichen (*Ramalina reticulata*) grows in abundance in this fog belt as an epiphyte, especially on oaks. It absorbs both moisture and nutriment from the air. About the surface of the lichen and between the hyphae of its fungal component there is a carbohydrate material which is very hygroscopic so that in the fog the lichen acquires an almost gelatinous consistency. Since this provides almost ideal conditions for the growth of bacteria which would decompose the lichen, the very existence of the plant implies its possession of some chemical defense against bacterial invasion for it appears to have no physical protection such as an epithelium. Exploratory investigations confirmed this hypothesis; finally a crystalline substance with marked antibacterial properties was isolated from the lichen and was shown to be d-usnic acid. This substance is present in a good many of the Uneaceae found throughout the world. It was first isolated by Stenhouse in 1848. Its structural formula has been tentatively established although not conclusively proved by Curd and Robertson and by Asanina in a series of papers, 1933-37.

When tested against bacteria *in vitro*, it shows some activity against gram positive bacteria and a few gram negative ones but is most active against mycobacteria and especially active against the human tubercle bacillus.

Work done at Woods Hole in 1947 with Miss Jane Harting showed that as little as 10 μg/ml would inhibit cleavage and 1 μg/ml would produce very abnormal cleavage in Arbacia eggs. No effect on O_2 consumption could be observed with 100 μg/ml although this concentration completely inhibited P^{32} uptake.

The inhibition of cleavage was investigated further and it was found that in the presence of 10 μg/ml the sperm nucleus reached the egg nucleus at the same time as in the controls, *i.e.*, between 8-12 minutes after fertilization. Some eggs were fixed in Bowin's solution for cytological observation, sectioned and stained with hematoxylin. Another portion was fixed in 5% citric acid, the remaining pigment extracted with 5% citric in 30% alcohol and finally lipids removed by boiling in acetone: alcohol. After the lipid extraction they were stained *en masse* by the Feulgen technique and examined with and without counterstain.

The egg nucleus did not stain with the Feulgen, neither did the nucleus of the unfertilized egg. Sperm were bright red as were the chromosomes in metaphase and anaphase. In the two-cell stage and subsequent interphases, the nucleus had a faint but definite magenta color. Later interphase nuclei had more color than earlier ones.

As the sperm nucleus approached the egg nucleus it became slightly larger and stained somewhat less intensely. On fusion with the egg nucleus, the intensity decreased and finally became indistinguishable from the egg chromatin which was colorless.

In the presence of usnic acid the sperm nucleus reaches the egg nucleus in the same time as in the controls but does not fuse with it; neither is there any decrease in intensity of its Feulgen stain. It may remain in this condition for at least four hours. This observation suggests that usnic acid may interfere with whatever mechanism in the sperm or egg is concerned with dispersion of desoxyribonucleic acid. To determine whether this might be desoxyribonuclease, experiments were conducted on the activity of *DNAse* in the presence and absence of usnic acid.

The *DNA* used was prepared by the method of Gulland, N/P = 1.66; another batch was prepared by the method of Greenstein, N/P = 1.87, containing some contaminating protein. Two

batches of *DNAse* were also used, one which was non-crystalline obtained from Dr. M. McCarty and another crystalline preparation obtained from the Worthington Chemical Company. The latter showed marked activity at 0.1 μg/ml; the former only at 1 μg/ml. The index of activity used was whether or not a minimal concentration of enzyme would or would not decrease the viscosity of a solution of *DNA*. Measurements were made in veronal buffer at pH 7.8 containing .001 M.

As little as 1 μg/ml of usnic acid will produce partial inactivation of the system *DNA-DNAse* and 10 μg/ml gives complete inactivation. It was found also that the inactivation requires the presence of cobalt in the reduced form ($CoCl_2$ .001-.002 *M*) and that this effect varies with the concentration of usnate. In the absence of usnate cobalt has no effect on enzyme activity. The inactivation cannot therefore be due simply to removal or inactivation of Mg by Co, but is a consequence of formation of complexes involving Co, usnate and enzyme or substrate.

Seymour Cohen showed that streptomycin forms a complex with desoxyribonucleic acid, resulting in turbidity of the solution. Sodium usnate was found not to produce such complexes as Cohen described. It was also found that streptomycin in concentrations up to 100 μg/ml in the presence or absence of cobalt will not inactivate the system *DNA-DNAse*. The results thus show that the usnate inactivation is produced by complex formation with the enzyme rather than the substrate.

Since usnic acid will also inhibit further cell division if added after cleavage has begun, it follows that desoxyribonuclease or a similar enzyme is involved in the mitotic process as well as in the fusion of the sperm and egg nuclei.

It is perhaps significant that the concentration required to inactivate the enzyme is the same as that required to inhibit cleavage in sea urchin eggs and to prevent the growth of tubercle bacilli. Although it may seem a far cry from sea-urchins to lichens, these findings suggest a possible rôle of usnic acid in the lichen in which it is found. The lichen is composed of an alga and fungus growing together, neither of which can outgrow the other without destroying the symbiont relationship. From the experiments described it is apparent that usnic acid could serve as a growth inhibitor of the alga, while the rate of fungal growth could of course be limited by the algae photosynthetic activity.

Note: Based on a paper presented at the Marine Biological Laboratory.

COLD AS A MEANS OF COMBATTING ASPHYXIA IN NEWBORN GUINEA PIGS

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Maternal mortality statistics associated with childbirth have been reduced in the last few years to a negligible figure. The medical profession has not succeeded quite as well in preventing death of the baby during the first year of its life, and in fact there has been no real improvement in mortality statistics for infants during the first 24 hours after birth.

The largest single cause of death of full term infants found at autopsy is asphyxiation with frequencies up to 68% reported by various authors. Therefore, any means which will enable fetuses and newborn babies to resist anoxia may result in an appreciable saving of lives during the most hazardous period of postnatal existence.

There are clinical indications and experimental proof in guinea pigs (Windle and Becker, 1942*a*, 1942*b*) that the sequelæ of neonatal asphyxia are not transitory but may persist into adulthood. They range from patent neurological symptoms to mental dullness which may be too subtle to be detected except in carefully controlled experiments. Accordingly, any treatments which enable the newborn to resist the effects of anoxia may aid in reducing the numbers both of individuals with frank neurological symptoms and of mentally subnormal individuals.

The standard treatment for anoxic babies includes, among other procedures, the maintenance of body temperature by placing them in a warmed bassinet or incubator until normal respiration is established. So far as they have been studied, all newborn mammals (including the human) are poikilothermic, with body temperature varying with ambient temperature. Since there is a close relationship between temperature and rate of chemical reactions, and since, according to van't Hoff's rule, rates of most reactions are doubled or trebled with each 10°C rise in temperature, it appeared to the writer that changing the temperature of the anoxic fetus or newborn might alter the metabolism of vital centers sufficiently to appreciably affect survival. This line of reasoning raised grave doubts concerning the rationale for maintaining the anoxic baby at a high body temperature and thereby increasing its need for oxygen at a time when its energy supplies are so low as to threaten life

itself. Accordingly, experiments were instituted to test the length of survival of anoxic animals at various temperatures.

Material: Guinea pigs were used because of their size and advanced condition at birth (more nearly resembling the human than do rats, mice, rabbits, or hamsters) and because they were used by Windle and Becker in their demonstration that asphyxia at birth produced lesions and generalized atrophy in the nervous system.

Methods: Litter mates 24 hours old or less were tested either at room temperature, after warming in an incubator, or after cooling by the evaporation of alcohol or by immersion to the neck in ice water. Cooling usually required five minutes or less. Temperatures were determined with a U.M.A. skin thermocouple modified so that is could be inserted 2 cm. into the colon. Animals were tested in pairs in a bell jar through which 95% N_2 + 5% CO_2 mixture was flowing. Complete records of the behavior of each animal were kept, including the time of each gasp. Autopsies were performed on all animals which died.

Results: Preliminary experiments (Miller, 1949) had shown that is was possible by cooling to save the lives of littermates of animals killed by 4½ minutes exposure to 95% N_2 + 5% CO_2. Likewise, when all animals in a litter were exposed until death the cooled lived longer than the untreated while the warmed animals died in the shortest time.

Next, 160 animals were tested in order to determine effects of temperature upon anoxic survival over a wider range than was used in the preliminary experiments. When averaged together in the 3° classes, a 185% increase in mean survival time is noted between 37.5°C and 11.0°C. This represents an increase of 7.1% for each degree or 71% for each 10° decrease in temperature. However, when the data are recorded in three groups according to the time of year during which they were obtained, there are striking differences in survival of animals at the same temperature. The averages in experiments 21 through 51 (when mean daily temperatures were approximately 80°F.) at every temperature were greater than either those in experiments 1 through 10 or 11 through 20. Similarly, experiments 1 through 10 (performed between March 5 and 28 when mean daily temperatures were in the low fifties) gave the shortest survival times. The means of experiments 11 through 20 in general were intermediate, as were the mean daily temperatures. These results are of interest in the light of the well known fact that the thyroid is larger and more active in cool weather, and that basal metabolism is higher in winter and lower in summer. Below 11°C cold itself begins to be lethal under conditions of these experiments.

Since it seemed possible that the temperature regulating mechanisms of the adult might be rendered inoperative by anoxia as are all visible reflexes, a series of 34 experiments was performed to test the effects of temperature upon young adult animals (291.4 - 321.2 gms.). At the temperatures tested these animals lived approximately one half as long as the day old animals. Decreasing colonic temperatures was not quite as effective in prolonging anoxic survival as it was in newborn animals. A 10° decrease in temperature increased survival almost 50%, a 16° decrease increased survival 75%. These results are in accord with the recent report on adult rats by Blood and d'Amour (1949). They found that the highest incidence of recovery from anoxia produced by simulated high altitudes was in the group subjected to low temperature. Thus, we may conclude that in spite of the presence of well developed temperature regulating mechanisms, adult mammals subjected to severe anoxia behave like poikilothermic animals, and reduction of temperature may be expected to prolong life.

Cooling a conscious animal stimulates great motor activity which reduces the supply of compounds available for anærobic metabolism during a subsequent period of anoxia. To reduce this activity nembutal was given one half hour before testing. In a series of 20 neonatal animals the mean seconds survival of nembutal treated animals was longer for each 2° class than that of the untreated series. This suggests that cold may be especially effective in protecting the asphyxiated human infant that is often partially anesthetized in addition.

A similar series of experiments to test the effects of temperature on survival of day old rabbits which was started this summer has been showing even more striking results. Another animal in which cold is effective in prolonging life of the anoxic newborn is the rat (Adolph, Dec. 1948). Neonatal animals of this species tolerate a temperature of 10°C and at this temperature will survive two hours exposure to nitrogen.

Though still not quite complete, these experiments are being reported at this time because they suggest that the present treatment of warming the anoxic baby may explain in part the lack of improvement, during recent years, in mortality statistics of infants during the first day or two after birth.

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NOTE: Based on a report presented at the Marine Biological Laboratory.

A SIMPLE, NON-INJURIOUS METHOD FOR INDUCING REPEATED SPAWNING OF SEA URCHINS AND SAND-DOLLARS

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This note is for the purpose of acquainting investigators, using sea urchins or sand-dollars, with a simple rapid method of obtaining eggs and sperm repeatedly without injury to the animals or the gametes. It involves simply injecting isosmotic KCl into the animals.

Palmer (1937), at the suggestion of L. V. Heilbrunn, tested solutions of various salts and extracts of various tissues of Arbacia and other animals for their ability to cause the gonads of ripe Arbacia to shed their gametes. Her method consisted of making two slits in the peristome and introducing measured amounts of the solutions through one of these slits. She found that isosmotic solutions of KCl and $CaCl_2$ were effective. E. B. Harvey (1940) described a method of determining the sex of Arbacia that involved injecting a drop of sea water saturated with KCl into one of the gonopores by means of a fine hypodermic needle (No. 27). Eggs or sperm exude almost immediately from the injected gonopore but the shedding can be stopped at once by placing the animal in a jar of still sea water. So, males and females can be separated and be made available for later use.

I have, for the past 12 years, used a slight modification of the above methods for the purpose of inducing spawning in various echinoids; including Arbacia and Echinarachnius at Woods Hole and Strongylocentrotus (2 species), Lytechinus (2 species), Dendraster and Lovenia, on the West Coast. The method involves simply injecting isosmotic KCl with a hypodermic syringe into the body cavity. It is preferable that the needle be inserted into the lantern coelom since it may pick up eggs or sperm if it enters the gonad in the perivisceral coelom and then require washing before use on another animal. A single injection of 0.5 cc. of 0.5 M KCl into an average size Arbacia, of about 30 cc. volume will induce shedding of virtually all the ripe eggs or sperm. For larger or smaller animals the dose should be proportionately larger or smaller. The shedding starts within a few seconds and is completed in five to fifteen minutes. If only a few eggs or a small amount of sperm are desired, correspondingly smaller amounts of KCl should be injected. The same animals will then be available for further material at later times. The eggs can be collected readily by immersing the aboral surface of the animal in a dish of sea water. The sperm can be removed "dry" or by similar shedding in sea water.

Sand dollars are conveniently injected by inserting the needle through the mouth in a direction as nearly parallel as possible to the oral surface of the animal.

The gametes are not injured by this method of inducing spawning. Also, the animals can regenerate eggs and sperm after the treatment. I have, with Lytechinus and Strongylocentrotus, been able to obtain successive new batches of eggs at two-week intervals for four weeks during their breeding seasons, after initial forced shedding of practically all their ripe eggs (as determined by opening control animals) and feeding them on eel grass, kelp, mussels and other marine plants and animals.

This method of obtaining the gametes is less troublesome than that of opening the animals. Also the eggs do not need to be strained from gonadal tissue and very few, if any, unripe eggs are obtained. Unripe animals generally fail to respond. The injection is conveniently made with a 2 ml syringe and the needles should be 27 to 25 gauge and ½ to ¾ inch.

It has been shown by Oshima (1921), Harvey (1939) and Pequegnat (1948) that the test of sea-urchins contains material that is inhibitory to fertilization. This material is liberated by washing the animals in fresh water, by drying the surface or by other procedures that injure the delicate epithelial covering of the test. The gametes, however, are not injured by this agent. Even when the material is present in high concentration (as indicated by a reddish-yellow color in Arbacia), a single washing of the eggs removes it sufficiently to permit 100 per cent fertilization and normal development.

At laboratories, such as the Marine Biological Laboratory at Woods Hole, where it is sometimes difficult to provide the tremendous numbers of sea-urchins that are requested, and where slaughter of the animals might lead to a local depletion, this procedure would alleviate supply problems by permitting repeated use of the same animals and their return to the natural habitat at the end of the season.

During the current summer Arbacia has also shown itself to be capable of regenerating eggs and sperm and of yielding, after two weeks of feeding on sea weeds, about as many gametes as upon initial forced shedding. Smaller amounts

are obtained if the time interval is less than two weeks. One lot of Arbacia has furnished four successive batches of eggs in nearly original quantity in a period of six weeks, with most of the animals responding each time.

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BIOLOGICAL SPECIFICITY AND PROTEIN STRUCTURE*

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Studies on the relation between biological function and biological form are already so far advanced that it is universally conceded that all biological specificities belong to the world of ångstroms. There is no longer any doubt that the fundamental questions can be formulated, and subsequently studied and elucidated, only by recourse to the nature of atomic patterns and electron density distributions.

The extent to which biological specificity depends upon the minutiae of atomic architecture (*cp.* Wrinch, *Australian J. of Sci.* **8**:103. 1946) is well illustrated by the work of Zentmeyer (*Science* **100**:214. 1944) on the inhibition of growth of micro-organisms whose enzyme systems depend upon the presence of metals. In this work use is made of the power of 8-hydroxyquinoline to form chelate salts with a number of metals. It is known that this compound loses this power at lower pH's and it is elegantly shown that its power to inhibit growth is restricted in a corresponding manner.

A second example may be taken from the work of Rubbo, Albert and Maxwell (*Brit. J. Exp. Path.* **23**:69. 1942). It is found that the antiseptic action of the 2-,3-,4- and 5- mono amino acridines is not present in the 1-mono amino acridine. The reason? The fact that in this one alone of the 5 compounds, an internal NH...N hydrogen bridge makes the amino group inoperative.

A third example may be taken from the studies of Chen and others on the glycosides, bufagins and bufotoxins. Such compounds share a common steroid ring system with a lactone ring on C_{17}. It is recognized that the unsaturated lactone ring and the hydroxyl on C_{14} are essential features and it is now found that local stereochemical changes in the ring system have a remarkable influence on the pharmacological action of these substances. Only in this way can the fact that any digitalis-like action is missing in such compounds as allocymarin and allstrophanthidin be explained.

These examples show the kind of issues which present themselves in these specific biological activities: chelate rings, hydrogen bridges, delicate stereochemical variations. They also point the way to a conclusion which accumulating evidence makes more and more inescapable: the view that the ultimate repository of biological specificity lies in the native proteins. The nature of the atomic patterns of native proteins must today be regarded as the major unsolved problem of biology and medicine—a problem whose solution is the necessary preliminary to any deep and satisfying progress in such vast fields as the effects of radiation on living matter, virus diseases and the cancer problem.

While it seems necessary to emphasize the important fact that the atomic pattern of proteins is today unknown, equal emphasis should be given to the fact that there is a great store of factual information in other fields of work which is available to guide protein studies. For the native protein, unique in its functions and in the subtlety and precision of its architecture, carries on its surface and largely operates through end groupings which occur throughout organic and inorganic chemistry. Practically everyone of these has already been the subject of studies in various domains of structure chemistry and crystallography. The main issue throughout seems to be the matter of favorable environments for various atomic groupings and for the various foreign components, *e.g.* water, sugars and metal ions which play so important a role in protein specificity. It so happens that a great deal is known about the atomic environments favored by many ions of the first importance in biological processes. What are the studies of the mica and clay groups of the silicates but a strikingly complete and vivid picture of the requirements of many such types? Isomorphous substitutions in the micas may well prove a valuable model for studies on the interchangeability (or the lack of it) of ions in systems involving enzyme systems. Permeability of cell membranes, primarily made up of native proteins in orderly network associations, attains new status, as a concept, when the data available are studied in close relation to knowledge of such framework structures as the ultramarines and noselite, with their variable ionic populations. Well understood today

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are the lithium and thallium ultramarines with a warm violet hue, the nearly colorless calcium- and zinc-containing structures, the silver variants, which are yellow or grey, the blood-red selenium-containing and the yellow tellurium-containing ultramarines (*cp.* Bragg's "Atomic Structure of Minerals"). In all these cases, the framework is the common background. In a similar manner, we may see the protein framework as the unifying theme in all the physiological situations which we have in mind.

With this viewpoint, the oligodynamic phenomena, so long studied at Woods Hole, fall into place. Perhaps the most striking case is that of the pining disease of sheep which for a century or more plagued farmers in Scotland and New Zealand. Extensive investigations finally uncovered the cause—a cobalt deficiency in the soil—and the simple cure, a result of great interest in connection with the new vitamin of the *B* group. The amount of cobalt responsible for the difference between a healthy and an unhealthy soil was found to be so small as to defy spectroscopic analysis. How then were such minute amounts detected? The answer is interesting: they were detected by the use of organic reagents, such as o-nitrophenol, capable of taking up cobalt ions. The balance to be maintained for health proved to be very delicate with a slight excess causing the sheep to contract polycythemia. But how can such minute amounts of cobalt be visualized as playing so vital a role? Evidently in terms of the concept of proteins having highly specific cavities or nests on their surfaces, which for stability must be suitably tenanted by a very few specific foreign components including vitamins and metal ions (*cp.* Glaser, Am. Sci. **33**:175. 1945).

For a fuller understanding of the nature of such active patches on native protein surfaces, at which key reactions are localized, we may turn to studies of molecular crystals which abound with examples of specific associations (*cp.* Wrinch, Wallerstein Comm. **11**:175. 1948). I would particularly stress the symmetry elements present in many such situations. It seems inevitable that the associations of the protein megamolecules should require what physiologists (*cp.* Findlay *et al. Biochem. J.* **36**:1. 1942) have called multipoint groupings. But multipoint groupings in association must in general depend upon a series of coincidences, since favorable environments for each of the atoms involve definite conditions. When a symmetry element is present, multipoint groupings are automatically permitted.

To understand biological specificity, not only problems of enzyme action but also problems of morphology must be elucidated by following them down through microscopic and sub-microscopic levels to the Ångstrom world. Here the work of many pioneers will finally attain its greatest fruitfulness. We may particularly bear in mind the work of Moll whose concepts are the forerunners of modern ideas on morphogenetic fields and Frey-Wyssling whose early use of crystallographic concepts in morphology opened new vistas. In the center of the picture today stand studies on the relations of symmetry in the developing embryo (*cp.* Harrison, *Trans. Conn. Ac. Arts and Sci.* **36**:277. 1945), and much work on polarity in general (*cp.* the invaluable review of botanical literature by Block, *Bot. Rev.* **9**:261. 1945). The major task in the next decades is the unfolding of the stages by which atomic patterns in biological materials, particularly the native proteins, determine both the gross morphology and the functions of living matter. Only when the atomic patterns of native proteins are known and this sequence has been established step-by-step will the nature of biological specificity be truly understood.

MOTION PICTURES SHOWING THE REACTIONS OF CELLS IN FROG TADPOLES TO IMPLANTS OF TANTALUM

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In recent years the metal tantalum has been increasingly used in various surgical procedures. It is regarded as being especially "kind to tissues." It provokes a minimum of inflammatory response. Surgeons have used it in the form of plates, wire, foil, gauze, and powder. Tiny tantalum bolts are used in the technique for the observation of living cells in the mouse (G. Algire).

Implants of tantalum in the form of both powder and fine caliber wire have been watched for long periods. The same individual implants have been observed and the day-to-day changes recorded by cine-photomicrography. Cellular movements are revealed best by pictures taken at low speeds.

In the implants of tantalum powder the chief features of interest are the early response of leukocytes, endothelial cells of lymph vessels and to a less extent those of blood vessels, fibroblasts

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and epithelium. Often there are developed outgrowths of the skin at or near the site of the implant. Such cutaneous papillæ may include a fair portion of the implanted powder. The papillæ become separated from the animal by pinching off at the base after a week or two.

A feature of cell behavior is the transportation of tantalum powder by macrophages. Tantalum-laden macrophages may enter either lymph vessels or blood vessels and thus be carried away from the site of the implant. Such activity may start as early as the second day and continue for many weeks. Other tantalum-laden macrophages, however, remain at the implant site for long periods (the motion pictures record this for as long as 84 days). Such cells exhibit continual slow movements and changes in their positions.

Frequently a process of encapsulation takes place. After about 12 days in some implants a closely packed group of tantalum-laden macrophages becomes encased by a definite capsule. In later stages the surrounding capsular substance may develop a fibrous appearance. Movements of the encapsulated macrophages are reduced to a minimum. Such capsules may persist indefinitely with little further change.

Implanted tantalum wire (of about 50 micra caliber) is surrounded within a few hours by a thin layer of leukocytes. This layer effectively isolates it from the adjacent tissues. There is very little disturbance of closely situated nerve fibres or other tissues. Short lengths of tantalum wire, thus walled off, persist indefinitely. Wherever the wire implant presents rough or jagged edges the ensheathing leukocyte shell becomes somewhat thicker.

NOTE: Based on a paper presented at the Marine Biological Laboratory.

GROWTH AND METAMORPHOSIS OF THE PLUTEUS OF *ARBACIA PUNCTULATA*

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The name *Pluteus paradoxus* was given by Johannes Müller in 1846 to what he thought was a new animal found in the North Sea near Helgoland, because it resembled an easel (*pluteus*) when drawn, as he drew it, upside down. In the same year he discovered his mistake that it was really the larval form of an Ophiurid, later identified as *Ophiura albida*.

The pluteus of *Arbacia punctulata* which is known to most of the investigators at the M.B.L. is the early pluteus of two or three days. This has four arms, two long anal arms and two short oral arms and bright red pigment spots. The later stages in its development are practically unknown to the investigators at Woods Hole. The reason for this is simple. For the later stages, it is necessary to feed the plutei. The best food has been found to be the diatom, *Nitzschia closterium*, and this must be cultured on Miquel's solution, a combination of a number of salts (Allen and Nelson 1910). Many years ago, in 1882, W. K. Brooks and two of his students, Garman and Colton (1883), raised the plutei of *Arbacia punctulata* at Beaufort, North Carolina, without any special feeding. The sea water there is rich in diatoms, and the plutei can probably obtain what they require for growth from the sea water, but this is apparently not the case at Woods Hole. This work was published in Brooks' "Handbook of Invertebrate Zoology."

Since the photographs shown at the Seminar cannot be given in the present article, a brief description of the development must suffice. In about ten days after fertilization, a new pair of arms, with red tips, grows out toward the base of the pluteus, and in about three weeks, another pair of arms, without red tips, grows out between the red tipped ones and the original pair of long anal arms. The animal now swims about by means of its cilia and tumbles about on its arms. All the arms grow much longer, and the body of the adult Arbacia is apparent as a yellowish green mass inside the pluteus. By six weeks, the pluteus has become quite complicated, with two pairs of additional short (oral) arms and two pairs of tubular processes (auricles), one pair dorsal and one ventral. Soon afterwards the five primitive ambulacral feet, with suckers at their ends, appear at one side of the body. These are continually contracted and expanded. In about two and a half months after fertilization, three flattened plates appear between each two ambulacral feet; these are the primitive spines. The pluteus has now reached its full development, and the arms their maximal length, about 1.6 mm. Now, or sometimes before this, the arms begin to go to pieces; the flesh peels off, leaving the bare skeleton. Several arms may be cast off together like a shell. The pluteus is metamorphosing into the adult *Arbacia*, the greenish

mass becoming larger, all the arms being gradually lost. The animal measures about a half millimeter in diameter. These last stages take place rapidly; the whole process of growth and metamorphosis took over four months in my cultures (July 12 to November 17). The animals now require another diet, a protozoon, Trichospherium, or a red alga, Corallina, these providing the calcareous matter needed for the development of the test and spines. This food was not available to me, and the four small *Arbacia* left in my cultures died. The further development has, however, been described by Miss Gordon (1929), with especial reference to the test.

The pluteus from the centrifuged egg develops in just the same way. The pigment spots which are at first unevenly distributed, become evenly distributed after three or four days, so that one cannot distinguish between the pluteus from a centrifuged egg and that from a normal egg. If the plutei are fed Nitzschia, they acquire the extra arms, continuing to develop like normal plutei.

The pluteus from the white half-egg, obtained by centrifuging, is at first colorless and smaller than that from the whole egg. It acquires the pigment spots in three or four days, and if fed, develops the first pair of new arms in about ten days. These are as heavily pigmented at the tips as in the normal pluteus; the animals are also of the same size at the same stages. The second pair of new arms (unpigmented at the tips) develops at the normal time. Owing to lack of material, these were carried no further, but presumably, since they are just like the normal, they would develop into normal Arbacia. Even normal plutei are difficult to raise; out of thousands of plutei fed from an early stage, comparatively few were alive after two months and only four survived through metamorphosis.

The complete paper is to be published in the *Biological Bulletin* for December, 1949.

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Note: Based on a paper presented at the Marine Biological Laboratory.

REVERSIBLE ENZYMIC REDUCTION OF RETINENE TO VITAMIN A

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An adequate intake of vitamin *A* is necessary for vision in dim light. Vitamin *A* deficiency results in a failure in the synthesis of visual pigments. The visual pigment of retinal rod cells, called visual purple or rhodopsin, bleaches in the light with the release of a yellow carotenoid named retinene by Wald. Retinene has been identified as vitamin *A* aldehyde by Morton.

If a freshly-excised retina is allowed to stand for an hour after bleaching, the vitamin *A* aldehyde is reduced to vitamin *A* independently of light. The speaker has shown (*Biological Bulletin*, 1946) that an enzymic process in fresh solutions of bleached visual purple likewise forms vitamin *A*. Morton's group has recently shown that vitamin *A* is also formed when synthetic retinene (vitamin *A* aldehyde) is fed or injected into rats. Since vitamin *A* is an alcohol, and can be dehydrogenated by the intact retina to a typical aldehyde, it seemed possible that the enzyme involved might be the well-known reversible *DPN*—specific alcohol dehydrogenase.

This hypothesis was tested with acetone and ammonium sulfate precipitates of rabbit liver prepared according to Lutwak-Mann, and showing high ethyl alcohol dehydrogenase activity. Since the equilibrium of the dehydrogenation is far toward the alcohol side, it is customary to drive the reaction toward the aldehyde side by removing the aldehyde as fast as it is formed. This may be accomplished by various substances which combine with the carbonyl group of the aldehyde, *e.g.*, cyanide and bisulfite.

In the present experiments crystalline vitamin *A*, dissolved with a detergent, Tween 80, was the substrate and coenzyme *I*, the hydrogen acceptor. At the end of the reaction the aldehyde formed was released by dilution or alkaline destruction of the addition compound (absorption maximum *ca.* 330 mu) and extracted with petroleum ether.

Experiments to date have shown up to 40% conversion to the aldehyde. Complete reversibility of the dehydrogenation was easily accomplished in the presence of enzyme and reduced co-enzyme.

The reversibility of the retinal dehydrogenase has likewise been tested in this laboratory. Wald has reported that retinene in retinal rods and extracts of whole retinas is irreversibly reduced by the retinene reductase of the rods in the presence of reduced coenzyme *I*. We have confirmed the activity of isolated rods. However, the reductase activity of extracts of whole retinas appears to be an artefact due to the large amount of reductase in the non-visual portion of the retina. Furthermore, we have found that vitamin *A* formation by isolated rods is freely reversible in the presence of cyanide.

We therefore need no longer assume a closed visual cycle to explain the formation of retinene from vitamin *A*. Instead, it is probable that the dehydrogenation is accomplished by alcohol dehydrogenase with the formation of visual purple which acts as the physiological trapping compound for retinene.

NOTE: Based on a paper presented at the Marine Biological Laboratory.

SOME EFFECTS OF ULTRA-VIOLET LIGHT ON THE CATALASE ACTIVITY AND ON PHOTOSYNTHESIS OF *CHLORELLA PYRENOIDOSA*

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It had been shown by Arnold (1) that ultra-violet light ($\lambda = 2537$ Å) inhibits the light reaction of photosynthesis. The percentage inhibition was observed to be the same in flashing light and in continuous light; thus the effect is similar to the one produced by narcotics.

In an attempt to obtain an action spectrum of the inhibition of photosynthesis by ultra-violet light, some observations were made to elucidate the mechanism of the inactivation process.

On the assumption that ultra-violet light could have inactivated the oxygen-liberating catalyst in photosynthesis, the catalase activity of Chlorella cells was tested before and after irradiation with ultra-violet light. In each case an increase in the catalase activity was found (Table I), a

TABLE I

Measurements were performed with .026 cm^3 of Chlorella cells suspended in 3 ml. of .035 M $KHCO_3$ and .065 M $NaHCO_3$ at 25°C.
Exposures to ultra-violet light were made using a quartz vessel, the gaseous phase was either air or nitrogen.

Time of exposure to ultra-violet light. Incident intensity 1.3 ergs/mm^2. sec.	Photosynthesis in % of control	Rate constants of decomposition of H_2H_2 by 1 cm^3 of Chlorella cells. (Sec^{-1})
0 minutes	100	1.3
3 ''	80	1.5
6 ''	50	2.5
11 ''	0	9.8

phenomenon which had been observed by Euler (2) in irradiated yeast. No catalase was released by the cells as the suspending fluid showed no activity after the cells had been centrifuged off.

At the present time we are not able to state if this enhanced catalase activity is due to an increased permeability of the cells to hydrogen peroxide (3), or to a photochemical activation of the catalase system (4), or due to the release of enzyme molecules from a structural configuration in which they were either inactive or only very slightly active (5).

According to Arnold the inactivation of photosynthesis by ultra-violet light follows a first order reaction. When we attempted to duplicate these results using a General Electric resonance lamp we found that the effect of this ultra-violet light (emission at 2537 Å rated at 95% +) was more effective when a definite amount of radiation of constant energy flux was given in one dose, than when given in several doses interspersed by dark periods or exposures to visible light. Thus the inactivation process appears to be complex, and not always of the first order.

Arnold had observed that the absorption spectrum of chlorophyll does not change after irradiation of Chlorella suspensions by ultra-violet light. However, it was noticed that in intact cells the transmission of the red chlorophyll peak increased after the ultra-violet light treated cells were exposed to visible light. This bleaching of chlorophyll is a function of the intensity and of the time of exposure to visible light. In some way the energy transferring mechanism in photosynthesis has become uncoupled and the light energy, directly, or through some photoperoxide, produces the bleaching of chlorophyll.

A test was made to determine if hydrogen peroxide would give results similar to those produced by ultra-violet light, but in agreement with Gaffron (6) it was found that inhibition of photosynthesis with hydrogen peroxide (10^{-4} to 10^{-5} moles per liter) was only observed at high light intensities and not at low light intensities, and thus hydrogen peroxide affects one or more of the dark reactions of photosynthesis in contrast to the inactivation of the light reaction by ultra-violet light.

Thus, to summarize, it can be stated that an amount of ultra-violet radiation which only imperceptibly affects the respiratory rate of Chlorella cells will not only decrease the photosyn-

thetic ability of these cells, but will also sensitize these cells so that bleaching of chlorophyll will occur subsequently in visible light. It also will cause, directly or indirectly, an acceleration of the catalase activity of intact Chlorella cells.

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SYNTHESIS OF ACETYLCHOLINE

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The ionic concentration gradients which exist between the inside of the nerve fiber and its outer environment have long been supposed to be the source of the electromotive force for the electric currents which propagate the nerve impulse. It has been postulated that during the passage of the impulse the resistance of the active membrane surrounding the axon breaks down. Due to the increased permeability, the ions can move freely and the concentration gradients may become effective. Recent investigations with radioactive sodium and potassium ions carried out in Cambridge, England, and in our laboratory, have shown that there are continuous ion movements across the axonal membrane even in rest. During activity the influx of sodium ions into the interior and the leakage of potassium ions to the outside are greatly accelerated. This could be expected from the respective concentration gradients of these two ion species in case of increased permeability. Some change in the active membrane must then obviously occur which is responsible for the rapid ion movement during the passage of the impulse and this change must be quickly reversible, since the resting condition is restored in a period of time less than one millisecond. A quickly reversible chemical process producing the change in the active membrane appears to be the most likely assumption for the interpretation of the mechanism underlying conduction.

During the last 12 years a great variety of facts have accumulated supporting the assumption that the release and removal of acetylcholine are essential events in the neuronal surface membrane and inseparably associated with the electrical manifestations during the conduction of the nerve impulse. The essential results have been recently reviewed and need not be discussed here (1, 2). For the understanding of the precise role of the ester, it appeared necessary to integrate the breakdown and the formation of the ester into the metabolism of the nerve cell and to correlate it with the electric currents. The extremely small amount of energy involved in conduction — the initial heat is of the order of magnitude of 1×10^{-8} gcal per gram nerve per impulse—offered a great obstacle to the correlation of electrical and chemical events. The difficulty was overcome by the use of electric organs, where by the special arrangement of a great number of cellular units in series chemical and electrical events occur on a scale which is within the range of measurement. In investigations using this material a whole chain of chemical reactions has been established and associated with the action potential. It was found that the energy released by the breakdown of phosphocreatine, a compound rich in energy, accounts for the total electrical energy released during the action potential (3). It is known from muscular physiology that the breakdown of adenosinetriphosphate (*ATP*) precedes that of phosphocreatine. The same sequence of reactions was assumed to occur in the conductive membrane. On the basis of the work of Meyerhof and Lohmann, Engelhardt and Lubimova, Needhams and their associates, Szent-Györgyi and his associates, it is today generally believed that in muscle *ATP* reacts directly with protein, this reaction being the primary process of contraction. However, it is difficult to conceive, for many reasons, that the reaction of *ATP* with the proteins or lipoproteins of the nerve membrane is the primary process in conduction. It was assumed that the release and breakdown of acetylcholine precedes that of *ATP* and that in the conductive membrane the breakdown of *ATP* is the primary *recovery* process yielding the energy for the synthesis of acetylcholine.

In accordance with this hypothesis an enzyme, choline acetylase, was discovered, which forms acetylcholine using the energy of *ATP* (4). The enzymatic system is rather complex but has been reconstructed in vitro during the last few years (5, 6). The enzyme requires for full activity, in addition to *ATP* and the substrates, acetate and choline, adequate amounts of a coenzyme, cys-

teine, and the following ions: potassium, calcium and magnesium. An inhibitor of acetylcholine esterase is always added to inactivate this enzyme completely, since a small fraction may be still present even if most of it is inactivated or removed.

By fractional ammonium sulfate precipitation a highly active and concentrated solution of choline acetylase has been recently obtained from acetone dried powder of rabbit brains (7). The fraction between 16 and 35 per cent ammonium sulfate was found to contain most of the enzyme protein. The yield of acetylcholine obtained with this enzyme solution was equivalent to about 2 micromoles of acetylcholine per ml. (350-400 μg), as tested with the usual bio-assay technique. The amount of acetylcholine formed per gram protein per hour was close to 200 milligrams.

It is remarkable that, in spite of the great physiological importance of acetylcholine, no chemical methods of determining the ester were available except after isolation with very tedious, time-consuming procedures. By necessity investigators were compelled to use bioassays of questionable specificity. Recently a very rapid and simple chemical method has been introduced by Hestrin (8). It is based upon the reaction of acetylcholine with hydroxylamine in alkaline medium. If the mixture is subsequently acidified and ferric chloride added, a brown-purple color develops. With the Beckman spectrophotometer less than 1/10 of a micromole of acetylcholine per ml. may be easily determined. The underlying process is a reaction of the acyl group with hydroxylamine forming stoichiometrically hydroxamic acid. The determination of acetylcholine may therefore be performed in excess of acetate and choline. It may be noted that the method may be applied to other short chain O-acyl derivatives and its usefulness may therefore be extended—if properly adapted—to other compounds containing O-acyl groups.

When the method was applied to the highly active enzyme preparation described it was found, surprisingly, that less than 50 per cent of the activity obtained in the bioassay can be accounted for by the chemical method. More than 50 per cent of the effect obtained must be attributed to a substance which appears to have the same biological activity as acetylcholine, but may be distinguished by chemical procedures. Acetylcholine added at the beginning or the end of the incubation was analytically recovered by both chemical procedure and bioassay. The substance is synthesized in the reaction mixture in the absence of added choline. In contrast, acetylcholine is not found to be formed by the chemical method if choline is omitted in the reaction mixture. In presence of added choline the synthesis of the compound occurs at a higher rate than in its absence. In the absence of acetate neither acetylcholine nor the compound are formed. Propionate and butyrate, but not formate and valerate may substitute acetate. The pharmacological properties of the enzymatically formed compound have been tested by Middleton and Middleton (9). These investigators found that the compound decreases the arterial blood pressure of cats and the amplitude of the isolated frog heart in the same way as acetylcholine. Atropine regularly suppressed both actions in the same concentrations as it suppressed the action of acetylcholine.

The chemical method of acetylcholine determination may be used for measuring acetylcholine esterase activity. The simplicity and rapidity of the technique make it most suitable for clinical investigations. Compared with the widely used manometric method it has the advantage that it may be readily applied over a wide range of pH. Using the colorimetric method with a highly purified acetylcholine esterase obtained from electric tissue Hestrin (10) was able to demonstrate an equilibrium of the following formula:

$$\frac{[\text{acetylcholine}^+]\ [\text{water}]}{[\text{acetic acid}]\ [\text{choline}^+]} = K$$

A considerable synthesis of acetylcholine is obtained in this system at pH 5.0. With the variation of pH K remains constant, thus confirming the assumption that acetic acid rather than acetate ion is the reactant species. From the data obtained the free energy of acetylcholine hydrolysis was calculated to be approximately —3100 gcals.

This figure is of considerable physiological interest. It has been recently shown that in frog sciatic nerves exposed to di-isopropyl fluorophosphate (*DFP*)—a potent inhibitor of cholinesterase—more than 90 per cent of the enzyme may be inactivated without affecting conduction. The remaining enzyme activity, however, is essential for conduction, which by further decrease is impaired and finally abolished. The minimum activity corresponds to 400-500 μg. of acetylcholine split per gram nerve per hour. Since the initial heat developed per gram per impulse is about 1×10^{-8} gcal., at most 0.0006 μg of acetylcholine could be split per gm. nerve per impulse. This figure is obtained on the basis of the free energy of acetylcholine hydrolysis if the initial heat is attributed exclusively to acetylcholine hydrolysis—a most unlikely assumption. It appears more probable that the release and removal of acetylcholine act as a trigger in a chain of reactions. On the assumption that one third of the initial heat may be ascribed to acetylcho-

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Lajtha, A., res. asst. Inst. of Muscle Research. Nucleic acids in muscular contraction.
Lansing, A. I., assoc. prof. anat. Washington Med. Calcium binding by cell surfaces.
Lazarow, A., assoc, prof. anat. Western Reserve. Experimental diabetes; cyto-chemistry of nerve tissue.
Lee, Lois E., asst. zool. Southwest Missouri State.
LeFevre, P. G., asst. prof. phys. Vermont Med. Complications of excitation theory in Squid axon.
Leikind, M. C., head biol. & med. unit, Lib. of Congress. Guide to the literature of the history of science.
Leonard, L., Lab. asst. chem. Haverford. Ion antagonism.
Levy, M., assoc. prof. biochem. New York Med. Quantitative study of denaturation of proteins.
Lillie, R. S., emerit. prof. phys. Chicago. Physiology of activation processes in marine eggs.
Ling, G. N., fel. phys. Chicago.
Litt, M., Rochester Med. School. Nature of bioelectric potentials of squid nerve.
Lochhead, J. H., asst. prof. zool. Vermont. Sources of calcium for newly molted Crustacea.
Loeffler, R., grad. asst. bot. Wisconsin. Study of flower primordia in commercial orchids.
Loewi, O., res. prof. phys. New York Med. Consultant in physiology; library reader.
Loud, A. V., grad. biophysics M. I. T. Ultra-structure of axoplasm.
Love, Lois, instr. phys. Pennsylvania. Physiology of erythrocytes.
Love, W. E., asst. instr. physiol. Pennsylvania. Physiology of erythrocytes.
Lovelace, Roberta, adjunct. prof. biol. South Carolina. Fertilization and cleavage.
Lucké, B., prof. path. Pennsylvania Med. Cell permeability; tumors in amphibia.
Luyet, B., prof. biophysics St. Louis. Life and death at low temperatures.
Lynn, F., res. asst. Stanford. Oxidotion-reduction studies.
Lynn, W. G., prof. biol. Catholic. Thyroid function in cold blooded vertebrates.
Malan, Martha, invest. genetics, Pennsylvania. Genetic studies on Mormoniella.
Marmont, G. H., asst. prof. phys. Chicago. Nature of nerve condition in Squid.
Marshak, A., res. assoc. biochem. New York Med. Nucleic acid metabolism of Arbacia eggs.
Marsland, D., prof. biol. New York. Temperature-pressure effects on fert. and cleavage.
Matoltsy, A., res. asst. Inst. Muscle Research. Histology and physiology of muscular contraction.
Matzke, E., prof. bot. Columbia.
Mavor, J. W., emerit. prof. biol. Union. Textbooks in general biology; invertebrate morphology.
Mazia, D., prof. zool. Missouri. Specificity of desoxyribonucleic acid.
McCay, P. B., grad. asst. zool. Oklahoma. Effect of bacterial toxins on cell respiration.

McColl, J. D., res. fel. biochem. Western Ontario Med. Comparative biochemistry of Myelin.
McCulloch, D., fel. embryology M.I.T. Techniques in connection with mitosis problem.
McDonald, Sister Elizabeth, prof. biol. Mt. St. Joseph-on-the-Ohio. Comp. biochem. body fluids of marine inverts.
McIntyre, Patricia, Johns Hopkins Med. Enzyme systems of Arbacia.
McKeehan, M. S., asst. zool. Chicago. Cytological phases of embryonic induction.
McLean, D., instr. phys. Vassar.
Menkin, V., assoc. prof. exp. path. Temple. Cytological work on Arbacia eggs.
Metz, C. B., asst. prof. zool. Yale. Role of specific substances in fertilization.
Meyerhof, O., res. prof. biochem. Pennsylvania.
Mihalyi, E., res. asst. phys, Inst. of Muscle Res. Electrochemistry of myosin and actin.
Miller, Faith, res. asst. Emory. Temperature effects on anoxic newborn guinea pigs.
Miller, J. A., assoc. prof. anat. Emory. Microinjection studies on Tubularia.
Miller, T. D., grad. bact. Amherst. Radiation induced mutations in bacteria.
Milne, L. J., assoc. prof. zool. New Hampshire. Visual physiology of invertebrates.
Mitchell, Constance J., instr. biol. Delaware. Developmental studies on Habrobracon.
Mitchell, R., grad. res. worker, Columbia. Biochemistry.
Moore, G. M., chrm. zool. New Hampshire. Structure and natural history of Nudibranchs.
Moos, C., res. asst. neur. M.I.T. Nature of bioelectric potentials of squid nerve.
Morrison, D., Flight Safety Foundation. Psychology.
Moskovic, S., fel. phys. New York. Temperature pressure effects on fert. and cleavage.
Moul, E., asst. prof. bot. Rutgers. Botany instruction; fresh water and marine algae.
Moulton, J. M., fel. biol. Harvard. Embryology of *Menidia* and *Fundulus*.
Mudd, H., student Harvard Med. Pigments in Homarus blood.
Musacchia, X. J., instr. biol. St. Louis. Role of lipids in animals.
Nachmansohn, D., asst. prof. neurology, Columbia. Chemical mechanism of nervous function.
Nadeau, L. V., grad. stud. phys. Dominican House of Studies (Ill.).
Nelson, L, instr. phys. Nebraska. Physiology of sea urchin sperm.
Neurath, H., prof., physical biochem. Duke. Crystalline proteolytic enzymes of the pancreas.
Noland, J. L., fel. biochem. Wisconsin. Determination of amino acids in invertebrate's blood.
O'Brien, J. A., Jr., asst. prof. biol. Catholic. Plastid development in germinating grains.
O'Brien, J. P., asst. prof. zool. Marquette. Biological effects of X-radiation.
O'Malley, B., grad. zool. Fordham. Growth factors in the protozoa.
Orsi, E. V., fel. Cancer Inst. Fordham. "Butter-yellow" and develop. of Fundulus and Arbacia.
Orski, Barbara M., Harvard Med. Rate of respiration of protozoa by cartesian diver.
Osterhout, W. J. V., emerit. phys. Rockefeller Inst. Behavior of marine eggs and algae.
Padykula, Helen, instr. zool. Wellesley. Formation of shell pigment by land gastropods.
Palay, S. L., instr. anat. Yale Univ. Sch. Med. Physiological aspects of neurosecretion.
Parmenter, C., prof. zool. Pennsylvania. Chromosomes in frog eggs.
Parpart, A. K., chrm. biol. Princeton. Permeability properties of cells.
Parshley, H. M., chrm. zool. Smith. Comparison of north and south New England Hemiptera.
Perkins, J. F., asst. prof. phys. Chicago. Temperature regulation in smooth muscle.
Pfister, R. R., asst. zool. Columbia Med.
Pick, J., assoc. prof. anat. New York Med. Anatomy, physiology of autonomic nervous system.
Pierce, Madelene, assoc. prof. zool. Vassar. Ecological survey of Rand Harbor, Megansett.
Plough, H. H., prof. biol. Amherst. Radiation induced mutations in bacteria.
Plummer, Jewel, fel. biol. New York. Arbacia egg antimitotic studies.
Proctor, N. K., grad. phys. Pennsylvania. Effects of various reagents on arthropod muscle.
Prosser, C. L., prof. zool. Illinois. Comparative physiology of muscle.
Provasoli, L., chrm. biol. St. Francis. Isolation and culture of flagellates.
Quastel, J. H., prof. biochem. McGill.
Rawley, June instr. zool. Kent State. Effect of bacterial toxins on cell respiration.
Reichart, Ruth, asst. biochem. Radcliffe.
Reid, W. M., chrm. biol. dept. Monmouth. Physiology of marine nemerteans, tapeworm parasites
Reiner, J. M., res. assoc. Tufts Med. Intermediates of peptide bond synthesis in egg.
Renn, C. E., assoc. prof. sanitary eng. Johns Hopkins. Toxicity of industrial wastes.
Rice, Mary, res. asst. phys. Oberlin. Eneuronimental effects on mineral composition.
Rieser, P., res. asst. phys. Pennsylvania. Mierurgical problems in cell physiology.
Root, R. W., assoc. prof. biol. City of New York. Protoplasmic ultra-structure.
Rose, S. M., assoc. prof. zool. Smith. Cellular transformations during regeneration of limbs.
Rosenbluth, Raja, res. asst. Rockefeller Inst.
Rosenthal, T., assoc. anat. zool. Washington Med.
Rossi, H. H., biophysics, Columbia. Biological effects of alpha radiation.
Roth, J. S., asst. prof. biochem. Rutgers. Uptake of radioactive phosphate by Tetrahymena geleii.
Rothenberg, M. A., res. asst. Columbia. Chemistry of nerve transmission.
Roy, S. C., lect. Calcutta. Muscular contraction.
Roys, C., grad. zool. Iowa. Insect physiology, senses.
Rudenberg, F. H., A.E.C. fel. phys. Harvard. Uptake and localization of Ca-45 in Arbacia eggs.
Rugh, R., assoc. prof. radiology, Columbia. Effects of Beta and X-rays on the embryo.
Saltz, M., med. student, Amherst. Radiation induced mutations in bacteria.
Sandeen, Muriel I., asst. zool. Northwestern. Comparative physiology of crustacea.
Sarkar, N. K., lect. chem. Calcutta. Muscle contraction.
Schaeffer, A. A., prof. biol. Temple. Leucocytes of rabbits.
Schallek, W. B., asst. prof. biol. Oregon. Glycogen in nerves of invertebrates.
Schmitt, F. O., head biol. dept. M. I. T. Nerve structure in squid axon.
Schmitt, O. H., prof. zool. biophysics, Minnesota. Nerve electrophysiology.
Scholander, P. F., res. assoc. Swarthmore. Heat regulation in arctic and tropical homo sapiens.
Schreibman, I., instr. phys. Pennsylvania. Physiology of cell division.
Schufer, Evelyn, grad. biol. Bryn Mawr. Embryology.
Scott, A. C., assoc. prof. biol. Union. Cytology of Anoxia in early development of marine eggs.

Scott, Sister Florence Marie, prof. biol. Seton Hill. Embryology of Amarocecium constellatum.
Scott, G. T., assoc. prof. zool. Oberlin. Environmental effects on mineral composition.
Seaman, G. R., fel. phys. Fordham. Enzyme systems in Protozoa.
Seki, S. Louise, grad. asst. phys. Mt. Holyoke. Effect of nitrous oxide on Arbacia eggs.
Shanes, A. M., assoc. prof. phys. & biophys. Georgetown Med. Potassium transport in invertebrate nerve.
Shwartzman, G., bacteriologist, Mt. Sinai Hosp.
Sheng, T. C., grad. zool. Columbia. Neurospora genetics.
Sichel, F. J. M., prof. phys. Vermont Med. Conduction in voluntary and cardiac muscle.
Slattery, L. F., electronic tech. Chicago. Nerve function in squid.
Slifer, Eleanor, asst. prof. zool. Iowa. Cytology of wax secretion—grasshopper eggs.
Speidel, C. C., prof. anat. Virginia. Cellular behaviour *in vivo*; cine-photomicrography.
Stein, O. L., grad. asst. Minnesota. Pollen ontogeny.
Steinbach, H. B., prof. zool. Minnesota. Distribution of enzymes in muscle and nerve tissue.
Stieglitz, Alice A., grad. phys. Pennsylvania. General physiology.
Stokey, Alma G., emerit. prof. bot. Mt. Holyoke. Gametophyte of homosporous ferns.
Stoudt, H. N., asst. prof. biol. & plant morph. Temple. Vegetative propagation in Oxalic ortgiesi.
Stout, Carolyn M., res. asst. Pennsylvania. Permeability of red blood cells.
Straus, W. L., assoc. prof. anat. Johns Hopkins. Somites and lateral plate contribution to body wall.
Atrittmatter, C. F., Harvard. Particulate systems of Arbacia eggs.
Stunkard, H. W., prof. biol. New York. Biology and life history of parasitic worms.
Suckling, E. E., instr. phys. Long Island Med. Single fiber recording of nerve impulses.
Sulkin, S. E., prof. bacteriology, Southwestern Med. Studies on the epidemiology of virus encephalitides.
Sutro, P. J., grad. phys. Harvard. Reading on problems in vision.
Szent-Gyorgyi, A., res. asst. Inst. of Muscle Res. Ionic influence on the contractile proteins.
Talpey, W. B., Washington Med. Calcium binding by cell surfaces.
Tannenbaum, S., grad. biol. Columbia.
Taylor, L. S., chief biophysics A.E.C. Nat. Bureau Standards. High energy radiation measurement.
Taylor, W. R., prof. bot. Michigan. Woods Hole and Bermuda marine algae.
Terry, R., asst. prof. biol. Union. Physiology of Arbacia eggs.
TeWinkel, Lois E., assoc. zool. Smith. Development of muscle in dog fish embryos.
Therman, P. O., physician, Inst. of Pa. Hospital. Properties of motor-nerve fibers.
Thomson, Betty F., asst. prof. bot. Connecticut Col.
Tietze, F., res. fel. biochem. Northwestern. Chromatography of hemocyanin.
Ting, T., res. assoc. biophys. Amherst. Studies on calcium uptake by Arbacia eggs.
Tracy, H., prof. anat. Kansas. Development of neural mechanisms in *Opsanus tau.*
Trinkaus, J. P., instr. zool. Yale. Mechanism of gastrulation in teleosts.
Truant, A. P., asst. prof. pharm. George Washington. Distribution of procaine in squid axon.
Tyler, A., assoc. prof. embr. Calif. Inst. Tech. Physiology of fertilization.
Varga, L., res. asst. phys. Inst. for Muscle Res. Thermodynamics of muscular contraction.
Vincent, W. S., A.E.C. fel. biol. Pennsylvania. Cytochemistry of nucleoli.
Vinson, C. A., grad. asst. zool. North Carolina. Cold treatment of fertilized eggs of *Nereis limbata.*
Vogel, M. L., res. asst. bact. Amherst. Bio-chemical varieties in *Salmonella typhimurium.*
Wainio, W. W., assoc. res. specialist, Rutgers.
Wald, G., prof. biol. Harvard. Chemistry, physiology of light reactions of organisms.
Walters, C. Patricia, res. asst. Lilly Res. Labs. Particulate systems of Arbacia eggs.
Warner, R. C., asst. prof. chem. New York Med. Rate of denaturation of proteins.
Webb, H. Marguerite, asst. zool. Northwestern. Comparative physiology of crustacea.
Weber, Patricia, res. asst. biol. St. Louis. Invertebrate biochemistry.
Wenrich, D. H., prof. zool. Pennsylvania.
West, Alice, Radcliffe. Oxidation-reduction studies.
Whiting, P. W., prof. zool. Pennsylvania. Genetics of Hymenoptera.
Wichterman, R., assoc. prof. biol. Temple. X-ray effect on mating in Paramecium.
Wilber, C. G., dir. biol. lab. St. Louis. Effect of environment on invertebrate body fluids.
Willier, B. H., dir. biol. labs. Johns Hopkins.
Wilson, Marie, asst. zool. Northwestern. Assistant on the invertebrate zoology course.
Wilson, T. H., instr. phys. Pennsylvania. Osmotic hemolysis of human erythrocytes.
Wilson, W. L., res. assoc. phys. Pennsylvania. Radiation on cell division and clotting.
Winblad, J. N., instr. anat. Kansas Med. Development of behaviour and motility in *Opsanus tau.*
Wittenberg, J., grad. biochem. Columbia.
Wood, R. D., instr. bot. Rhode Island. Instruction in botany.
Woodward, A. A., asst. prof. zool. Brown. Localization of enzymes in protoplasmic granules.
Woodward, A. E., asst. prof. zool. Michigan. Effects of some vitamins on Eichinoderms.
Wrinch, Dorothy, lect. physics, Smith. Structure of native proteins.
Wulff, V. J., asst. prof. physiol. Illinois. Physiology of optic pathway of Limulus and Loligo.
Zeiokar, M., res. fel. Calif. Inst. Tech. Fertilization studies on Nereis.
Zeuthen, E., lect. Copenhagen. Respiratory metabolism of cell division.
Zorzoli, Anita, asst. prof. Sch. of Dentistry, Washington.

THE M.B.L. CLUB IN 1949

DR. ROBERTS RUGH
President, M.B.L. Club for 1949; Columbia University

The Marine Biological Laboratory Clubhouse has a long history, dating back to when it was a yacht club. Never has it been so used and useful as this summer. Some 483 full-term members have entered its portals and have benefited by its existence. In addition there were 53 weekly members.

The M.B.L. Club is maintained for the pleas-

ure, convenience and recreation of laboratory personnel. Its membership is therefore limited to those connected with the laboratory or their immediate adult relatives. Workers at the Oceanographic Institution or the United States Bureau of Fisheries are admitted to special membership and a very limited number of patrons are admitted, upon favorable action of the Executive Committee. The dues in these special cases are somewhat more than for the M.B.L. personnel, as it rightly should be. Unfortunately, the physical facilities of the Clubhouse make it necessary to limit the membership to adults, but it is hoped that the Laboratory will soon provide recreational facilities for the younger people as well.

The Club sponsors four regular weekly events: the Sunday Evening Home Talent Concert, the onday Evening Classical Record Concert, Thursday Evening Square Dancing with instruction, and Saturday Evening Ballroom Dancing with refreshments. The dances were under the direction of the Social Chairman, Miss Ruth Alsher. These events have all been very well attended—in the case of one of the concerts fifty-five members were seen lying on the grass around the building within hearing distance. All floor and stair space was occupied. For the square dances Dr. Bartlett and Connie Mitchell have had as many as six groups for instruction and dancing.

The Home Talent Concerts were under the direction of Dr. Walter Wainio, the president of the Club for 1950. These concerts attract some members who may not participate in any other activity of the Club. Fortunately, the club had the talented service of Max Pepper, a most accomplished pianist who not only carried a heavy burden of accompanying but also of solo work. Miss Withrow, Mrs. Kisch and Dr. Wainio sang, and there were violin, flute and other instrumental solos.

The Monday Evening Classical Record Concert was of very high caliber due to the generosity of townspeople and friends of the Oceanographic Institution who loaned their valuable records. Mrs. Daniel Mazia organized the concerts and Dr. M. Brust directed them.

The dances were so well attended that the new Vice-President, Dr. Bartlett, is beginning a campaign to raise funds for the construction of a hall measuring about twenty by fifty feet which would adjoin the present building along the waterfront to the West. This large room would be used for the dances, relieving the present quarters for the more sedentary activities such as bridge, chess, checkers, reading, etc. It will probably also have a large sundeck on top. There is no question but that the membership has far outgrown the physical facilities of the present Clubhouse.

During the present season the Executive Committee purchased a piano aided by a gift from Mrs. Frost. They also purchased a Sonomaster record player and speaker for both regular and long-playing records, equipped with a microphone for calling at the square dances and for announcements. With a slight increase in the dues and four boat excursions (around the Islands, Edgartown Regatta, Gay Head, and Cuttyhunk) for which there was a nominal charge, the Executive Committee left several hundred dollars in the treasury for the use of the incoming officers, to the pleasure of the new Secretary-Treasurer, Dr. Ralph Cheney.

The major credit for the very successful season goes to the Hostess, Miss Mary Lou Failla. She opened the Club on June 15 and closed it on September 15; during the interim she became acquainted with everyone who used the Clubhouse. Never before has the Club had such an atmosphere of friendly welcome as it has through her good humor and fine sense of balance. It was Miss Failla's generous spirit and her wholehearted cooperation, far beyond the requirements of her position, that made the M.B.L. Club such an integral part of the lives of so many laboratory people this summer.

SYNTHESIS OF ACETYLCHLORINE

(*Continued from page* 26)

line hydrolysis the minimum amount of cholinesterase would be adequate to split the acetylcholine released by two to three million impulses per gm per hour. Both the chemical and the thermodynamic data are thus in agreement with the assumption of the necessity of the acetylcholine-esterase system for conduction.

NOTE: Based on a paper presented at the Marine Biological Laboratory.

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THE REACTIVATION OF THE NARRAGANSETT MARINE LABORATORY

Dr. Donald J. Zinn, *Research Associate*

In July of this year, the Narragansett Marine Laboratory of Rhode Island State College, newly reactivated under the directorship of Dr. Charles J. Fish, opened its doors for the first time since the summer of 1941, to scientists interested in research in marine biology. The Laboratory is situated about three miles from the entrance to the western passage of Narragansett Bay, at Fort Kearney. It is less than thirty-five nautical miles from Woods Hole.

Working with Dr. Fish on the life history of *Venus mercenaria* in Narragansett Bay are Dr. David M. Pratt, assistant professor of marine biology at Rhode Island State College, and Dr. Donald J. Zinn, assistant professor of zoology at the State College. Marie P. Fish is in charge of a Navy project on the production of sound in marine animals, and is being assisted by Alton Kelsey of the Woods Hole Oceanographic Institution and John Kelley of Rhode Island State College. Dr. D. Eugene Copeland of Brown University is continuing work started last summer at the Marine Biological Laboratory on the methods of adaptation of anadromous and catadromous fish. The U. S. Fish and Wildlife Service is working on a problem concerning the variation in population of *Venus mercenaria* in Narragansett Bay. Warren Landers, formerly of the Marine Biological Laboratory, is in charge of this project and is assisted by Thomas Kane.

The Laboratory, through Rhode Island State College, has established in cooperation with the Woods Hole Oceanographic Institution a graduate student training program in biological oceanography and marine fisheries biology. Instruction and supervision of research will be provided by members of the staffs of the two institutions, emphasis being placed on open ocean investigations. Arrangements are being made for a limited number of students who will enroll as candidates for the degree of Master of Science in marine biology. The first year will be spent in Kingston and at the Narragansett Marine Laboratory. It is expected that students will spend the second year either at the Woods Hole Oceanographic Institution or at the Woods Hole Station of the U. S. Fish and Wildlife Service.

THE INVERTEBRATE ZOOLOGY COURSE AT THE MARINE BIOLOGICAL LABORATORY

Dr. F. A. Brown, Jr.
Instructor in Charge; Professor of Zoology, Northwestern University

The invertebrate zoology course, the oldest of the courses at Woods Hole, began another season Tuesday evening, July 26. Fifty-six students were enrolled. The demand for this course continues to climb upwards, with the number of applications now exceeding the number of available places nearly threefold. This pressure has resulted in a gradual reduction in the number of undergraduates admitted.

In charge of the course was Dr. F. A. Brown, Jr., professor of zoology at Northwestern University. Other members of the Senior Staff were Dr. W. D. Burbanck, professor of biology, Drury College; Dr. C. G. Goodchild, professor of biology, Missouri State College, Southwest; Dr. Libbie H. Hyman, the American Museum of Natural History; Dr. L. H. Kleinholz, associate professor of biology, Reed College; Dr. J. H. Lockhead, assistant professor of zoology, the University of Vermont; Dr. Madelene E. Pierce, associate professor of zoology, Vassar College; Dr. W. M. Reid, professor of biology, Monmouth College, and Dr. T. H. Waterman, assistant professor of biology at Yale University. Members of the Junior Staff were Robert S. Howard, assistant in zoology, University of Miami and Marie Wilson, assistant in zoology, Northwestern University.

The course opened with a lecture by Dr. G. L. Clarke, entitled "The Sea as an Environment," which began a series of six lectures on oceanography presented by members of the staffs of the Woods Hole Oceanographic Institution and the Woods Hole United States Bureau of Fisheries. Other lectures in the series were: "Tides" by Dr. William S. von Arx; "Chemical Problems of the Sea" by Dr. A. C. Redfield; "Oceanic Plankton" by Dr. Mary Sears; "Geographical Distribution of Marine Animals" by Dr. Louis W. Hutchins; "Natural Resources of the Sea" by Dr. Paul S. Galtsoff. These lectures provided the students with an unusual opportunity to learn basic problems of the ocean from individuals especially qualified to speak about them.

The more conventional portion of the course involved a series of about thirty lectures and approximately an equal number of laboratory periods presented by members of the regular staff. In addition there were nine field expeditions.

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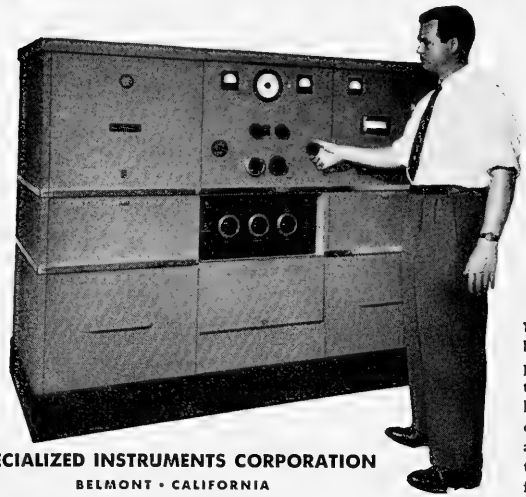

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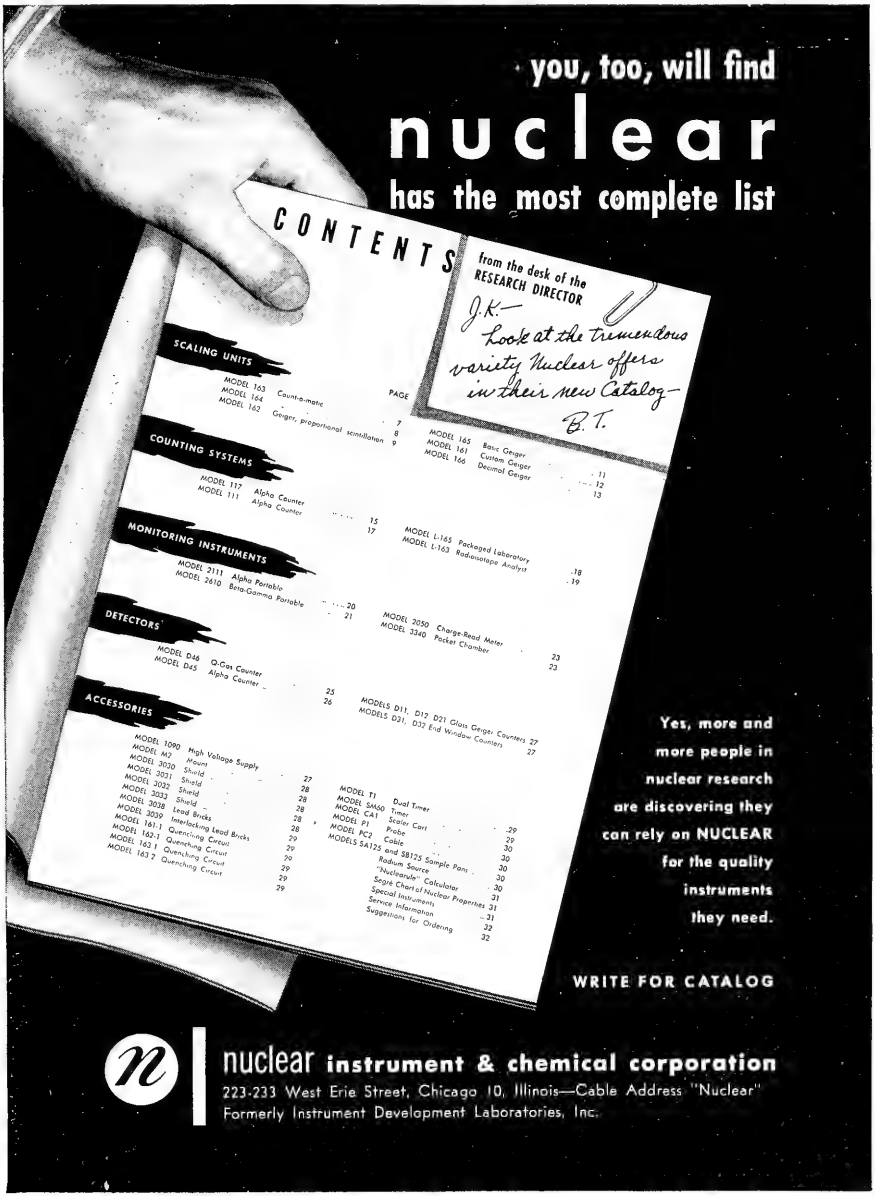
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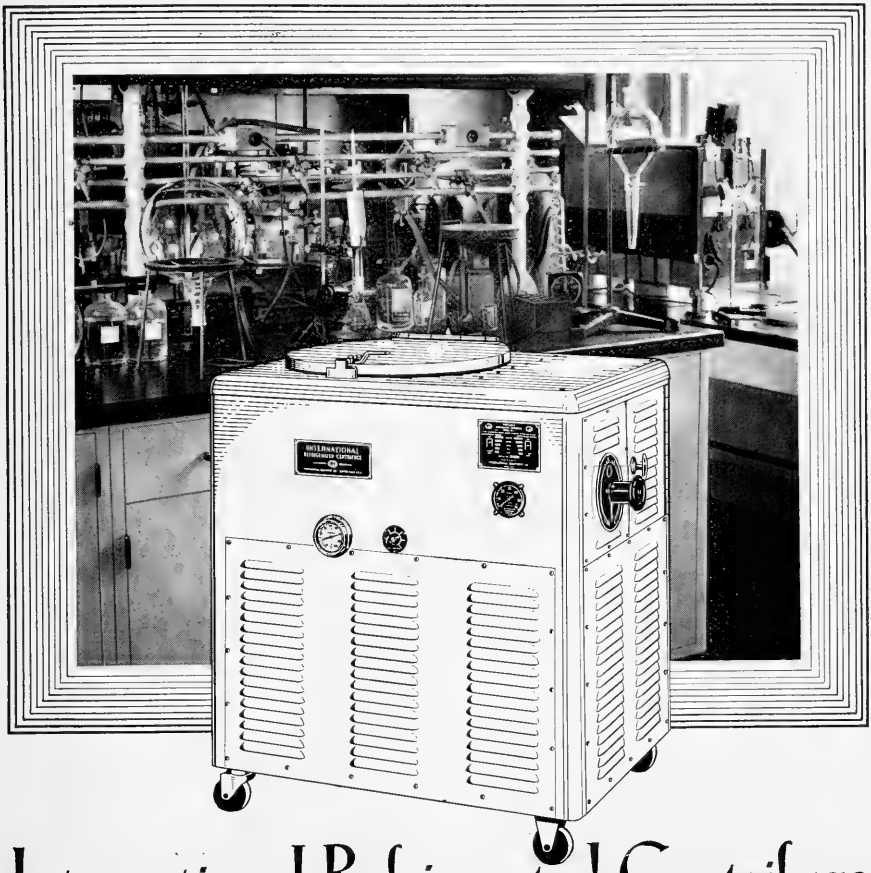

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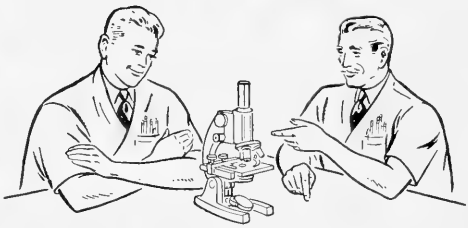

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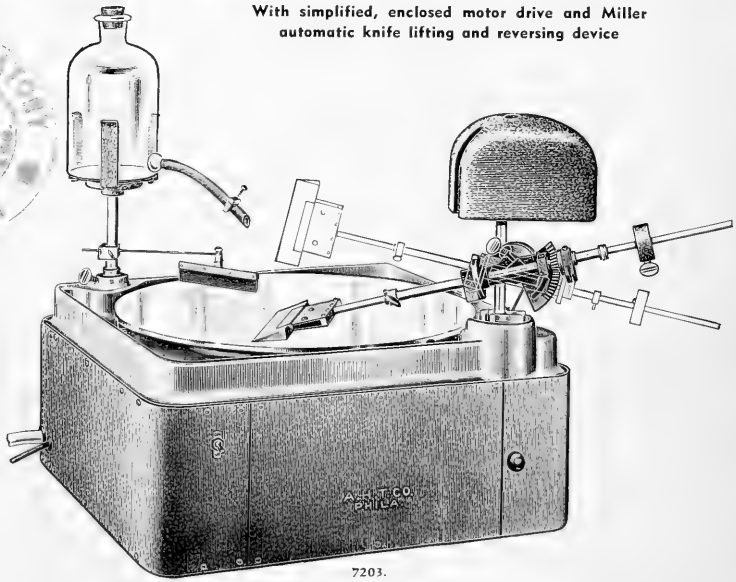

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