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INSTITUTE OF MOLECULAR EVOLUTION

UNIVERSITY OF MIAMI CORAL GABLES, FLORIDA

FIRST ANNUAL REPORT

1 June 1965

OUTLINE

RELATIONSHIP TO OTHER REPORTS

This document is the first annual report of the Institute of Molecular Evolution of the University of Miami. It is also partly a termination report of the Institute for Space Biosciences of The Florida State University. Two annual reports had been issued from the Tallahassee institution, one on 1 November 1962 and one on 1 November 1963. Many of the project reports contained in this volume represent work conducted entirely at the Miami site. Other paragraphs describe work carried out at both laboratories. Little attempt is made to distinguish the locale in these reports. The section on Principal Accomplishments - - - deals, however, mainly with developments at the Institute for Space Biosciences.

"MOLECULAR EVOLUTION"

The term molecular evolution is open to broad or to rather narrow interpretation. The term was first used in print by the director in a paper titled "A Correlation of Observations Suggesting a Familial Mode of Molecular Evolution as a Concomitant of Biological Evolution", published in 1953 in the American Naturalist.

A narrow interpretation of the term can be stated more specifically as prebiological molecular evolution, i.e., the processes preceding and leading to Darwinian evolution of organisms. The broader interpretation embraces and spans in a continuum both molecular evolution as thus defined and molecular evolution in organisms. This broader interpretation is implicit in the 1953 paper referred to.*

The interpretation applied here to the term, molecular evolution, is however conceptually broader. The broadest context embraces in one continuum astronomy, geology, chemistry, biology, psychology, and sociology. The phenomena treated in each of these sciences are increasingly recognized to have their roots in phenomena described in a more basic science. Full understanding of the origin of molecules of

*The terms molecular evolution and chemical evolution have been used almost interchangeably. Molecular structure is implicit in chemical behavior and chemical behavior is implicit in molecular structure, so that either term can serve. The structural aspect is considered to be more fundamental; therefore the choice of the former.

biological significance requires an orientation in astronomy and geology as well as in chemistry and biology. The molecular level is the one at which a large number of significant problems come into focus. The premise that the evolutionary continuum extends to mental powers and to social faculties was first clearly spelled out by Charles Darwin in his "Descent of Man", Chapters 3, 4, and 5. The unified total concept is treated in a modern manner by Herbert H. Ross in his "Synthesis of Evolutionary Theory" (Prentice-Hall, 1962). At a somewhat more philosophical level, the Jesuit anthropologist, Theilard de Chardin, has arrived at a similar conceptual synthesis in his "Phenomenon of Man" (Harper and Brothers, English translation, 1959). On p. 71, for example, he states, "In the world, nothing could ever burst forth as final across the different thresholds successively traversed by evolution (however critical they be) which has not already existed in an obscure and primordial way" (italics de Chardin's). The Institute will consider itself quite fully manned when a proper balance of scientists representing astronomy, geology, organic and physical (surface) chemistry, and evolutionary and developmental biology has been convened. Psychologists and a sociologist of an appropriate sort may be added later.

The concept of a continuum of natural phenomena offers promise in education for the sciences. The traditional forces of education have resulted in fragmentation of the sciences for purposes of assimilation by students. What is needed is the integration of these fragments. Such educational unification rarely occurs; it is essentially left to chance and the

individual student. The evolutionary continuum can provide a pedagogical basis for a natural and meaningful structure to the reunification of many sciences. Teaching of this sort, through faculty of the Institute of Molecular Evolution, has already begun.

Needless to say, the integrative outlook is also most effective in the solution of problems with many disciplinary interfaces, e.g. problems of the origin of life, fundamental problems of memory processes, and various biological problems in the context of outer space.

PERSONNEL OF THE INSTITUTE OF MOLECULAR EVOLUTION

Faculty

Sidney W. Fox, Ph.D., Professor and Director [a/] [b/]

Charles B. Metz, Ph.D., Professor [b/] [c/]

Oktay Sinanoglu, Ph.D., Visiting Professor [d/]

Kaoru Harada, Ph.D., Assistant Professor [e/]

Gottfried Krampitz, Ph.D., Assistant Professor, University of Bonn [f/]

David Durant, Ph.D., University of Leicester, 1963, Research Scientist

Luther Franklin, Ph.D., Florida State University, 1964, Research Scientist

Heinz Hardebeck, Ph.D., Research Scientist, University of Bonn [f/]

Emmett Durrum, Ph.D., Durrum Instruments Corp., Palo Alto, Calif. [f/]

Tadayoshi Nakashima, Ph.D., Kyushu University, 1961, Research Scientist

Thomas Waehneldt, Ph.D., University of Göttingen, 1963, Research Scientist

[a/] Also professor in the Biochemistry Department, University of Miami

[b/] Participant in the Cellular and Molecular Biology program, University of Miami

[c/] Also professor in the Zoology Department of the University of Miami

[d/] Professor in the Chemistry Department of Yale University

[e/] Also assistant professor in the Chemistry Department of the University of Miami

[f/] Activity in the Institute under a subgrant from the University of Miami

Robert McCauley, Research Assistant

Charles Ray Windsor, B.S., Research Assistant

(Mrs.) Margret Soppelt, Research Assistant, University of Bonn

Miss Leonie Wulf, Research Assistant, University of Bonn

Graduate Students

George Brown

Priscilla Everett

Peter D. Hoagland [a/]

David Joseph

Duane L. Rohlfing [b/]

Gerd Röttgen, University of Bonn

Alan Schwartz [c/]

Samuel Stern

Kent K. Stewart [d/]

Fusae Suzuki

Pamela Tyler

[a/] Ph.D., 1964, now at Eastern Regional Research Laboratory

[b/] Ph.D., 1964, now at Ames Research Center, Moffett Field, California

[c/] Ph.D., now at Molecular Radiobiology Department, Los Alamos National Laboratory

[d/] Ph.D., 1965, USPHS Postdoctoral Fellow at Rockefeller Institute

Office Personnel

M. E. Dockendorf	Executive Officer
Virginia Bowser	Secretary
Susan Riggs	Secretary
Rosalie Peterson	Typist
Dorothy Butterbrodt	Typist

PROGRESS IN RESEARCH

Evolution of Chymotrypsin and Trypsin (Fox)

This section involved no work during the year, but rather bears on one culmination of studies of evolution of organismic protein, studies which earlier led to a principal program of the institute.

In Proceedings of the National Academy of Sciences, Vol. 52, p. 884-889 (1964) Walsh and Neurath have pointed to the many ways in which chymotrypsin and trypsin have been found to be homologous. According to these authors, "The only really striking difference between the two enzymes lies in their substrate specificity". In Archives of Biochemistry and Biophysics, Vol. 63, 352-367 (1956), Hurst and Fox pointed to the structural similarity of chymotrypsin and trypsin, but especially to the similarity in action on lysozyme as substrate. The exception noted by Walsh and Neurath can be explained as due to the unrepresentative data accumulated by the Bergmann school in studies of proteases on small, synthetic peptides. Also, in Comparative Biochemistry IV, 202 (1962), Vegotsky and Fox have made the point that chymotrypsin and trypsin may be homologous rather than heterologous.

Extraterrestrial Macromolecular Sampler (Durrum, Fox)

A number of soil sample hydrolyzates have been studied by both ion exchange column chromatography and paper electrophoresis. Paper electrophoresis analysis of desalted soil samples is readily carried out in periods of one hour with complete and clear resolution of aspartic and glutamic acids,

with a third band of unresolved monoaminocarboxylic acids, and a fourth partially resolved band representing the basic amino acids. Prior desalting is definitely required for both paper electrophoresis and column separations. Paper, resins, and detective reagents (ninhydrin and trinitrobenzenesulfonic acid) have all been shown to be stable under conditions of heat sterilization.

Incorporation of the most favorable process into an automatic device is being considered.

The Origin of Optical Activity from Physical Particles
(Sinanoglu, Harada)

Many reviews of new suggestions for the origin of optical activity have appeared from this laboratory in recent years. Dr. Harada has developed new plausible experimental demonstrations for other answers to this problem, and some of these results are reported in this document. A more fundamental explanation for the roots of the phenomenon has been sought and the suggestion has been made that the nonparity of physical particles might supply such insight [Fox, J. Chem. Education, 34, 472 (1957)]. While a visiting professor at the Institute, Dr. Oktay Sinanoglu has looked into this possibility. Although no conclusion has emerged, initial review of the possibility has encouraged further scrutiny.

Studies in this laboratory have tended to indicate that order at one level, e.g. the macromolecular, is a derivative of order at a lower level, the molecular. Perhaps the asymmetric nature of small molecules in the biochemical world is similarly

a reflection of asymmetry at the level of physical particles.

Optical Resolution of an Amino Acid by a Stereoselective Ligand Exchange Reaction (Harada, Fox)

DL-Aspartic acid was successfully resolved by a reaction in aqueous solution with the copper complex of L- and D-glutamic acid, the copper complex of L- or D-alanine, and the copper complex of L-proline. Optically active L- or D-aspartic acid copper complex precipitated from the reaction mixture. Optical purity of the resolved aspartic acid was in the range of 90-100%. The mechanism and the prebiological meaning of the stereoselective reaction have been considered [Nature, 205, 590 (1965)].

Optical Resolution of DL-Aspartic Acid, DL-Glutamic Acid, DL-Asparagine or DL-Glutamine by Inoculation (Harada)

Several amino acids were totally, or almost totally, resolved by seeding supersaturated solutions with each of the optically active amino acid crystals. Ammonium formate solution was successfully employed to produce supersaturated solutions.

In the primitive sea, before life emerged, many kinds of organic compound could have accumulated in relatively high concentrations (ca. 10%, H. Urey). In a lagoon or in a pond, some organic compounds might have crystallized from the primitive sea by evaporation of water. Upon crystallization of the "primitive soup and chance seeding", some racemic organic

compounds could have been resolved to their optically active isomers. Dissymmetric crystals such as quartz might also have acted as seeds for crystallization of organic compounds. This postulated set of conditions in the primitive sea is similar to that employed for the optical resolution of several amino acids mentioned here. Ammonium formate, which induces supersaturation of other compounds, can be rationalized as one of the most abundant compounds on the primitive Earth [Nature, in press, and Bull. Chem. Soc. Japan, in press].

Formation of Amino Acids by Pyrolysis of Ammonium Formate or Formamide (Harada)

Thermal decomposition of ammonium formate or formamide resulted in several amino acids: glycine, alanine, and aspartic acid. Seven to nine other ninhydrin-positive materials were observed on the automatic amino acid analyzer and in two dimensional paperchromatography. Glycine, alanine, and aspartic acid were identified by several methods: automatic amino acid analyzer, two dimensional paperchromatogram, high voltage electrophoresis; dinitrophenylation, column chromatography, and u.v. absorption spectra of isolated DNP amino acids.

The mechanism of the amino acid formation was studied.

Synthesis of α-Amino Acid Menthyl Esters (Hayakawa, Harada)

Continuing from the previous study [Bull. Chem. Soc. Japan 39, 191 (1963)], the synthesis of α-amino acid menthyl esters by use of α-amino acid N-carboxy anhydride and l-menthol was studied. This is a basic study for asymmetric

synthesis of α-amino acids by the use of l-menthol as an asymmetric source (Bull. Chem. Soc. Japan, in press).

Analyses of Proteinoid Synthesized in Presence and Absence of Terrestrial Crustal Materials (Krampitz, Windsor, McCauley, Fox)

The composition of proteinoids has been found in both Tallahassee and Bonn to vary as the polymers are made in the presence of materials from the crust of the Earth (Table I).

These results are of interest in two main ways. One of them is that they demonstrate the feasibility of the laboratory reactions under a greater variety of geological conditions. The other is that the analyses reveal amino acid compositions more like those of natural proteins than the ones obtained in glassware, e.g. the content of aspartic acid is lower.

Table I

Composition of Proteinoids Prepared in

Presence of Various Materials

Amino Acid	1:1:1-Proteinoid prepared in glass	1:1:1-Proteinoid prepared on lava	1:1:3-Proteinoid prepared in presence of 1 part granite
Lysine	3.3%	3.0%	5.2%
Histidine	1.4	1.3	1.4
Ammonia	3.8	1.3	3.2
Arginine	0.7	1.6	6.2
Aspartic Acid	62.0	45.8	11.5
Glutamic Acid	12.6	29.7	8.9
Serine	---	0.1	0.3
Threonine	---	0.2	0.5
Proline	---	0.8	0.7
Glycine	2.1	1.1	5.3
Alanine	3.6	2.3	8.9
Valine	1.1	2.1	5.6
Half Cystine	1.7	1.6	10.2
Methionine	1.7	---	4.9
Isoleucine	1.3	1.6	2.4
Leucine	1.2	1.7	3.8
Tyrosine	1.1	2.2	10.4
Phenylalanine	2.0	2.9	7.4
Cysteic Acid	---	0.7	

Degree of Heterogeneity of Thermal Proteinoids (Nakashima, Fox)

Probably the most astonishing of the results to emerge from this program is the finding that the heterogeneity of the thermal proteinoids is sharply limited. Many indications were earlier at hand of a somewhat limited heterogeneity, e.g. by partition, by moving boundary electrophoresis, by fractionation at the ultracentrifuge, and especially by comparison of the amino acid compositions of thermal proteinoid before fractionation from hot water, after such purification, and after two such purifications [Fox, et al., Archives of Biochemistry and Biophysics, 102, 439 (1963)].

The latest results have been reviewed with several protein chemists including Dr. Fred Sanger (Cambridge), Dr. Theodor Wieland (Frankfurt), Dr. Emil Smith (UCLA), and Dr. H. Michl (Vienna), and the unanticipated nature of the results was reaffirmed.

In the latest and most extensive study reported here, 1:1:1-proteinoid was first converted to proteinoidamide by standing in liquid ammonia. This treatment was designed to convert imide groups to amides with the thought that variation in structure due to a theoretical variation in mode of opening of imide rings would be avoided. The polymer obtained proved to be neutral in character.

The proteinoidamide was distributed on DEAE-cellulose. Five major peaks were obtained on elution. These are shown in Fig. 1. Several of these peaks are seen already to have a high degree of symmetry; the entire pattern is far removed from being

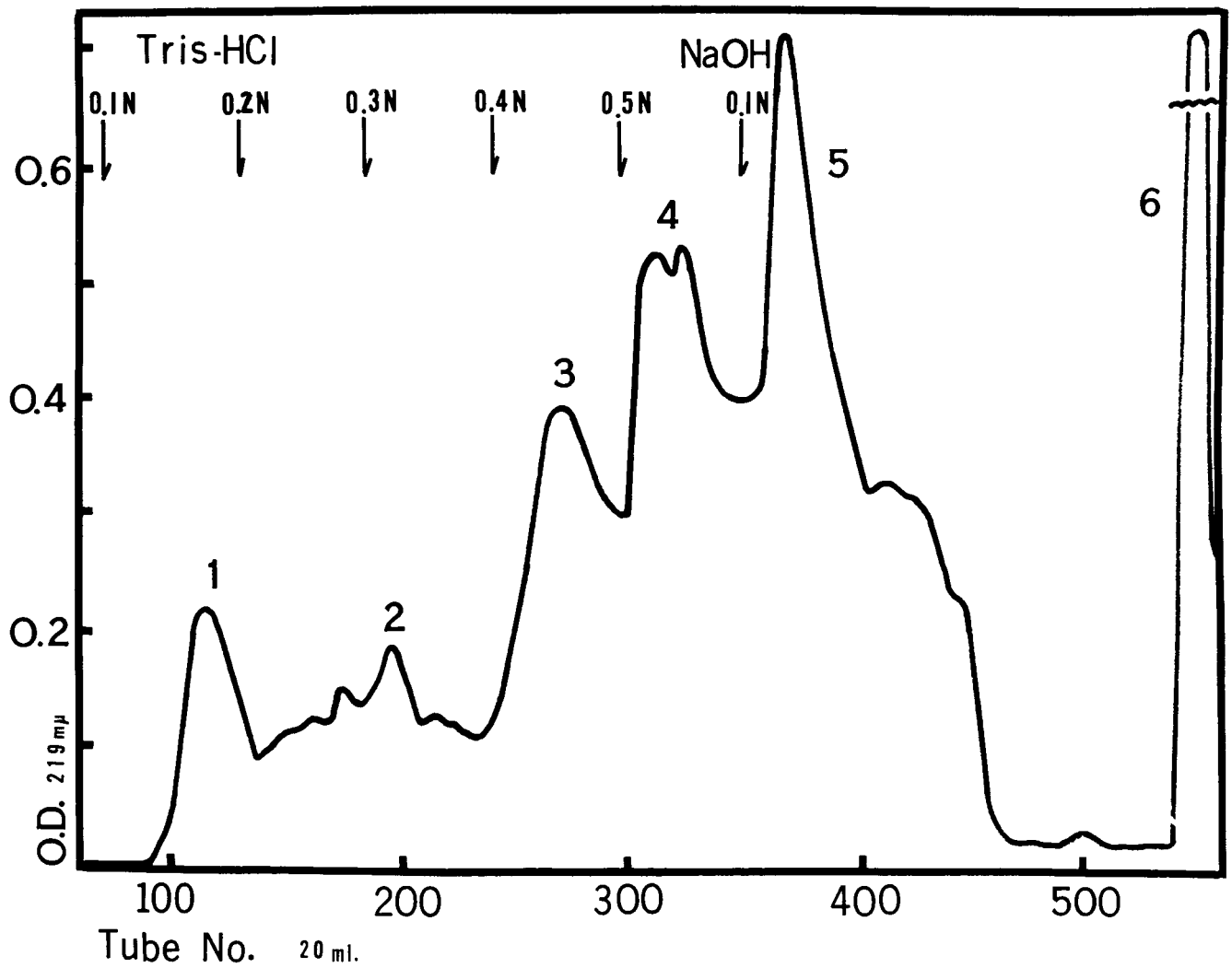


Fig. 1. Elution pattern of 1:1:1-proteinoidamide on DEAE-cellulose.

a random smear.

The material represented in each peak was next purified by partial dialysis. Peaks 1 and 2 were lost by diffusion; they undoubtedly constitute material of molecular weight lower than those in other fractions. Fractions 3, 4, and 5 were further purified on Sephadex G-25 and each was found to be homogeneous in this case also.

When hydrolyzed by heating with 6 N HCl for 24 hr. at 105° C. and then analyzed on the Phoenix automatic analyzer, fractions 3, 4, and 5 displayed remarkably similar amino acid compositions (Fig. 2). The results show also that all fractions contain all amino acids (except serine and threonine).

When fractions 3, 4, and 5 were partially hydrolyzed by 12 N HCl at 37° C. for 72 hr., "fingerprints" were obtained (Fig. 3). The number of peaks observed were 39-41. No more than 17 of these peaks can be free amino acids (tryptophan being destroyed on hydrolysis by mineral acid). The remaining peaks are thus undoubtedly peptides, as indicated by the reactivity to ninhydrin. These peaks were distributed also in a fashion similar to that of the total hydrolyzates of fragments 3, 4, and 5 from the DEAE-cellulose elution. The inevitable conclusion is that both the composition and sequence within the polymers is sharply limited by internal processes. The information within the polymers is derived from information within the monomers only. This behavior is in keeping with a general suggestion made by Professor George Wald in 1954 (Scientific American, Aug., p. 50).

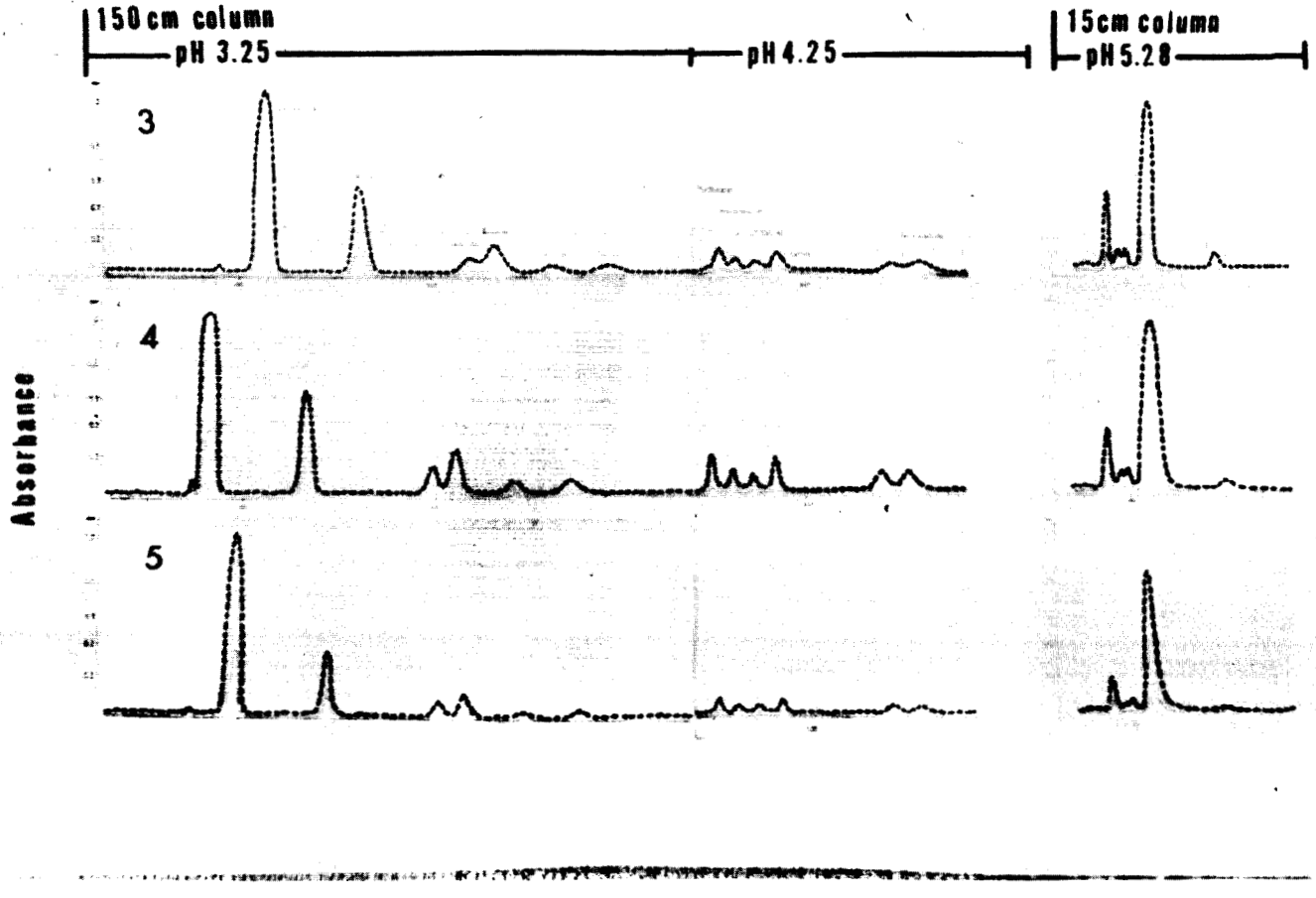


Fig. 2. Amino acid compositions of polymer fractions corresponding to peaks 3, 4, and 5.

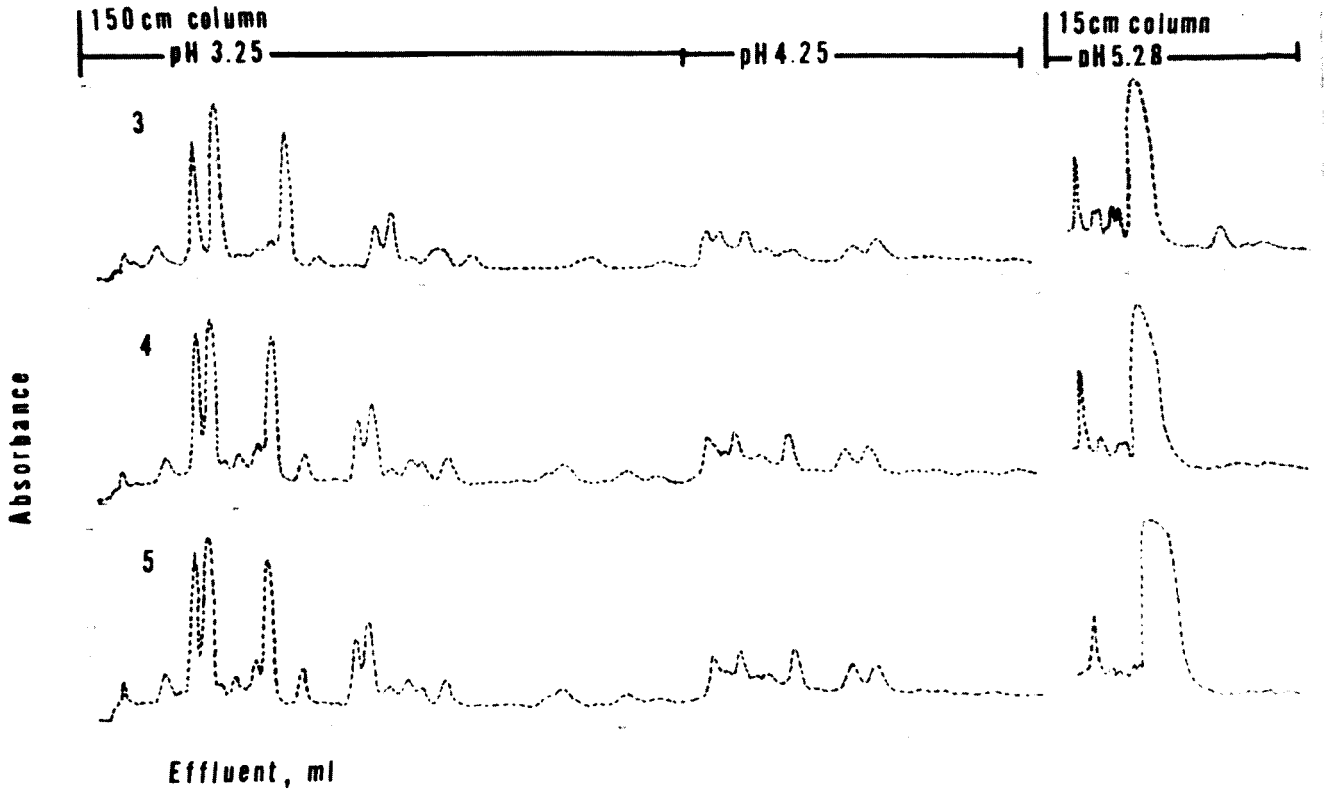


Fig. 3. "Fingerprints" of partial hydrolyzates of fractions of proteinoid obtained from DEAE-cellulose. Tracing from the Phoenix automatic amino acid analyzer.

Order is thus observed at a rudimentary, molecular level, and the many other phenomena which have emerged from these thermal poly-α-amino acids are more easily understood in the light of this degree of order.

Evolution at the molecular level in a prebiological phase is thus visualized to be in this way, as in others, sharply self-limiting.

Also of interest is the fact that systems containing such information can arise so easily from simpler systems having information at another level, and that none of the information has been imparted from outside of the macromolecular system itself. In this model of original protein, for instance, no information has been obtained from nucleic acid. The premise that nucleic acids had to appear before protein has been held by some. While that premise may yet be supported, rigid acceptance of it would have precluded the experiments here described. Also of interest is the work of Pattee who has inferred that primitive proteins contain their own information [H. H. Pattee, Biophysical Journal, 1, 683 (1961)] on the basis of computer theory, and recent reports that some syntheses of polypeptides in cells of primitive forms are non-template in character [Lipmann, in Fox, "The Origins of Prebiological Systems", p. 271, 1965; Mach and Tatum, Proc. Nat'l Acad. Sci., 52, 876 (1964)].

Finally, the high degree of order in the proteinoids, as models of primitive protein, contrasts with the almost random

nature of amino acid residue sequences in contemporary protein [Gamow, Rich, and Yeas, Adv. Biol. Med. Phys., 4, 23 (1956); Sorm and Keil, Adv. Protein Chem., 17, 167 (1962); Vegotsky and Fox, Comparative Biochem., IV, 185 (1962)]. The order sometimes imputed to contemporary proteins is not order in a sequential sense but repeatability of sequences of single proteins. We are thus led to infer that highly ordered primitive protein evolved to statistically disordered contemporary organismic protein.

Assuming that the proteinoid model accurately represents primordial protein, one may conclude that the evolution of generic protein from relatively ordered polymers to less ordered polymers is consistent with the second law of thermodynamics.

Catalytic Activity of Hematin (Stewart, Fox)

The activity of the hemin-enzyme, tryptophan pyrrolase, has been found in the hematin group alone. The pH optimum is 11.8 and the activity is several orders of magnitude less than that of natural tryptophan pyrrolase which contains the specific protein. At lower pHs such as 6.0, the activity of the hematin is that of catalase, when hydrogen peroxide is present. At intermediate values such as 8.6, hydrogen peroxide is decomposed and tryptophan is oxidized. In the oxidation of tryptophan, the same major product as is found biologically, kynurenine, is also found. With ferric or ferrous iron instead of hematin, no kynurenine was obtained.

The hematin system is thus of considerable interest for the study of the effects of poly-α-amino acids on the catalytic activity of this prosthetic group, the specificities, the pH maxima, etc. Also of interest will be the tracing of oxidative activity through many organic derivatives of iron as these might have evolved.

Catalytic Activity in Proteinoids (Krampitz, Durant, Harada, Joseph, Wiggert, Hardeback, Röttgen, Rohlfing, Fox)

Semantic Considerations

The definition used here for catalysis is the original one by Ostwald which describes as catalysis the increase in rate of reaction by a substance foreign to the reaction. Modern views on catalysis and enzymes have tended to require in addition that a catalyst be unchanged after the reaction. Whether the proteinoid behavior meets this latter requirement is in most cases not yet known. Whether the activities meet a modern definition of catalysis, they demonstrate ways in which metabolic pathways might have originated.

Hydrolysis of p-Nitrophenyl Acetate

The relatively high activity of thermal proteinoids, as compared to free histidine, for the hydrolysis of p-nitrophenyl acetate has been shown to depend upon the simultaneous presence in the macromolecule of histidine and anhydroaspartic acid residues. As the imide is progressively opened, the activity is progressively destroyed [Rohlfing, Ph.D. dissertation, 1964].

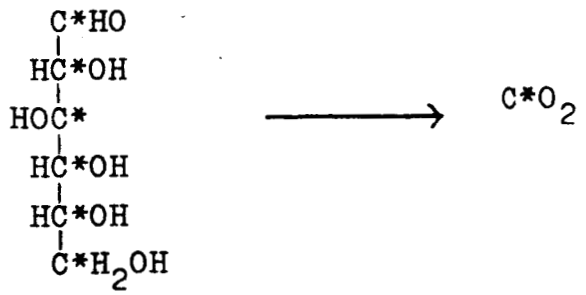
Hydrolysis of ATP by Zinc-Proteinoid

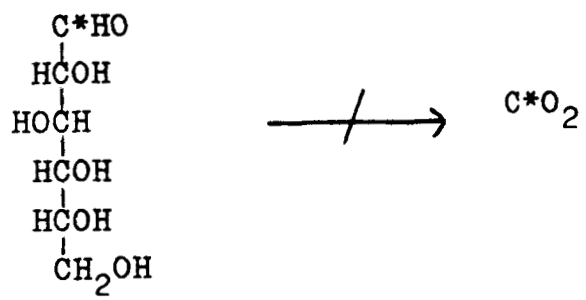
With radioactive ATP, the previously reported breakdown of ATP by zinc-containing proteinoids (Fox, "The Origins of Prebiological Systems") has been shown to yield in some cases ADP, in others AMP, in some cases each simultaneously. The conditions which favor one type of catabolism or another have not been established.

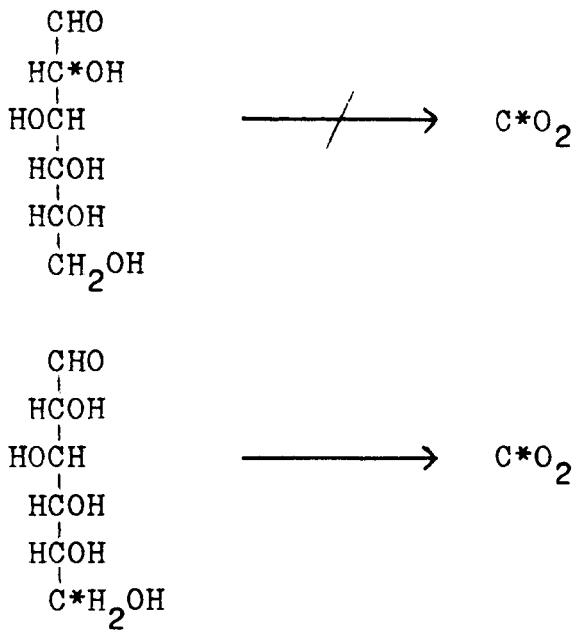
Conversion of Glucose to Glucuronic Acid and Its Decarboxylation

With uniformly labelled D-glucose, thermal proteinoid has been shown to catalyze in aqueous solution the breakdown of glucose to glucuronic acid, and the decarboxylation of the latter. Production of radio-CO_2 was shown first.

When 1-labelled, 2-labelled, and 6-labelled glucose were each tested, only the last yielded radioactive CO_2.







These results suggested that the intermediate was the 6-carboxy analog of glucose, D-glucuronic acid. Glucuronic acid was isolated as the p-bromophenylhydrazone and identified by isotope dilution and in other ways. Radioglucuronic acid was also shown to be converted, at a relatively high rate, to C^*O_2.

The turnover number in moles of glucose per mole of acid proteinoid was calculated roughly to be about 25 for 3 days at 37.5°. The estimated rate for lysine proteinoid is about 100 times as high [Fox and Krampitz, Nature, 203, 1362 (1964)].

The activities so far recorded are however extremely low when compared to those of enzymes of contemporary terrestrial organisms. Especially is this true for production of carbon dioxide which is a minor conversion product of glucuronic acid, which is itself a minor conversion product of glucose. These results do not necessarily affect tests planned

for detecting microbes on Mars, but the enzymic power of hypothetical Martian microbes is unknown, and the possibilities that these results suggest should not be ignored.

Hydrolysis of Urea by Lysine Proteinoid

Urea is broken down by lysine proteinoid with a velocity greater than that observed in the catabolism of glucose. For this substrate, acid proteinoid is inactive. The pH optimum for lysine proteinoid is found to be 7. Above 9, alkaline hydrolysis occurs.

Lysine proteinoids are often more active than acid proteinoids. Accordingly, the relationship of lysine content to activity, and the effect of lysine residues on removal of protons from imidazole residues needs to be investigated.

Many lysine proteinoids were tested. One group, arising entirely from one bottle of free L-lysine, showed no activity. This anomalous behavior is being investigated.

Exposure to ultraviolet light for 30 min. has been shown to eliminate urea-splitting ability entirely.

Hydrolysis of AMP to Adenosine by Lysine Proteinoid and by Zinc-Proteinoid

Lysine proteinoid catalyzes the breakdown of AMP to adenosine, according to radioautograms. This breakdown is substantial, occurring to an extent of 70-80% in three days. This reaction has been shown to follow Michaelis-Menten kinetics, as has the reaction with zinc-proteinoid.

A helpful circumstance is that the lysine proteinoid has been found in the laboratories of three expert microbiologists (Dr. Lutz Wiese, Florida State University; Dr. Bennett Sallmann, University of Miami; Dr. Bernd Stille, University of Bonn), to be bacteria-free. This behavior can be understood on the basis of the known antibacterial power of polylysine (M. Stahmann, "Polyamino Acids, Polypeptides, and Proteins", Univ. Wisconsin Press, 1962). A sample of zinc-proteinoid was found also to be bacteria-free.

New catalytic results with copolylysines have in each case been demonstrated with preparations that were also shown to be free of bacteria. For the hydrolysis of p-nitrophenyl acetate, for the conversion of glucose to glucuronic acid and its decarboxylation, and for the activity of lysine proteinoid on urea, not only were key preparations shown to be free of bacteria. Entire aseptic syntheses were carried out with sterile, catalytically inactive free amino acids and the preparations were monitored at key stages of the preparation, purification, and testing of catalytic activity to insure the absence of microbes throughout. This added precaution was taken

to insure that microbes were absent at all stages, so that any activity observed might not be due to organisms which died and released enzymes. This (tedious) kind of testing has not yet been applied to studies of activity on pyruvic acid, acetic acid, or AMP.

Catalysis of Decarboxylation of Pyruvic Acid

The decomposition of pyruvic acid in aqueous solution is catalyzed more strongly by acid proteinoid than by lysine proteinoid. The C-1 labelled pyruvic acid showed the activity while the C-2 and C-3 labelled acids were found not to yield radioactive CO_2. For CH_3COC^*OOH, the decarboxylation in the presence of 2:2:1-proteinoid was 50% in 48 hr. at pH 8. This result has been obtained in more than 40 experiments without fail, but the reproducibility is being intensively studied.

Conversion of Acetic Acid to Other Acids

Initial experiments with acetic acid were focused on liberation of CO_2. This process was found with C^{14}-2-acetic acid but not with C^{14}-1-acetic acid. The formation of CO_2 was low in amount and was not observed in all experiments.

In all of more than thirty experiments, the conversion of C^{14}-2-acetic acid to other compounds was established by two-dimensional chromatography. Among the compounds thus identified was malic acid. An origin of more extensive metabolism in pre-cellular units can be visualized from some of the other products.

The examples assembled in this section on catalytic activity thus permit understanding in principle how metabolic activities could have appeared in the first prebiological systems.

More strictly, the activities would not have arisen in these systems but would rather have been properties of the polymeric material in which the first organization arose.

Nutritive Quality of Thermal and Leuchs Proteinoids (Everett, Fox)

The preparation of the thermal and Leuchs proteinoids has been fully described and their potential nutritional utility has been discussed at the conference on Nutrition in Space and Related Waste Problems, Tampa, Florida, 27-30 April 1964 (NASA SP-70).

Nutritional studies have been conducted in two laboratories, and a new investigation has begun in collaboration with Professor George Lewis in the Department of Biochemistry. The thermal proteinoids had earlier been shown to have nutritive quality for Lactobacillus arabinosus, Proteus vulgaris, and rats. In the present study, their value has been shown for Tetrahymena pyroformis R. Such activity is entirely absent unless the Tetrahymena are simultaneously primed with free amino acids. One possible explanation of this need is that the cells must first multiply and that those that then happen to have proteolytic power can proliferate. Under these conditions, some of the thermal proteinoids are found to be more nutritious than casein.

The Leuchs proteinoids contain the L form of amino acids almost exclusively, and the contents of most of these polymers is very favorable to nutritive quality. In these studies, Leuchs proteinoids were found to be considerably

superior to casein, nor did the cells require supplementation by free amino acids in the culture.

The potential fields of application of Leuchs proteinoids are a) the provision of supernutritional proteinaceous foods for long-term missions permitting maximum food value per kg. and b) a controlled elucidation of amino acid requirements in protein nutrition. These Leuchs proteinoids are capable of functioning in a way originally sought by Mendel, Abderhalden, and by Rose.

The thermal proteinoids are produced much more directly and easily than the Leuchs type. They open an investigational avenue to a simple chemical synthesis of proteinaceous food under extraterrestrial conditions, with however many problems to be solved. Most of the free amino acids can be produced, however, from simple gases. Also, as Scrimshaw has pointed out (p. 338 in NASA SP-70), the content of "essential" amino acids need not be high in order for proteinoids to be useful.

The total results of feeding to date suggest that future tests should be carried out on rats or mice.

Phosphorylation of Nucleosides (Waehneldt, Fox)

The phosphorylation of adenosine, guanosine, cytidine, and uridine each with polyphosphoric acid has been studied. Preliminary evidence has been obtained for the formation of ATP, ADP, AMP, GTP, GDP, GMP, CTP, CDP, CMP, UTP, UDP, and UMP. The yield in nearly all of these reactions is substantial.

The formation of ATP has been reported from other laboratories, but in a yield of only 0.1%. The present studies, particularly in view of the likelihood of polyphosphate on the primitive Earth, are demonstrating how substantial quantities of ATP and of analogous nucleotide triphosphates might arise.

Thermal Oligonucleotides (Schwartz, Waehneldt, Fox)

Further characterization of oligonucleotides has led to a relatively large-scale preparation which has been found to have the structural characteristics of earlier preparations. This material is not only attacked by ribonuclease, indicating 3' linkages; a major fraction of the theoretical number of linkages is attacked by venom phosphodiesterase, which is specific for 5' linkages. A major fraction of the linkages in thermal oligocytidylic acid ($5\frac{1}{2}$ residues long) thus obtained are of a natural type.

An earlier observation about copolymerization of mononucleotides has been confirmed. No oligopolymer of adenylic acid has been obtained by heating this nucleotide in polyphosphoric acid at 65°. When adenylic acid is heated with

cytidylic acid both polymerize simultaneously, very probably into a copolymer. Analyses reveal 70% C, 30% A.

Yields of solid, white oligocytidylic acid have not exceeded a few percent. A systematic investigation of all fractions indicates, however, that cytidylic acid is largely converted to polymers. These experiments are particularly being scrutinized for results which may open the way to thermal polynucleotides of higher molecular weight.

The establishment of natural linkages in these thermal oligonucleotides is of particular significance in view of the fact that the polymers reported by Schramm and coworkers [Angew. Chem. International Ed. 1, 1 (1962)] have been claimed by others to have unnatural linkages only [e.g. Kotchetkov, et al., Biochim. Biophys. Acta, 80, 145 (1964)]. The solvent, polyphosphoric acid, and temperature used in this laboratory are the same as have been used here for many years in promoting the polymerization of amino acids. Schramm's study modified these conditions only by substituting ethyl metaphosphate, a derivative of polyphosphoric acid, for polyphosphoric acid. Perhaps this explains why Schramm's products have not been proved to have natural linkages. Ethyl metaphosphate is furthermore difficult or impossible to explain as a geological material. The reagent is made from chloroform and diethyl ether, neither of which can be plausibly explained as occurring on the primitive Earth (Fox, in "The Origins of Prebiological Systems", p. 314). Miller [Miller and Parris, Nature, 204,

1248 (1965)] has made the same point, but also has advanced assertions that while salts of pyrophosphoric acid were likely, polyphosphoric acid was not. This assertion is regarded here as arbitrary, and it especially fails to take into account the fact that the most predominant constituent of polyphosphoric acid is pyrophosphoric acid (Harada and Fox, in "Origins of Prebiological Systems", p. 290). Furthermore, the entire argument of Miller in the paper cited tends to obscure the fact that the idea of a prebiological phosphoric medium of one kind or another was first expressed from this laboratory [Fox and Harada, Science, 133, 1923-1924 (1961), last sentence]. Considerations introduced by Schramm or Miller are derivatives of this central emphasis.

Ultraviolet Photomicrographic Evidence for a Membrane in Proteinoid Microspheres (Fox, McCauley, Windsor)

By use of the ultraviolet microscope set-up of Dr. Philip O'B. Montgomery of the University of Texas Southwestern Medical School, new evidence has been obtained that the boundary of proteinoid microspheres is selective (Fig. 4). After the pH is raised, the material can be seen progressively to disappear, while the boundary remains. Due to aromatic amino acids, as in protein, the proteinoid absorbs u.v. light. Amino acid assays show some difference between the boundary and the interior but not much. The boundary is thus selective.

This evidence supports similar evidence from the optical microscope, which is however open to interpretations of optical artifacts. The electron microscope pictures were

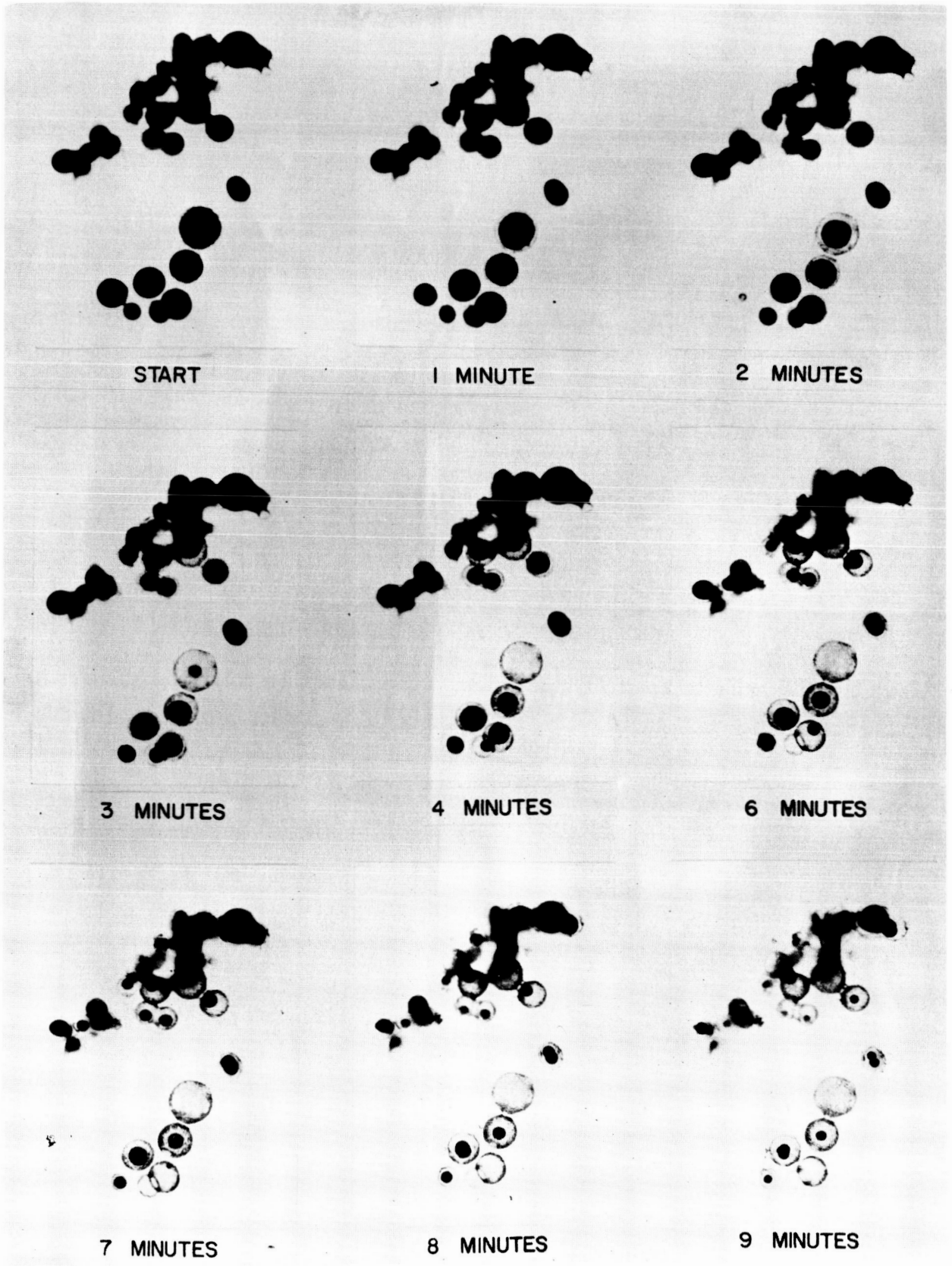


Fig. 4. A suspension of proteinoid microspheres to which buffer has been added to raise pH. Photographed with 2557 A° light. Like protein, the proteinoid absorbs in the ultraviolet. Polymer within the microspheres diffuses through the boundary, which however remains.

however unequivocal in the same way as the u.v. pictures are. The selective retention of polysaccharides by the microspheres, as contrasted to monosaccharides (Fox and Fukushima, earlier) in aqueous suspension is consistent with the photographic evidence.

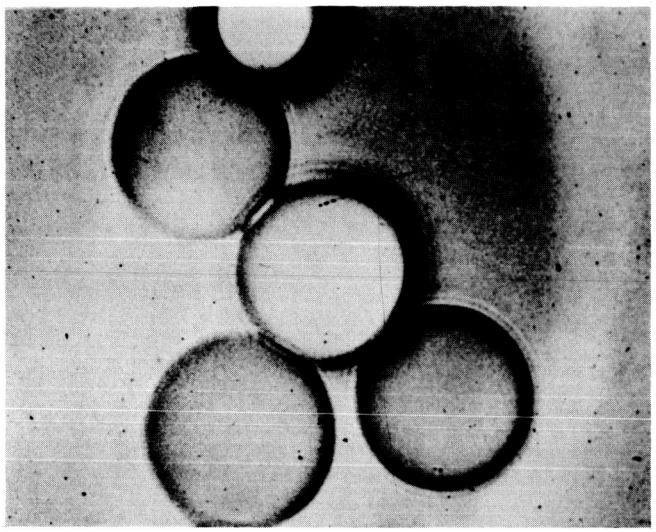
The thermal model thus provides a demonstration not only of how primitive protein might have arisen and how such protein may have formed the structure of the first cells but, also, how such structures could have spontaneously formed selective membranes. In other words, a way in which membranes could first have emerged terrestrially is explained by experiments. The key reaction is the one of appropriate polymerization by heat of amino acids, formerly regarded as a brutal process. The process is brutal when not carried out under appropriate conditions.

Growth in Size of "Buds" (Fox, Joseph, McCauley)

One of the properties not previously described for the proteinoid microspheres is that of growth in size, or enlargement. In a time-lapse sequence published in 1964, one of the "buds" appears to have enlarged to a size comparable to that of its parent microsphere [Particle C in Figs. 1-16 facing p. 319 in Comp. Biochem. Physiol., 11 (1964)]. However, that observation could be merely one of an attached microsphere coming into sharper focus. In the context of interpretation of telemetered recognition of such phenomenon occurring extraterrestrially, the observation is nevertheless significant in that it suggests that similar phenomena could be misinterpreted in this way.

Meanwhile, instances of growth in size of "buds" have been observed; some of these have been recorded in time-lapse cinemicrography. The clearest pictures have been obtained when the microspheres were made in the presence of cholesterol. The proportion of cholesterol is unknown. Fig. 5 represents cholesterol-containing units. The microspheres in these figures remain in the mother liquor in which they were produced.

The mechanism of this growth in size is not understood. The phenomenon is one of those which arises in the course of simulated natural experiments in the empirical mode that is imputable to natural experiments. Whether the growth of "buds" is fed through the microspheres or directly from the medium is scheduled for study through the use of radioactive proteinoid. The finding in telemetered pictures of such data would not distinguish between a terrestrial type of organism of the same general description and the result of spontaneous experiments. The probable imperfections of such pictures would make any fine distinction more difficult.



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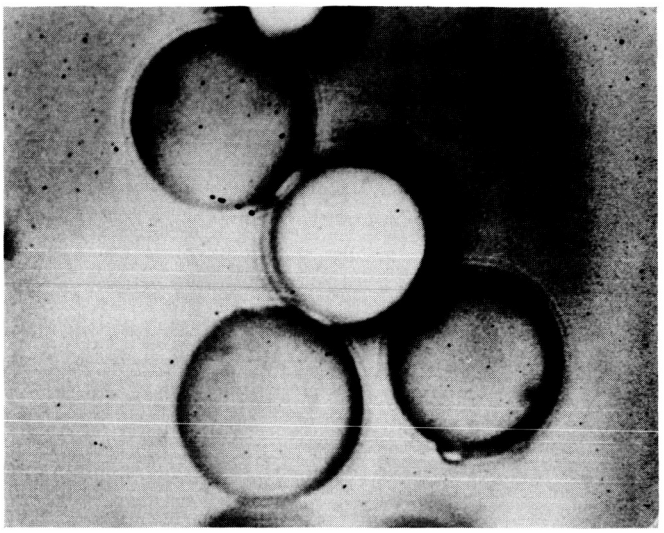

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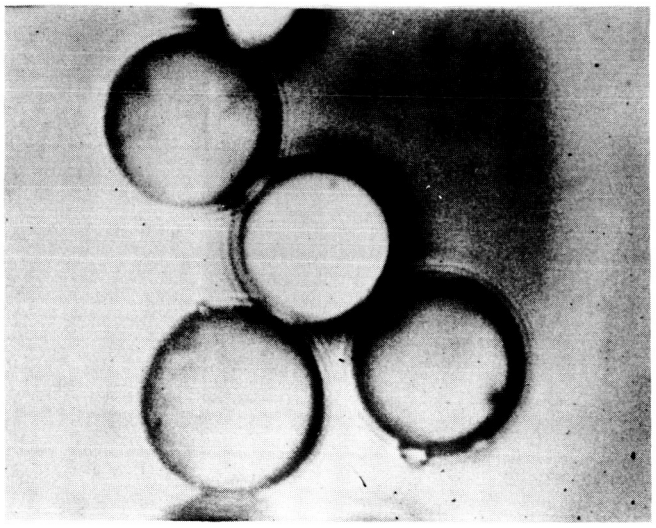

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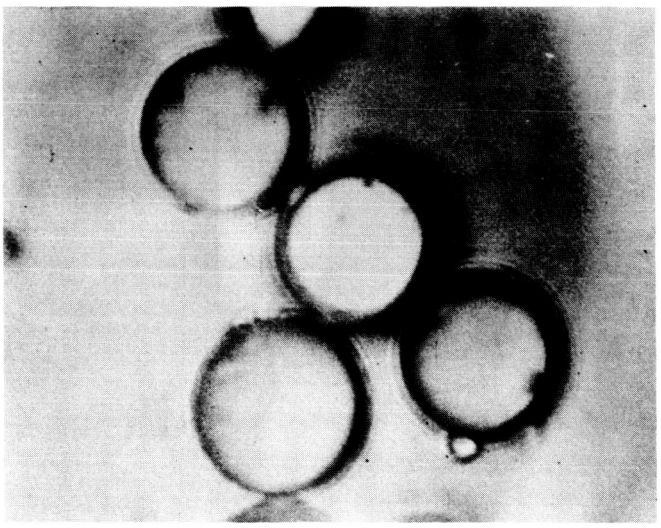

68 HOURS

Fig. 5. Growth in size of a "bud" on a proteinoid microsphere.

Simulation of Motility of a Proteinoid Microsphere (Fox, Joseph, McCauley)

The observation described in this section is one that has been recorded in a number of cinemicrographic sequences. Selected frames to illustrate the effect are presented in Fig. 6.

The sufficient conditions are the presence of at least one asymmetric microparticle in a field of many microparticles. In the sequence reproduced, the asymmetric microparticle consists of a small microsphere attached to a large one. In other sequences, the attached structure is usually filamentous in nature. In the sequence shown, the many uniformly sized microspheres are undergoing a Brownian kind of jiggling with little net movement. The asymmetric particle, however, is moving about in a nonrandom fashion, from field to field and within fields.

Other conditions appear to be, besides the presence of an asymmetric particle, a content of zinc and the addition of ATP to the suspension. The asymmetric particle also rotates. This behavior was first noted by Dr. Oktay Sinanoglu.

As in other phenomena of microspheres, the cinemicrographic sequence is needed to bring out fully the sense of the phenomenon. The rendition of selected frames from the motion picture is however shown in Fig. 6.

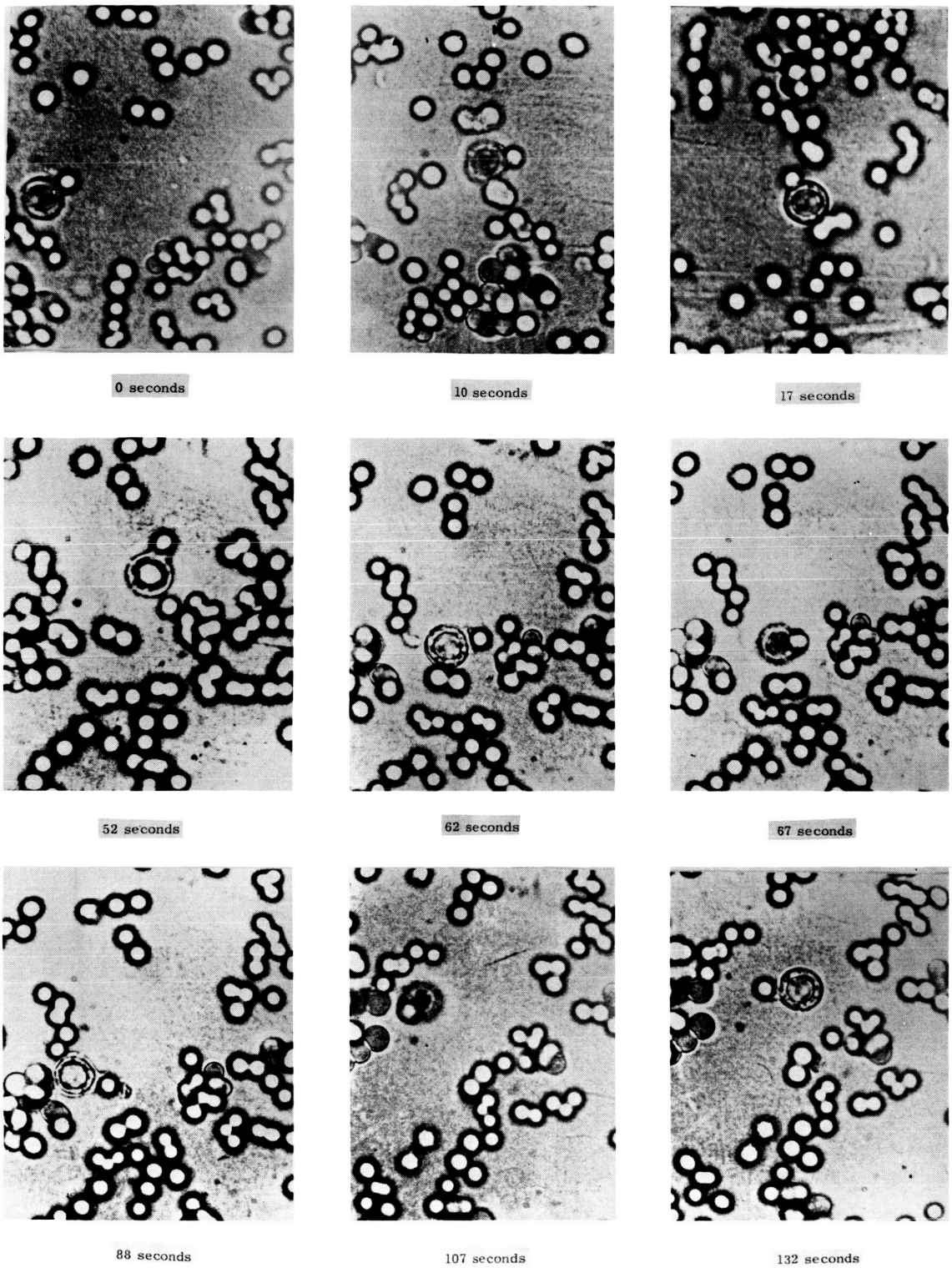

Fig. 6. "Motility" of an asymmetric zinc-containing proteinoid microparticle in a suspension to which ATP has been added. The zincous particles are known to split ATP.

Rotation within a Microsphere (Fox, McCauley)

A number of instances of streaming of contents within proteinoid microspheres have been observed. One instance of rotation of residual center has been caught in time-lapse studies (Fig. 7).

Catalytic Activity in Proteinoid Microspheres (Fox, Joseph, Krampitz, Wiggert)

The associated properties of the proteinoid microspheres have been reviewed (Second Annual Report, ISBS). The way in which salient metabolic activities might arise in these, as in a protocell, is now discernible.

The ability to split ATP has been demonstrated in proteinoid microspheres which are formed in the presence of zinc hydroxide gel. The ability of inorganic zinc salts to catalyze the hydrolysis of ATP at 80° has been reported by Tetas and Lowenstein [Biochemistry, 2, 350 (1963)]. Attempts to localize such activity in proteinoid microspheres have been made and this was accomplished by heating in water the proteinoid with zinc hydroxide gel, and allowing the clear liquid to cool. The zinc-containing microspheres, after washing, split ATP at room temperature. Questions of resistance of these units to solubilization in ATP have yet to be answered.

The ordinary microspheres, lacking zinc, have been shown to break down glucose to glucuronic acid and to decarboxylate this compound or a conversion product.

The microspheres reflect also the ability of the

proteinoid to convert acetic acid to malic acid. An origin of more extensive metabolism can be visualized from some of the other products.

The assembled examples thus illustrate in principle how metabolic activities could have first arisen. More strictly, the activities would not have arisen but would rather have been properties of the polymeric material from which the first organized units arose.

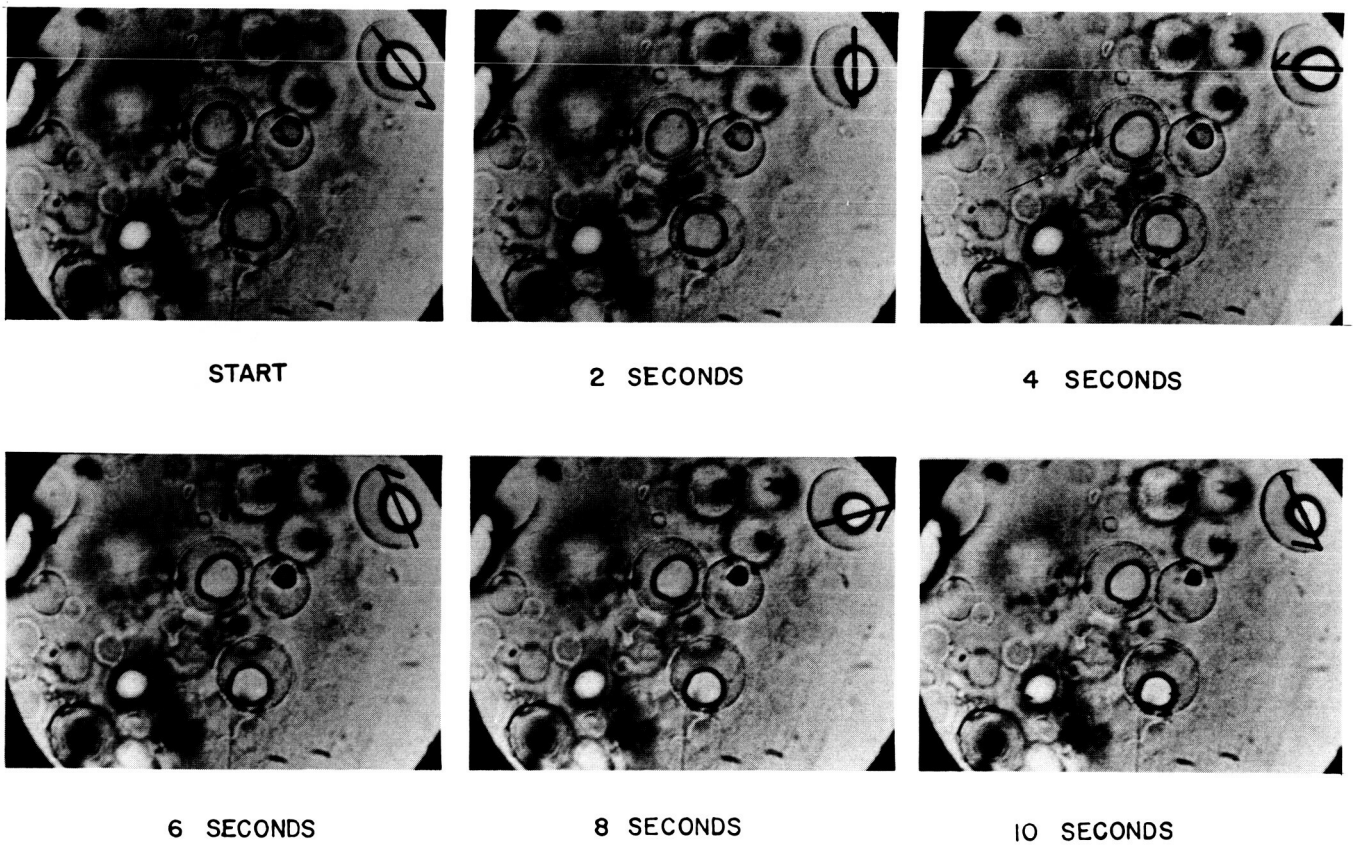

Fig. 7. Rotation of center material in a proteinoid microsphere in aqueous suspension.

Polynucleotide in Proteinoid Microspheres (Fox, McCauley)

Many experiments have been performed to ascertain if morphological or other effects can be observed with the addition of salient biocellular components such as nucleic acid, lipid, histone, etc. to the thermal proteinoid subsequently incorporated into microspheres. Marked changes in morphology have been observed with lecithin and with histone or protamine; some of the results have been published (Young, 1965; Fox and Fukushima, 1964). Effects of added nucleic acids have not been so obvious as with those additants. The selection of experiments of this sort has tended to wait on polynucleotides which would be more appropriate to primitive systems than are nucleic acids of contemporary organisms.

More recently, observably different characteristics have been found in proteinoid microspheres produced in the presence of enzymically synthesized polycytidylic acid (Fig. 8). The microparticles are larger; these are in the range of 10-20 μ in diameter. The granularity of the contents, when the pH has been raised with McIlwain buffer as in this case, is more marked than in proteinoid particles prepared in the absence of polynucleotide.

In an earlier visit to the Virus Laboratory of UCB as guest investigator, Dr. Fox planned with Dr. C. A. Knight similar experiments with TMV. During that visit, emphasis shifted to abiogenic synthesis of uracil which was first identified as a product at that time. The invitation to renew the collaboration has been accepted for summer 1965, at which

time much more knowledge of microsphere behavior is at hand. Fig. 8 represents a study to provide background.

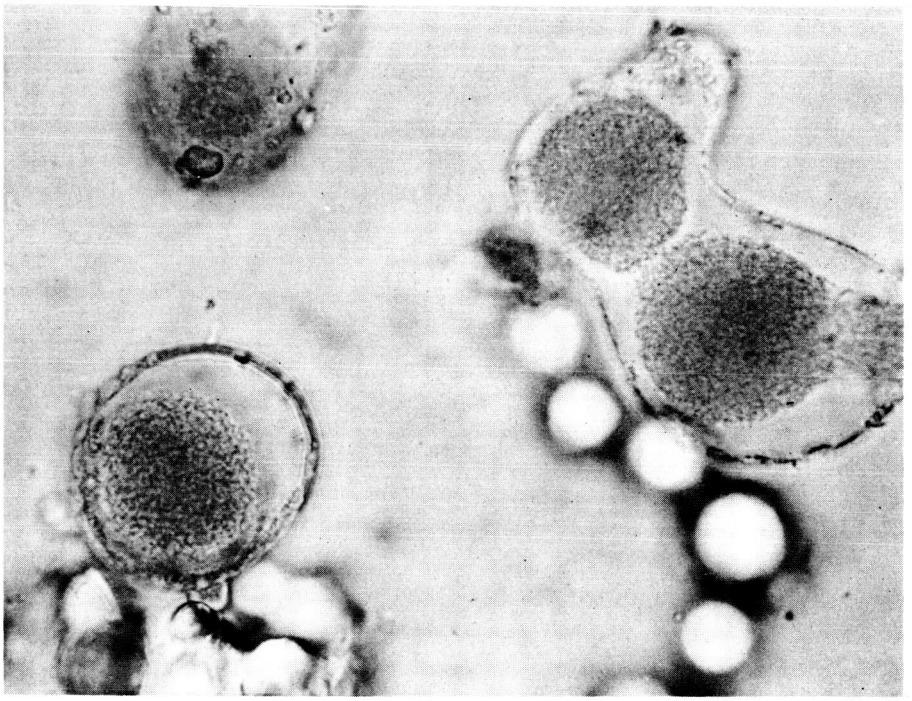

Fig. 8. Proteinoid microspheres prepared in presence of enzymically synthesized polycytidylic acid, followed by increase in pH.

Clarification of Concepts Relative to the Thermal Theory of Origins*

The thermal theory, like any other theory, has been developed from a particular background of orientation and premises. The results have in some cases evoked theoretical discussions for which the following treatments appear to be timely.

Temperatures of Polymerization Condensation and Formation of Organized Units

Two recent books tend to prolong a confusion on the formation of polymers from which the first living units arose, and the temperature which such units could have tolerated. The theoretical sequence consists of two successive steps which should be conceptually disentangled. A high temperature (170° C) is most conducive to polymerization of α-amino acids, for theoretical thermodynamic reasons and on the basis of experimental results. Polymers such as proteins are in fact anhydropolymers. The second step could consist of cooling of the hot polymer by addition of water (e.g. rain) or the polymer could cool spontaneously, and then merely be subjected to contact with cold water. The propensity of such polymers to form a

*The theory developed in this laboratory is referred to as thermal, but not all stages of it are thermal. Some of the reactions are capable of being activated by thermal energies, and some stages involve a drop in temperature, or no change. The term thermal is used for convenient distinction of the total theory.

type of unit having coccoidal appearance and some coccoidal properties is great and can occur at 20° C.

The sequence of steps is very simple, and imputable to the primitive Earth. A moving picture demonstrating these steps on a piece of lava in the laboratory has been on view in the exhibit, "The Origin and Structure of Life", at the American Museum of Natural History in New York.

Ubiquity of Thermal Terrestrial Regions

The widespread nature of thermal regions of the Earth both contemporaneously and in its long history has been documented by Bullard in "Volcanoes", University of Texas Press, Austin, 1962 and in Fox [Nature, 201, 336 (1964)].

More than 450 active volcanoes are known on the surface of the Earth. Volcanic temperatures attain 1100° C, whereas 200° is more than high enough for the polymerization. Accordingly, individual regions at 200° or above would appear to be many times the number of volcanoes (e.g. many hot spots are found in Yellowstone National Park, where no volcanoes are present).

Many, if not all geologists, believe that the crust of the Earth was hotter in earlier times. Bullard, for example, (p. 55) refers to "the great plateau basalts such as those which make up the Columbia River Plateau - - -. Here, - - - with an area of 200,000 square miles, are basaltic lavas reaching a thickness of 3,000 feet and representing hundreds of flows superimposed one upon another. When one realizes that this is but one of many such areas - - -".

These considerations have led to the inference that the polymerization, requiring only a few hours, and spherulization, requiring but minutes, must have occurred innumerable times on the surface of the Earth in its long history.

Consistent with this reasoning, polyamino acids have been found in thermal zones (cinder cone at Kilaeua-Iki). The polymers have thus been demonstrated geologically; demonstration of the emergence of the requisite amino acids geologically has not been offered, and will be more difficult to establish as not arising from organisms already here.

Thermodynamic Considerations of Prebiochemical Reactions

Thermodynamic concepts can be useful in suggesting research in this area and in evaluating the results. In fact, the entire program has developed from recognition of the difficulty of the thermodynamic barrier associated with formation of peptide bonds in aqueous solution [Fox, Vegotsky, Harada, and Hoagland, Ann. N.Y. Acad. Sci., 69, 328 (1957)]. The use of thermodynamic concepts unduly to inhibit consideration of or experimentation with possibilities of prebiochemical reactions should however strictly be avoided.

In particular, thermodynamic considerations have especially been developed for, and are relevant to, single-phase closed systems. Geochemical systems and biological systems are, however, typically multiphase, open systems to which thermodynamics of homogeneous systems are not directly applicable.

Other criticisms of limiting thermodynamic analyses are relevant but the above are crucial. In particular, the visualization of feedback systems, so essential to the processes of life, is easier when homogeneous equilibrium is not attained.

The late Professor Hugh M. Huffman often said, "Thermodynamics will tell you what you can't do, but it will not tell you what you can do."

Synthesis of Peptide Bonds in Water

The simple synthesis of peptide bonds in significant degree in aqueous solution is contraindicated by thermodynamic theory. This statement is not an unduly limiting concept such as referred to in the preceding section; it is a duly limiting concept. The free energy relationships involved in the formation of the peptide bond are such that a few percent of amino acids can be expected to form small peptides such as dipeptides or tripeptides (Fox in Fox, "The Origins of Prebiological Systems", p. 378). The need however is for substantial amounts of large peptides, large enough to yield many organized microstructures. Organisms have developed a means for imparting the necessary free energy to the synthesis of peptide bonds in quantity. The effect can also be attained acellularly by reactions in non-aqueous systems in which the thermodynamics is not prohibitive [Fox, et al., loc. cit.; Meggy, J. Chem. Soc., 1444 (1956); Borsook and Huffman, in Schmidt, "Chemistry of the Amino Acids and Proteins", 1944, p. 822]. No assurance that cells synthesize protein in aqueous

solution is at hand. Intracellular lipid surfaces are a conceivable cytolocale. Cells are organized so that phosphate energy can be fed into synthesis.

Polyphosphoric Acid as a Medium

Phosphoric media have been suggested as the media through which life arose [Fox and Harada, Science, 133, 3468 (1961)]. This medium constitutes a nonaqueous solvent.

The argument that pyrophosphoric acid is likely as an early geochemical substance and that polyphosphoric acid is unlikely has recently been advanced. This kind of contrast overlooks the fact that the constituent of polyphosphoric acid that represents the largest single constituent is pyrophosphoric acid (Harada and Fox, in Fox, "The Origins of Prebiological Systems", p. 290).

Were Nucleic Acids First in Geological History?

The position of genic material in the contemporary cell appears to lead easily to the inference by some that nucleic acid had to appear before protein. Variations of the thermal syntheses are being studied to test several sequences of events, including two in which nucleic acids emerged first. Reasons for questioning the latter type of preorganismic sequence more seriously than before have appeared. The proteinoids, for example, are highly uniform products in repeated syntheses. This "order" arises without nucleic acids. Strominger, Tatum, and others have demonstrated that some primitive organisms make polypeptides by syntheses without template control and Lipmann (in Fox, "The Origins of Prebiological

Systems", p. 271) has emphasized that primitive polypeptides might be so produced. The thermal model demonstrates that the self-organizing properties of poly-α-amino acids are sufficient to explain the origin of a number of subtle phenomena such as coccoidal shape and size with divisional properties, double layers, selective diffusion, motility, internal streaming, etc. These results are consistent with a prediction made by Professor George Wald, in 1954, to the effect that the first organism might form as a result of the properties of the material from which it was composed. Both the prediction and the results are consistent also with the now extensively demonstrated self-organizing properties of bioprotein (Schmitt, Gross, Reed, etc.).

While the several possible sequences of events are being investigated in variations of the model, the results to date suggest that reliance on the one premise of an initial nucleic acid could have obviated experimental progress such as the finding of the limited heterogeneity of thermal poly-α-amino acids or of their extensive morphogenic properties. One position with respect to nucleic acids was expressed by S. W. Fox in Jan. 1959 (Bull. Amer. Inst. Biol. Sci., 9, 20-24) as follows: "We may thus visualize that the complex administrative substances, the nucleic acids and/or nucleoproteins, have appeared at a late stage when the business is complete, and thus available for effective administration". Since this is neither a conclusion nor an unquestioned premise, experiments based on each of the premises are in process or are planned.

Relationship to other Similar Studies Reported in Literature

Other similar studies have been reported by Oparin, Bahadur, Perti, Kalinenko, Briggs, and by Young.

Oparin's work has been with a cell model, the coacervate droplet. Oparin uses the materials introduced in the late 1920s by the Dutch colloid chemist, Bungenberg de Jong. These are lacking in stability, as Oparin himself points out. The stability can be increased by inclusion of other materials, e.g. nucleic acids. The crucial difference between coacervate droplets and proteinoid microspheres is that the former are produced from biological polymers. The proteinoid microspheres arise in an entirely acellular series of processes, in a way that can be imputed to the primitive environment.

Bahadur has reported results involving the generation from amino acids of units having the powers of growth, multiplication, and metabolism. In this area Bahadur received his orientation in this laboratory. Documented statements of the unjustified interpretations by Bahadur and of other aspects of the nature of the reports are found in the Second Annual Report of the Institute for Space Biosciences.

O. N. Perti, a colleague of Bahadur's, has reported (Agra Univ. J. of Research) on a theory of the origin of life. While he acknowledges some of the early work of this laboratory, he states that no explanation has been advanced for the way in which cells might have arisen. This statement ignores

the main emphasis in papers published as early as 1960.

M. H. Briggs has reported a confirmation of some of the processes described by Bahadur (J. British Interplanetary Society). The report of Briggs mentions the pioneer aspects of proteinoid microspheres in the context of protocells. The microspheres obtained are stated to have some of the properties of cells; they are not presented as Jeewanu (Sanskrit for particles of life), as Bahadur referred to them.

In the reports of Kalinenko (NASA translation no. TTF-9239) photomicrographs resembling many of those of the proteinoid microspheres have been published. The papers suggest that the material arises electrically but no chemistry to explain the results is offered.

The work of R. S. Young and colleagues (Young in Fox, "The Origins of Prebiological Systems", p. 347) represents a confirmation and extension of the studies on proteinoid microspheres and is regarded as arising from sound investigation and interpretation.

In the total context of the reports referred to in this section a central contribution of this institute is the laboratory demonstration that by the thermal condensation of amino acids and the effect of added water, microscopic units having many of the properties of biocells emerge spontaneously. The combinations of amino acids and the range of conditions yielding such combinations have been shown in this institute to be wide. The structure of the polymers and the properties of the formed units have been extensively and carefully examined.

Rigorous evidence on the relationship of any sequence of planetary chemical processes and the origin of life may be accumulated only after many years beyond the period in which relevant hard planetary data first become available.

The experiments spanning a conceptual sequence involving primordial gases, amino acids, primitive protein, and organized prebiological systems demonstrate what might occur on a planet, as these results might influence judgments from a distance. In addition, they suggest salient phenomena to seek extraterrestrially, such as particular balances of amino acids, the identity of forms of phosphorus, regions with a thermal history, regions having a history of alternating dry - moist conditions, etc.

The experiments also demonstrate the possibility of an eventual total theory of how life originated, and they represent the first unified route which has been advanced on the basis of laboratory studies. The essence of some of the contributions has been presented in other ways by authors who are, relatively, experts exercising perspective. Some of these treatments which have appeared recently are: Molecules to Man (AIBS, 1963), The Cell (Life Science Library, 1964), and The Origin and Structure of Life (American Museum of Natural History, 1964).

Tests for Life which are Met by Proteinoid Microspheres and Other "Spontaneously" Synthetic Systems

Although the selection of criteria for life has been regarded by some (Pirie, Calvin, Keosian, etc.) as unsatisfactorily subjective, subjectively selected tests will undoubtedly have to be used in attempts to recognize life by devices placed on Mars or on other planets. The validity of these tests can in part by judged by the ease with which synthetic particles possessing such attributes are produced in the laboratory under conditions that could exist on the planet.

A listing of such tests (details are referenced in the bibliography at the end of the report) and the finding of positive or negative results with synthetic materials or particles follows:

Attribute	Result of Test
Small Molecules	
Net optical activity (from racemic molecules)	+
Biochemical staples (within an organized unit)	+
Proteinoids	
Net optical activity in poly-α-amino acids (derived from optically active monomers)	+
Susceptibility to proteases	+
Limited heterogeneity by several tests	+
Repeatability of total composition	+
Repeatability of terminal (sequential) composition	+
Some catalytic activities	+
Other catalytic activities	-
Ordered sequence within proteinoids	+
Alterability of sequence within macromolecules by outside agent	+
Thermal Polynucleotides	
Susceptibility to ribonuclease (3' linkages)	+
Susceptibility to venom phosphodiesterase (5' linkages)	+
Organized Microparticles	
Structured coccoidal particles	+
Variety of morphologies in microparticles ("buds", filaments)	+
Growth in size of coccoidally shaped units	+
Bilamellarity	+
Streaming within unit	+

Ability of units to proliferate	+
Ability of units to divide in simulation of terrestrial organisms	+
Osmotic responses	+
Selective diffusion of molecules through boundaries of microparticles	+
Motility	+
Energy transfer	?
Metabolic pathways	+
Cyclic metabolism ("feedback")	-
Death (cessation of dynamic activity)	+
Antigenicity	-
Cyclic growth and division	-

The above list is not exhaustive. It could be greatly lengthened by inclusion, for example, of some dozen other tests for protein, all of which tests are positive for thermal proteinoids. Other attributes for which tests are negative could also be listed. The absence from the table of positive tests for some properties, e.g. antigenicity, indicates not that the proteinoids are necessarily nonantigenic, but that studies have so far failed to reveal how they might be antigenic. Some proteins, incidentally, are nonantigenic. All organisms, however, appear to have metabolic cycles.

Such key attributes as information transfer, in this context, need to be reexamined in the light of the fact that proteinoids are unexpectedly and with high repeatability limited in the degree of their heterogeneity. Also of relevance is the fact that the balance between total composition and terminal composition is altered to, again, reproducible analyses by the outside agent phosphoric acid, which does not enter into the product in significant proportions.

The conditions under which any of the designated attributes emerge in the laboratory are so simple that their relatively easy spontaneous origin on a planetary surface cannot be dismissed. The mechanism of the phenomenon is not understood in each case. The significance of the phenomena relative to evaluation of the extraterrestrial tests is however essentially not altered by the fact that the knowledge was gained empirically. Although refinements of some of the tests

might presumably lead to an ability to distinguish spontaneous chemical experiments from life, utilization of such refinements in telemetered tests may require special study.

Direction of Studies of Physiology and Reproduction (Metz)

Research and instruction in this field proceeded on several fronts. These included ultrastructural, biochemical and immunochemical studies on marine invertebrate gametes and sperm-egg interaction and physiological studies on gamete interaction in the alga, Chlamydomonas.

Ultrastructural Studies

Sperm-egg Interaction in the Sea Urchin (Franklin and Metz)

Electron microscopical studies of early stages in sea urchin sperm-egg and sperm-oocyte interaction have been completed. The study constitutes the Ph.D. thesis of Dr. Luther Franklin [J. Cell Biology 25, 81-100 (1965)]. This study reveals that initial contact and subsequent fusion of gamete membranes involves the "reacted" acrosomal membrane of the sperm. Sperm were observed and sections were photographed in various stages of entry into oocytes. Membranes disappeared from the nucleus and middle piece of the sperm during entry into the egg. Some of the photographs obtained in this study were reproduced in the Second Annual Report.

This study confirms the "end-on" sperm-egg membrane fusion as a regular process in the sea urchin, comparable to that described by the Colwins in Hydroides. However, the ultimate fate of the sperm cell membrane following sperm entry remains to be determined.

Comparative Ultrastructural Studies on Crustacean Sperm Morphology and Sperm-egg Interaction (Brown, Metz)

Spermatozoa of many crustacea are atypical in that they are non-motile, lack a conventional flagellum and mitochondria and they frequently have arm-like extensions. This relatively little studied group is under intensive investigation in this laboratory to elucidate the complex structure of these cells, to determine their mode of interaction with the egg and, if possible, to find a sequence of possible evolutionary steps leading from conventional flagellated sperm to the bizarre forms illustrated in Figures 9-12.

So far, ultrastructural studies supported by phase-contrast optics and cytochemistry have been carried out primarily on morphological and functional aspects of the Callinectes sapidus and Menippe mercenaria spermatozoa. The complex acrosomal region (Figs. 9-12) is an organelle 2.5 - 3.0 microns in diameter which consists of a PAS-positive acrosomal vesicle and a centrally located PAS-negative acrosomal tubule. This region is partially bounded by a lamellated membranous central region and is embedded in a nuclear cup with only its apical cap exposed to the sperm surface (see especially Figs. 9 and 11). The Feulgen-positive nuclear cup has eight radial processes or arms. In sperm penetration of the egg, these arms attach to the egg chorion (Fig. 13), a process bringing the apical cap into direct contact with the chorion. Under certain experimental conditions (slight pressure on coverslip or certain osmotic concentrations) the

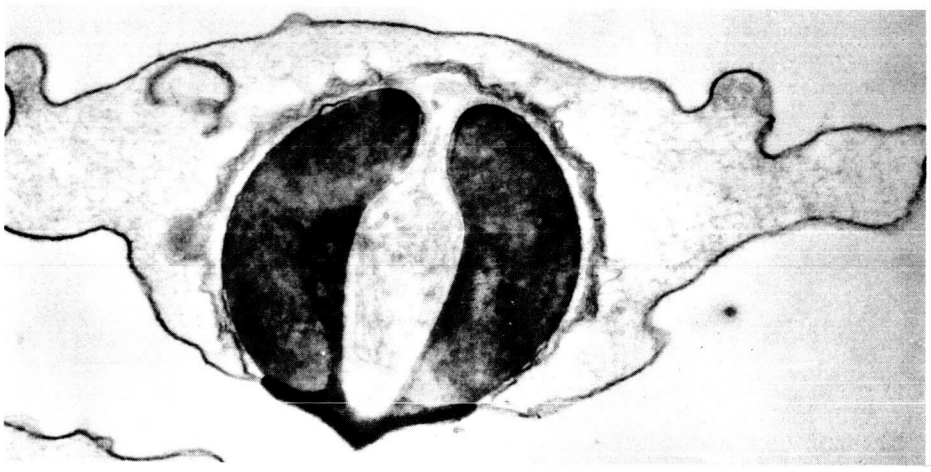

Fig. 9. The relationship of the acrosomal region to the central region and nuclear cup. Callinectes sapidus. Glutaraldehyde plus OsO_4. Electron micrograph, X 24,000

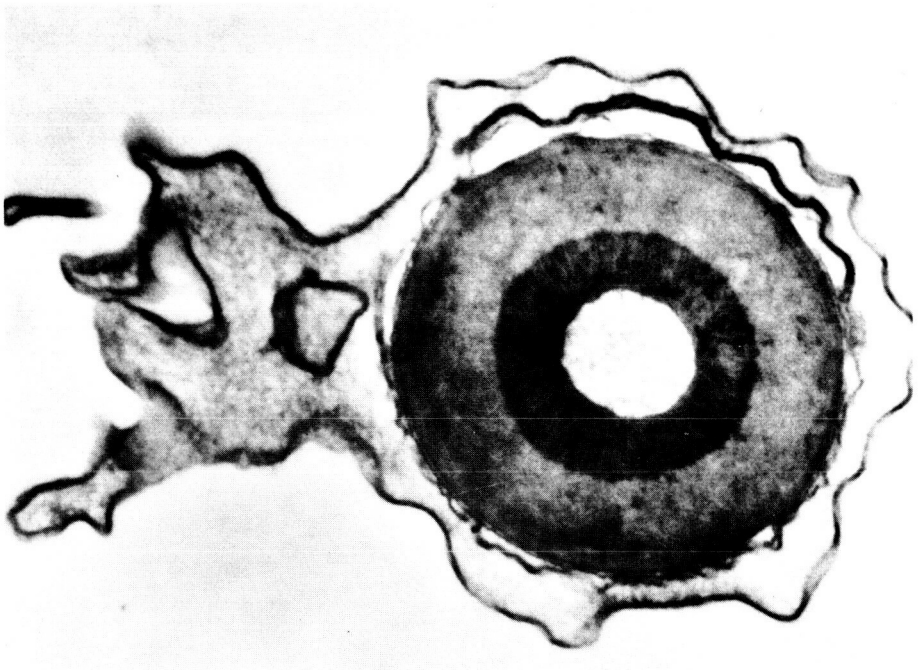

Fig. 10. A cross section of a C. sapidus sperm. OsO_4. Electron micrograph, X 33,000

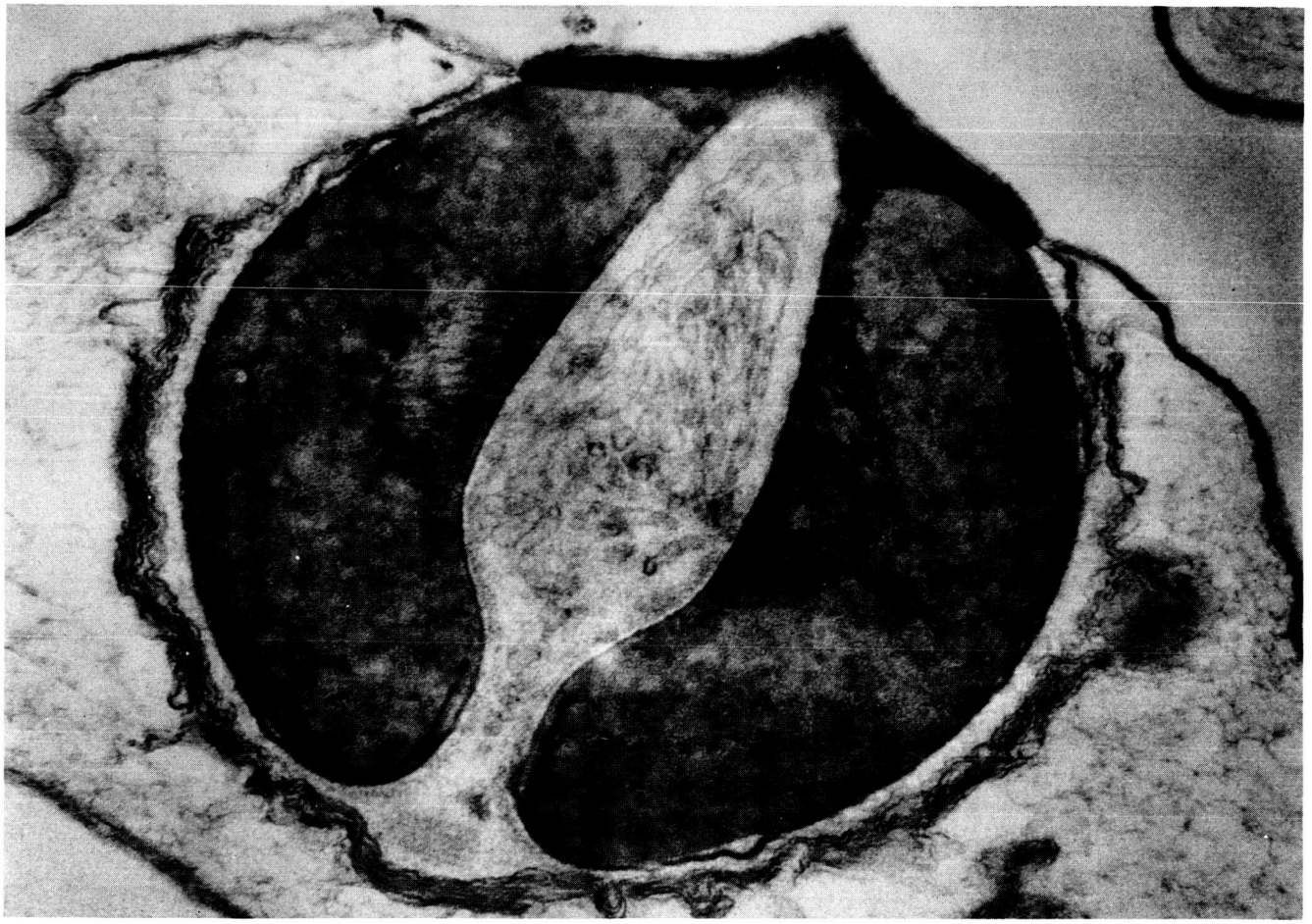

Fig. 11. A longitudinal section of a C. sapidus sperm revealing ultrastructural details of the acrosomal region. Glutaraldehyde plus OsO_4. Electron micrograph, X 68,000

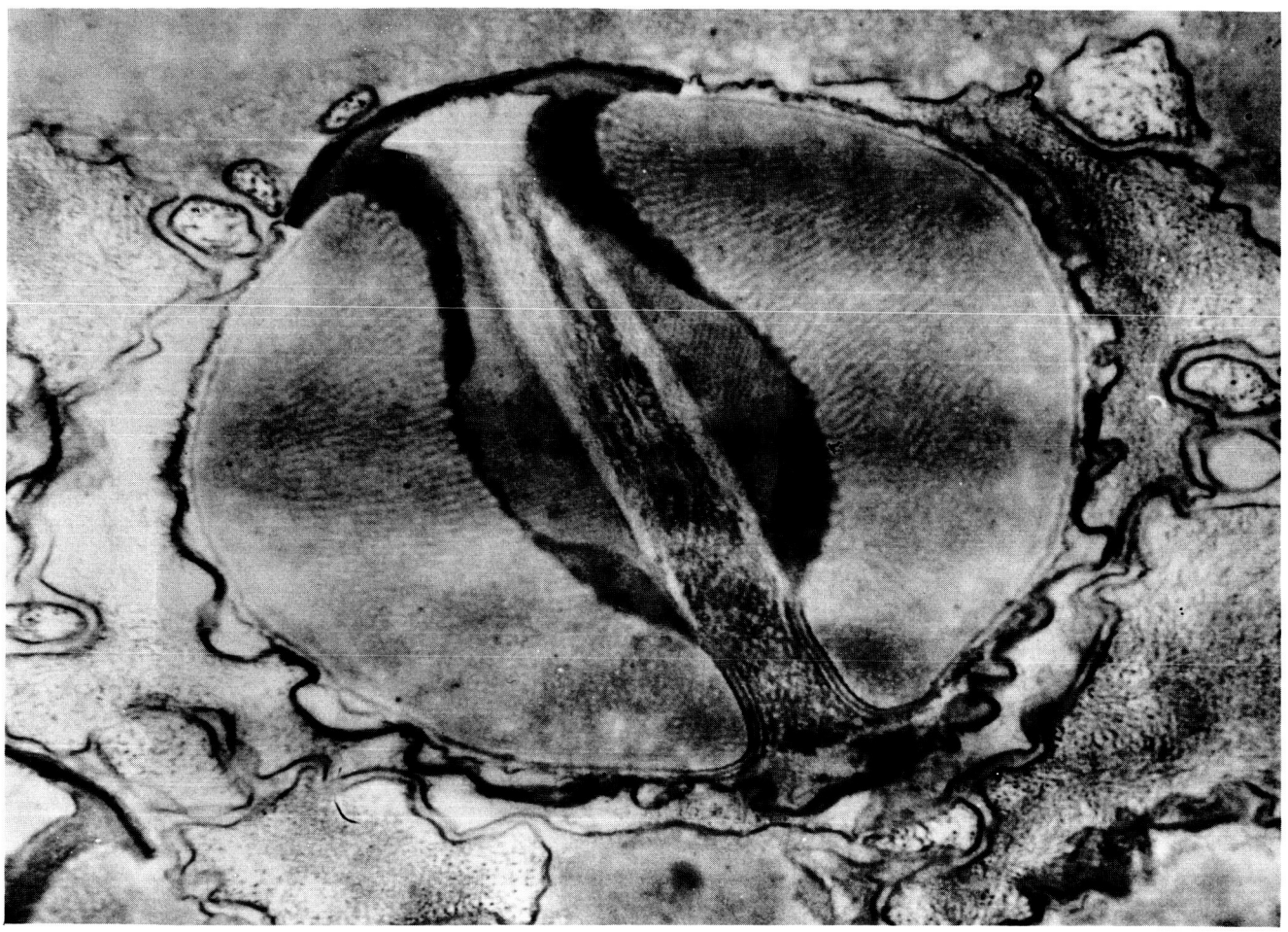

Fig. 12. A longitudinal section of a Menippe mercenaria sperm in a spermatophore. Cross sections of the radial arms wrapped around the sperm body are observed. OsO_4. Electron micrograph, X approx. 60,000

acrosomal reaction occurs. This converts the spherical acrosomal region into a complex extension as shown in Figs. 14 and 15. Simultaneously, a rounding up of the nucleus with disappearance of the arms occurs.

In fertilization following attachment of the sperm to the egg chorion the acrosome reaction is initiated and the acrosomal region is everted (Figs. 14 and 16) through the chorion causing a cup-shaped depression in the egg plasma membrane (Figs. 14 and 16). The acrosomal tubule comes into direct contact with this membrane and possibly initiates the entry of the nuclear material into the egg cytoplasm. The aspects studied present a basis for comparing the C. sapidus (e.g. Fig. 11) and M. mercenaria (Fig. 12) spermatozoa (previously regarded as atypical) with conventional spermatozoa. For example, these ultrastructural studies suggest that the arms-chorion attachment may involve "attachment substances" and penetration of the chorion may involve lysins from the sperm.

Further studies have involved ultrastructural and functional aspects of gametes of the freshwater shrimp Palaemonetes paludosa. The sperm of this decapod is quite unusual in consisting of a discoidal nucleus bordered on the concave side by numerous large vesicles and on the convex side by a long spike containing collagen-like fibrils which is non-motile (Figs. 17 and 18). This sperm undergoes a reaction which involves the rounding of the nucleus (Fig. 17),

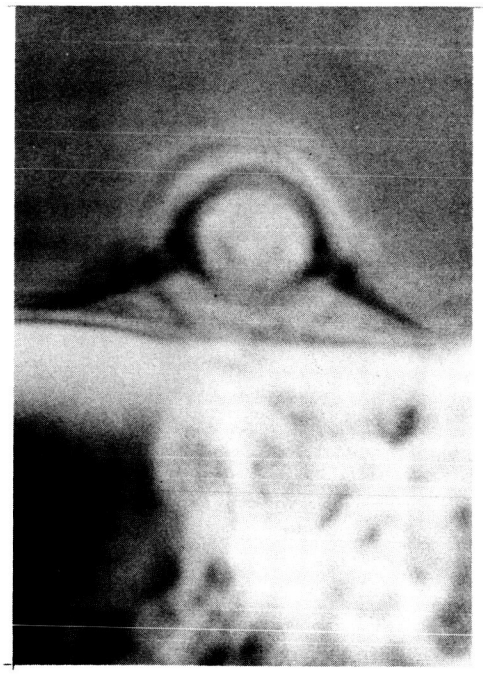

Fig. 13. A sperm attached by arms with the acrosomal region adjacent to the egg. This is the sperm position before the acrosome reaction and penetration of the chorion. C. sapidus. Living material. Phase-contrast, X 5,700

Fig. 14. Sperm penetration through the chorion of the egg. The cup-shaped depression is the egg plasma membrane pushed inward by the acrosome reaction. The acrosomal tubule is against the egg membrane. C. sapidus. Living material. Phase-contrast, X 5,700

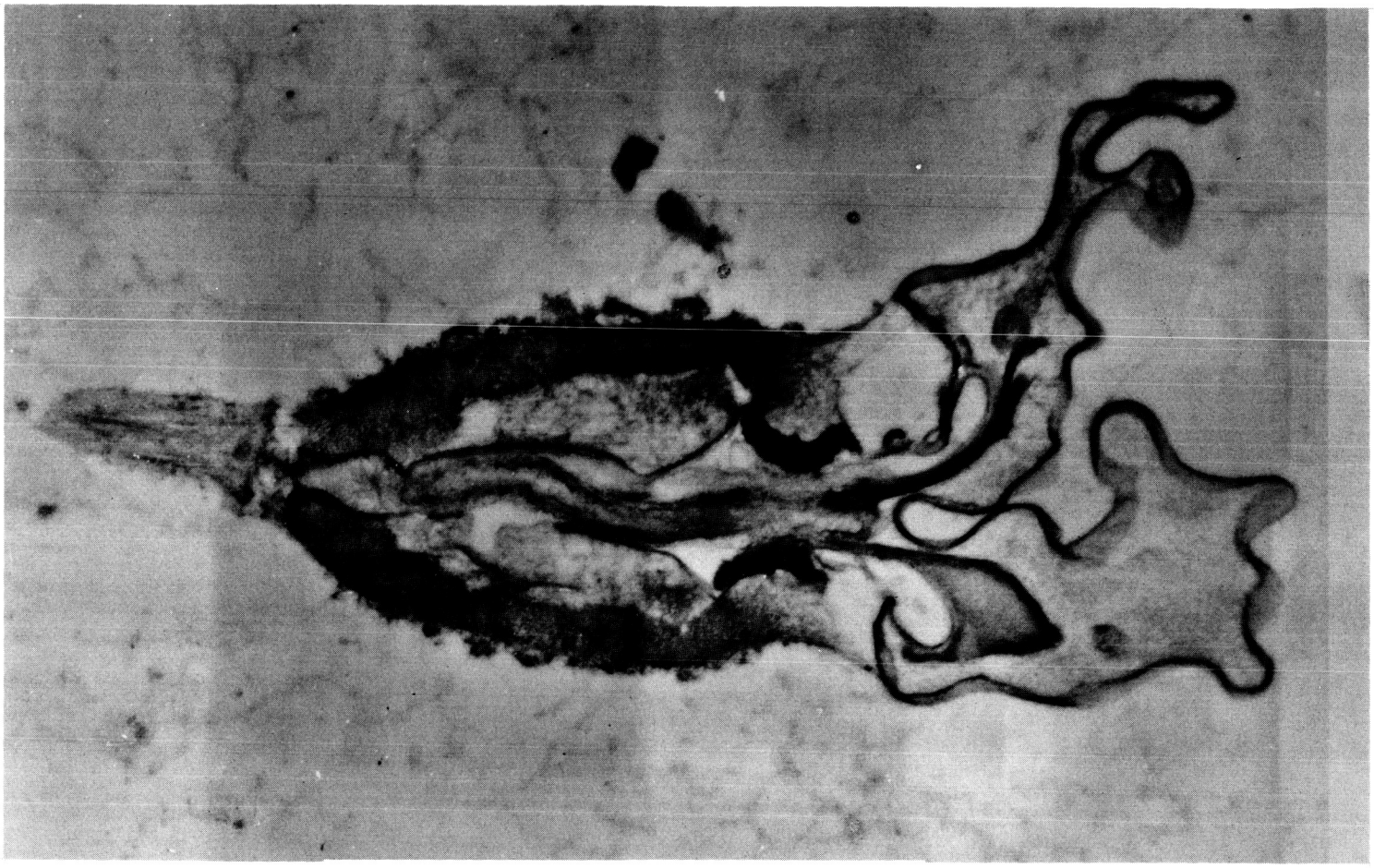

Fig. 15. A longitudinal section of a reacted C. sapidus sperm (acrosome reaction). The everted acrosomal material is condensed due to the preparatory method. OsO_4. Electron micrograph, X 23,400

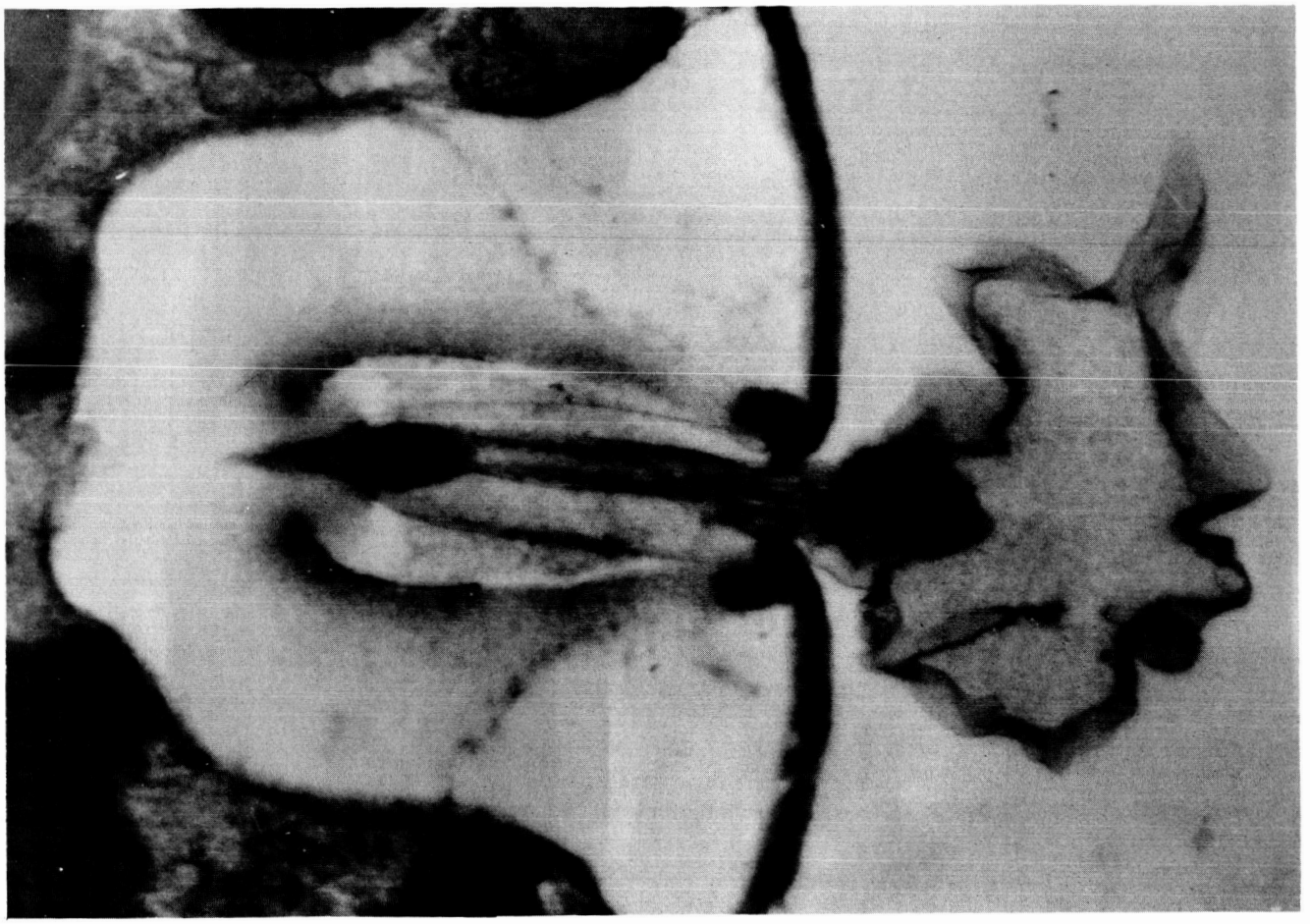

Fig. 16. A longitudinal section of sperm penetration of the egg chorion. The everted acrosomal material is in contact with the egg plasma membrane forming a cup-shaped depression. C. sapidus. OsO_4. Electron micrograph, X 23,000

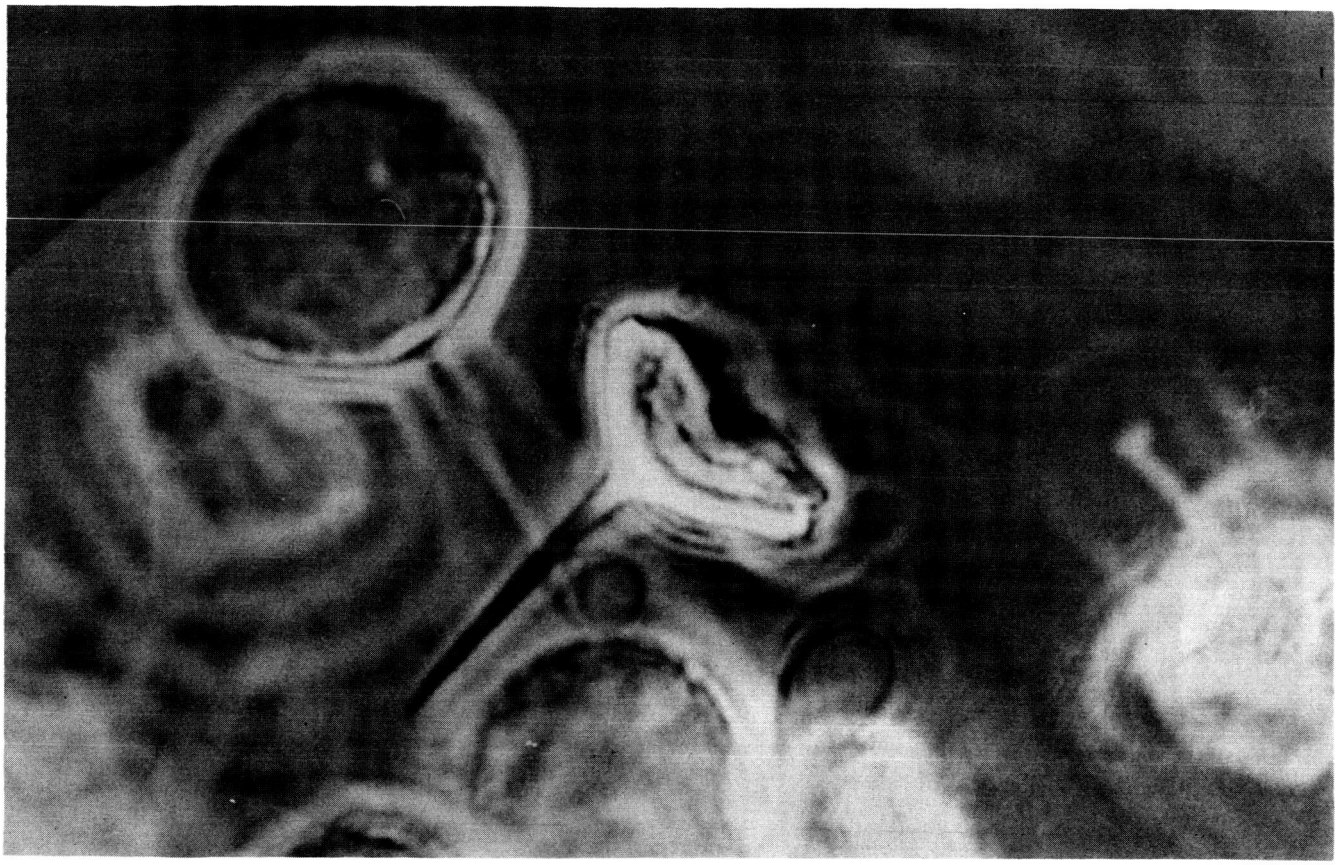

Fig. 17. The unreacted and reacted spermatozoa of the freshwater shrimp Palaemonetes paludosa. Living material. Phase-contrast approx. X 3,500

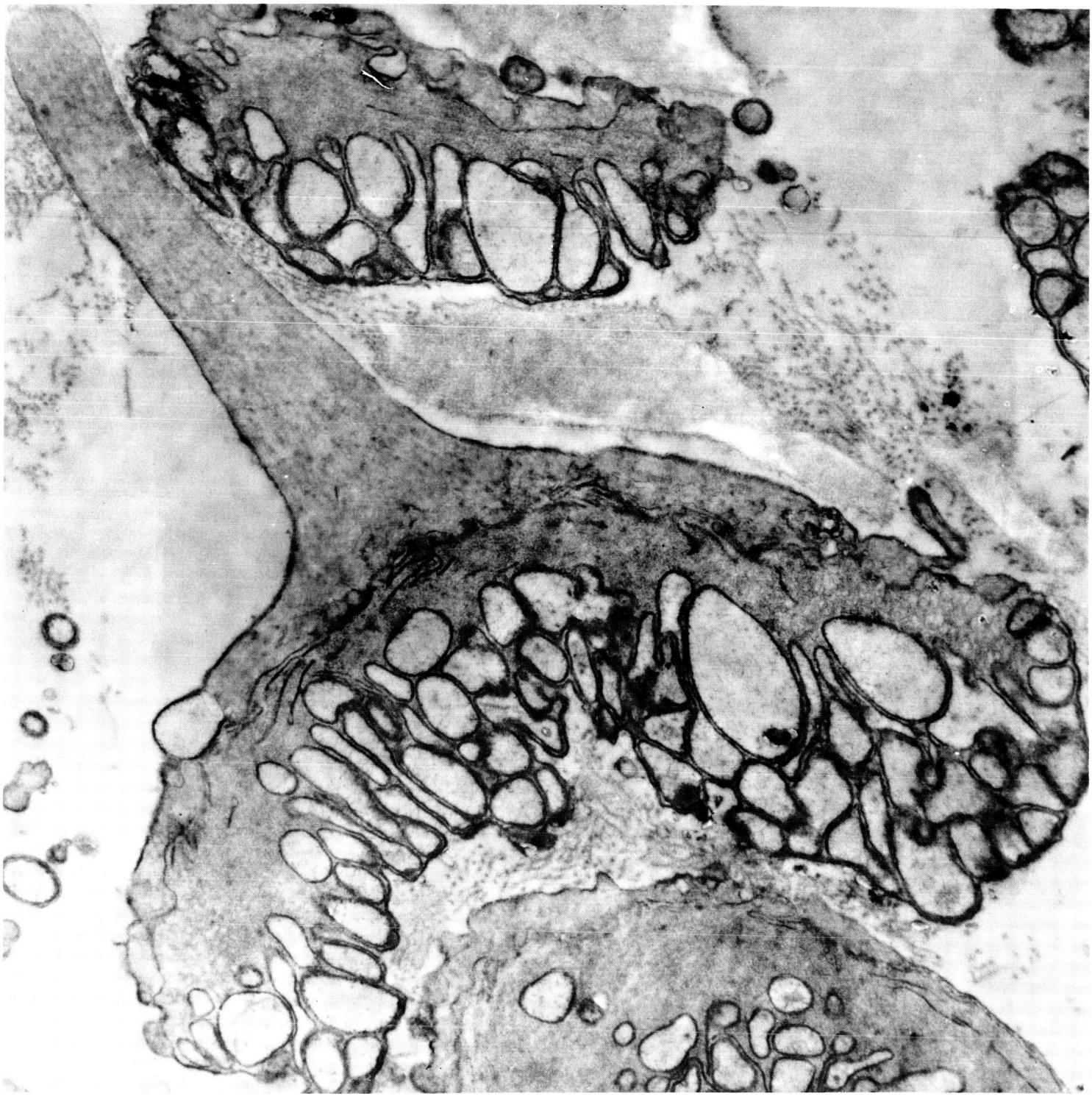

Fig. 18. Longitudinal section of a P. paludosa sperm. OsO_4. Electron micrograph, X 23,000

however, the functional aspects of this reaction have not been determined.

Comparative Ultrastructural Studies on Fish Sperm (Tyler, Metz)

Some fish sperm and especially teleostian sperm have been reported to be atypical. For example, toadfish sperm are reported to have two tails. This has been confirmed in this and other laboratories. Several reports indicate that teleost sperms lack an acrosome. Since this cellular organelle, derived from the Golgi apparatus at spermatogenesis, plays significant roles in fertilization in many forms, its absence in teleosts presents interesting problems. In view of the wealth of fishes both fresh water and marine (some of which are quite primitive, e.g. Amia and gar pike) in the Miami area it seemed worthwhile to conduct a comparative study of representatives of some groups locally available. Amia, the holostian which occupies an intermediate position in bony fish evolution, is currently being examined.

Chemical and Immunological Studies on Fertilizin, the Sperm Isoagglutinin from Eggs (Stern, Metz)

The jelly surrounding eggs of sea urchins and a variety of other organisms dissolves on standing in water. The solution specifically agglutinates sperm of the species and appears to be a glycoprotein (see Second Annual Report for more details).

This jelly material (called fertilizin) from sea urchin eggs has some unusual properties. For example, the agglutinin

readily dissociates to a univalent, non-agglutinating form. Hathaway, working in this laboratory, showed indirectly that the conversion resulted in the release of an "inert" fraction which failed to combine with sperm. In addition, one or more fragments of the molecule retained the capacity to bind to sperm.

The nature of the fertilizin molecule is of considerable interest because it evidently plays a significant role in fertilization and, in addition, it has inherent interest in relation to the structure of large molecules.

Fertilizin from the eggs of the sea urchin Lytechinus variegatus were used. The material was converted to the univalent form by oxidation with H_2O_2. Such oxidized material no longer agglutinated sperm but still combined with the sperm surface.

Immunodiffusion tests were performed using rabbit antibody prepared against multivalent fertilizin. In such tests the univalent and multivalent preparations yielded a common precipitin band. In addition, the multivalent material produced an additional unique band. These results suggest that the multivalent material was partially degraded to the univalent form and was a mixture of univalent and multivalent material.

Analysis of the carbohydrate portion of fertilizin was done utilizing both paper chromatography and the cysteine HCl method of Diesche and Shettles. The only monosaccharide found was fucose, which accounts for about 30% of the material. No significant difference was found in the amount of fucose

present in the native and univalent form. Amino acid analyses done on the Phoenix amino acid analyzer show the presence of all of the most common amino acids, except for the tryptophan (destroyed by acid hydrolysis) and no detectable amounts of cysteine or methionine. The amino acid patterns were qualitatively and quantitatively the same for both univalent and multivalent fertilizin. These analyses indicate that conversion of fertilizin to the univalent form does not result in release of low molecular weight, dialyzable fragments such as monosaccharides and individual amino acids.

The sedimentation value (uncorrected for temperature and solvent) of a 1 mg/ml solution of fertilizin in 0.1 M NaCl is 5.6 Svedberg. A solution of univalent fertilizin made from the native material and containing an equivalent amount of fucose has a value of 3.4 Svedberg.

Viscosity measurements of multivalent and univalent fertilizing carried out in a Cannon-Ubelohde semi-micro dilution viscometer indicate that the native material is extensively depolymerized in the formation of univalent fertilizin.

Electron microscope studies of the native material showed at relatively high concentrations (0.2 mg/ml in H_2O), threads of a long (10-20 μ) highly branched nature. With dilution, the length of the strands decrease as does the branching. This was carried out using copper grids coated with a carbon film, streaked across the fertilizin solutions, shadowed with platinum and viewed in the Philips 100B.

Toluidine Blue combines with the $SO_4^{=}$ groups of fertilizin giving a metachromatic reaction. The extent, i.e., sensitivity of the reaction, is the same with both the native and univalent forms. As little as 5 gamma can be detected by this method.

To further compare the multivalent and univalent materials, these were subjected to electrophoresis on cellulose acetate strips and stained with Toluidine Blue. The univalent material yielded three well defined bands some distance from the origin whereas the multivalent preparation produced a single strong band at the origin and a diffuse staining reaction in the region of the bands in the univalent material.

Univalent fertilizin that had previously been absorbed with sperm produced only one of the three Toluidine Blue staining bands (Fig. 19).

These studies then show that conversion of fertilizin to the univalent form results in a lowering of viscosity, a drop in molecular weight and increased electrophoretic mobility. In addition, the electrophoresis study suggests a splitting of fertilizin into three fragments, two of which retain combining sites for reaction with the sperm and one fragment which does not bind to sperm. This interpretation is consistent with the observations of Hathaway.

Finally, these results on fertilizin structure provide an interesting parallel with antibody structure. As is known from the work of Porter (1958) rabbit antibody (7S gamma

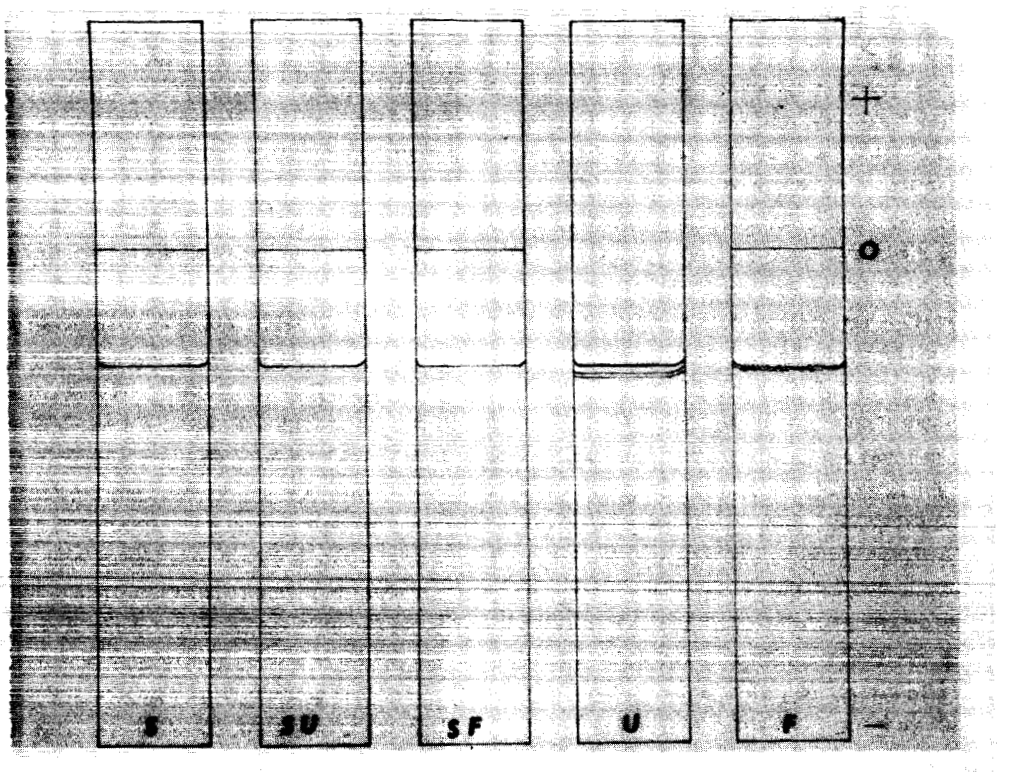

Fig. 19. S = Supernatant of sperm and sea water
U = Univalent fertilizin and sea water
F = Multivalent fertilizin and sea water
SU = Supernatant of sperm and univalent fertilizin
SF = Supernatant of sperm and multivalent fertilizin
O = Origin

One cc of a 20% sperm suspension was added to three different test tubes containing 1 cc of sea water, univalent fertilizin, or multivalent fertilizin. One cc of sea water was added to 1 cc of univalent fertilizin and multivalent fertilizin. All mixtures remained at room temperature for 30' and then were centrifuged at 4° C. The supernatants were collected and small aliquots applied to separate strips of cellulose acetate. Current was applied for 20 minutes (200 V., pH 8.6). The strips were stained in Toluidine Blue and the distance of migration of each metachromatic band was measured.

globulin) can also be fragmented into one inert (Porter fraction III) and two reactive (Porter fractions I and II) fragments.

Immunochemical Studies on Sperm Antigens (Metz, Hampson)

Previous studies (see Second Annual Report for details) have demonstrated that sea urchin sperm have at least one sperm surface "fertilization antigen". It now becomes of interest to localize and characterize this and other sperm antigens. Preliminary experiments with fluorescein-conjugated antibody show that use of this reagent combined with fluorescence microscopy does not have sufficient resolution. Therefore recourse is now being made to the use of ferritin-conjugated antibody combined with electron microscopy. Initial results indicated that an extensive purification of the ferritin-antibody conjugation mixtures was needed to obtain a reagent relatively free of competing unconjugated ferritin and antibody. Separation of ferritin from unconjugated antibody was readily achieved by sucrose gradient ultracentrifugation.

In attempts to achieve separation by column chromatography, it was found that 2X crystallized horse spleen ferritin from commercial sources is not homogeneous. The ferritin produced five peaks in DEAE Sephadex chromatography (Fig. 20). These were indistinguishable when tested with antiferritin antibody in gel diffusion precipitin tests. The peaks retained their identity when rerun through DEAE Sephadex indicating that the material is not an equilibrium of molecular aggregates. Furthermore, electron microscope examination

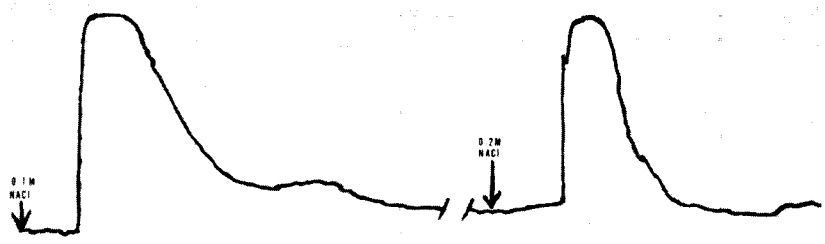

Fig. 20. Step gradient elution of ferritin (2 X crystallized, NBC) from DEAE Sephadex (A50M); column equilibrated with 0.02 M $PO_4^{\equiv}$ containing 0.025 M NaCl, pH 7. Elution with 0.1 M NaCl yields a major peak followed by a minor "bump". Subsequent addition of 0.2 M NaCl yields a major peak preceded and followed by single minor peaks. These minor peaks can be resolved better by more gradual increase in NaCl concentration.

failed to show any consistent aggregation relationship of ferritin molecules to the DEAE Sephadex peaks. However, preliminary sucrose gradient centrifugation experiments suggest some separation into components of varying density. Although this problem is now beyond the area of the main effort, it seems of sufficient interest to warrant some further analysis, for example, examination of crystalline ferritin freshly prepared from a single horse. It seems possible that there may be individual variations among ferritins from different horses.

In an attempt to fractionate soluble sperm antigens, sea urchin sperm extracts were subjected to column chromatography. So far, several protein fractions have been demonstrated. It is expected that these may be obtained in sufficient quantity to provide material for standard immunological procedures such as absorption and immunization.

Fertilization Studies on Microorganisms (Wiese, Metz)

Some of the apparently simplest sexual mechanisms are found among microorganisms. Of these, the unicellular alga Chlamydomonas is unusually favorable since it can be cultured in sterile, defined medium and gametes can be produced in large numbers by appropriate control of the environment.

Sexes are isogamous (e.g. gametes morphologically indistinguishable) in the species used in our studies. However, gametes from a stock are sex specific since they will only mate with gametes of certain other strains. When "male"

and "female" gametes are mixed they agglutinate in large clumps. Adhesion is by the tips of the paired flagellae. Subsequent to agglutination, the clumps dissociate into copulating pairs, consisting of one male and one female gamete. The gametes finally make contact by adhesion of papillae at the base of the flagellae. Papillar adhesion is followed by papillar fusion, the gametes so fused finally become a single cell, the zygote. The mechanism involved in the initial agglutinative flagellar adhesion is reasonably well understood. It evidently is the interaction of specific, complementary macromolecules. In fact, these have been obtained in isolation from cultures of the two sexes. The second stage of mating in Chlamydomonas, namely papillar fusion, is not well understood. In an attempt to determine if the latter interaction depends upon the same mating substances as flagellar interaction, the effect of trypsin was examined on the two processes. Trypsin affects the copulation of Chlamydomonas gametes in two ways. In low concentrations it rapidly suppresses pair formation by interfering with papillar adhesion and fusion. Trypsin can even split the protoplasmic bridge formed at the time of fusion. In higher concentrations, trypsin treatment gradually destroys the capacity of female gametes to agglutinate with male gametes. The agglutinating capacity of male gametes does not appear to be affected by trypsin. Trypsin does not affect the species specificity of copulation within the system tested. This differential

sensitivity of the two processes to trypsin indicates that they depend upon different mechanisms.

Principal Accomplishments in First Three Years of Operation of the Institute

The principal advances during the first years of operation of the institute are listed below. Those listed were selected by the criterion of judged significance.

Models of Abiogenesis

A laboratory demonstration of how the protein molecules of which living cells are composed could first have come into existence without cells to produce them.

Extensive demonstration of how the self-organizing properties of such large molecules, produced under geological conditions, could have generated precursors of contemporary cells.

Demonstration of how various attributes of biocells could arise spontaneously.

The demonstration, through experiments, of how a selective membrane could arise spontaneously from amino acids.

The finding of sharply limited heterogeneity (near-homogeneity) in the thermal poly-α-amino acids, in both composition and sequence.

The finding of a variety of metabolic types of activity in thermal proteinoids, and in the units organized therefrom.

Experimental demonstration of how most of the eighteen amino acids common to protein might arise from volcanic reactions in the presence of silica.

The demonstration of many new modes for the spontaneous origin of net optical activity acellularly.

The finding of how mononucleotides might be polymerized under geologically plausible conditions.

Demonstration of how primordial gases could be spontaneously converted through amino acids and proteins to protocells under layers of protecting water in a few hours.

The finding of amino acids, entirely in polymerized form, in bacteria-free volcanic material at 150-200° C.

The demonstration that units resembling quite exactly in appearance "organized elements" of carbonaceous chondrites are produced easily in the laboratory under planetary or meteoritic conditions.

The organization, and publication of the proceedings, of a symposium on The Origins of Prebiological Systems.

Comparative Biology and Reproductive Physiology

Demonstration of a "capacitating" action of frog egg jelly on frog sperm.

The demonstration through the use of univalent antibodies that specific frog egg jelly components are essential in fertilization.

Partial restriction of the members of sea urchin sperm surface antigens essential in fertilization rising antibody dissociated from sperm.

Electron microscopal demonstration of end-on membrane fusion as part of the sperm entry mechanism in sea urchin fertilization.

Demonstration that hybrid cross fertilization involves membrane fusion.

Clarification of the ultrastructure of the complex non-motile spermatozoa of crustacea.

Clear-cut homologization of the "explosion" reaction of crustacean spermatozoan with the acrosomal reaction of conventional spermatozoa.

Demonstration of the antigenic similarity of pig ovarian follicular fluid and pig plasma.

Demonstration that initial union of flagellae and cell attachment leading to fusion of gametes in the alga Chlamydomonas depend upon different mechanisms.

The Educational Program of the Institute

Contributions to education are being made at the postdoctoral, predoctoral, undergraduate, and secondary levels.

A principal emphasis is the postdoctoral one, which is most appropriate for the interdisciplinary subject matters represented. Traditional educational channels seldom train students in a balanced way to understand, attack, and solve problems of molecular evolution or other interfield problems. Until newer avenues of education are developed, and perhaps always, a most pragmatic approach is to broaden the outlook and abilities of men trained in a traditional discipline. The postdoctoral appointees are, accordingly, a relatively large proportion of the trainees in a small institute. In the past few years, financing of postdoctoral appointees in the institute has increased. All postdoctoral appointees have been selected as the result of initial inquiry by the appointee. The number of inquiries has been many times that of the number of appointees; accordingly those receiving education at the postdoctoral level are quite highly selected.

The predoctoral education is being carried out through the Cellular and Molecular Biology program. Both Dr. Metz and Dr. Fox participate in the review of students and in the preparation and grading of curricular examinations. Both deliver many lectures and both lead seminars in the elementary cycle and the advanced cycle of the program. For education of students in the institute, the CM Biology program is most

appropriate, and each faculty member mentioned is able uniquely to bring to the course and program subject matter in molecular biology not previously included.

A curriculum in molecular evolution is being constructed and early versions have been submitted to faculty members in related areas for comment.

Dr. Fox has been called upon for many special lectures and seminars. This included eight hours to graduate students in microbiology; this presentation began with planetary information and dealt with the evolutionary continuum in the way that the curriculum in Molecular Evolution is managing informally.

The seminars to which contributions have been made include Cell Aging, Physiology, Biochemistry, Quantitative Biology, Chemistry, Physics, Sigma Xi and Phi Beta Kappa.

Dr. Harada contributes regularly to Chemistry seminars.

Lectures in undergraduate courses have consisted of five hours presented by Dr. Fox on the subject of the origin of life to students in the natural science course.

A contribution to secondary education was made in the presentation of a kinescope on "The Origin of Life" for Dade County Educational TV. This material elaborates the extensive treatment of the subject included in the Blue Version of the Biological Sciences Curriculum Study of the American Institute of Biological Sciences.

Presentations to community groups such as the Women's Guild and the Unitarian Fellowship have been made.

Ph.D. Dissertations Completed by Graduate Students in the Institute

Joseph Branham (1963)	Inhibition of Fertilization by *Fucus* Extracts
Luther Franklin (1963)	Morphological Aspects of Gamete Contact and Fusion and of Sperm Entry into Oöcytes
Peter D. Hoagland (1964)	The Hydrolysis of Five-Member Cyclic Imides and Polyimides
Duane L. Rohlfing (1964)	The Catalytic Activity and Heat Inactivation of Thermal Poly-α-Amino Acids
Alan W. Schwartz (1965)	Condensation of Cytidine-2'(3')-Phosphate in the Presence of Polyphosphoric Acid
Kent K. Stewart (1965)	I. The Hemin-Catalyzed Oxidation of Tryptophan II. Conductometric Titration of Polyaspartic Acids

Funding of the Institute

The funds for the buildings and grounds of the Institute, and their renovation, have been provided by the University of Miami, as are essential services such as telephone, custodial, utilities, purchasing, library and other services.

The funds for full professorships are underwritten by the private endowment of the Science Education Fund of the University.

The research program of the Institute has been or is being supported by grants from the National Aeronautics and Space Administration, the National Science Foundation, The U.S. Public Health Service, the General Foods Corporation, and Eli Lilly and Company.

Some Visitors since the Last Report

Dr. T. A. Welton	Oak Ridge National Lab.
*Dr. M. Nirenberg	NIH
Dr. Carl Sagan	Harvard
*Dr. N. O. Kaplan	Brandeis
*Dr. Albert Tyler	Caltech
Dr. J. Finamore	Oak Ridge National Lab.
Dr. E. C. Pollard	Penn State
Dr. Lloyd Berkner	Southwest Center
Dr. John Keosian	Rutgers
*Dr. Edward Anders	U. Chicago
Dr. Alberto Monroy	U. Palermo
*Dr. Gordon Tollin	U. Arizona
*Dr. Verne Schumacher	U. Penn.
Dr. Adolf Hochstim	I.D.A.
Dr. H. Tani	U. Osaka
Dr. A. H. Brown	U. Penn.
Dr. John French	UCLA

*Lecturer

Activities and Recognitions of Faculty of Institute During Period of 1 November 1963 - 1 June 1965

S. W. Fox

Inclusion in Current Volume J (1960-1963) of the National Cyclopedia of American Biography (one of three biochemists included).

Commemorative meeting of the American Museum of Natural History on the occasion of opening the exhibit on The Origin and Structure of Life, 18-20 May 1964. (Believed to be the first museum exhibit on the origin of life.)

The Brian Priestman Lectures, University of New Brunswick, Canada, 12-14 March 1964.

The John Bard Lecture in Natural Science, Bard College, 30 April 1965.

Friday Evening Lecture at Marine Biological Laboratory, Woods Hole, 24 July 1964.

Guest lectures at Stanford, UCLA, UCB, and Indiana University in the U.S.A. during the report period and also at Cambridge University, England and the Universities of Bonn and Frankfurt and the Technical Hochschüle at Hannover in May, 1965.

American Chemical Society Lecture Tour in California and Far West, November, 1963.

Invited participant in Conference on Theoretical Biology, Princeton University, 23 and 24 November 1963.

Invited participant in American Institute of Biological Sciences Symposium on Space Biology, Boulder, Colorado, August 1964. Lecture on "Experiments in Molecular Evolution and Criteria for Life on Mars".

Invited participant in Rutgers Institute of Microbiology Symposium on Evolving Genes and Proteins, 16-18 September 1964. Formal paper, "Experiments Suggesting Evolution to Protein".

Invited lecture on Origin of Life in four-man symposium of Northeast Section of American Chemical Society on Current Trends in Chemistry, 5 February 1964, Brookline, Massachusetts.

Invited lecture in Symposium on Novel Nutrient Sources, University of South Florida, OART-NASA auspices, 26-30 April 1964, with coauthors.

American Chemical Society Lecture Tour in Ohio and Pennsylvania, February 1965.

Participation in COSPAR VI meeting, Mar Del Plata, Argentina, 17 and 18 May 1965. Formal paper, "Simulation of Organismic Morphology and Behavior by Synthetic Poly-α-Amino Acids", with coauthors.

C. B. Metz

Vth International Congress of Animal Reproduction and Artificial Insemination, Trento, Italy, September 1964 (Vice President - Section on Pathology of Gametogenesis).

NASA - Gemini Urchin Experiment - Cape Kennedy, March 22, 1965 - Consultant.

Japanese American Conference on Developmental Biology - Tokyo, March 27-April 3, 1965. Chairman of Section on Fertilization.

National Institute of Child Health and Human Development, Special Program Planning Committee, National Institutes of Health, New York City - May 16 and 17, 1965. Consultant on Fertilization.

Mote-Bartel Foundation Meeting, Cape Haze, Florida, May 15.

Southeastern Regional Developmental Biology Conference, Wakulla Springs, Florida, March 1964.

Guest lecture on immunochemical studies on fertilization, Zoology Department, Tulane University, April 1964.

Cell Biology Fellowship Study Section, National Institutes of Health (meetings July and December 1964 and June and July 1965).

L. E. Franklin

Lectures at:

Department of Biology, University of Houston - Fertilization in Sea Urchins

Department of Zoology, University of Kentucky - Fertilization in Sea Urchins

Delta Regional Primate Research Center, Tulane University - Fertilization in Sea Urchins

G. G. Brown

Mr. Brown was awarded the $100 Association Research Prize for his paper presented at the meeting of Southeastern Biologists at Charlottesville, Virginia, on Ultrastructural studies of sperm morphology and sperm-egg interaction in the Decapods Callinectes sapidus and Menippe mercenaria.

PUBLICATIONS FROM THE INSTITUTE

Book

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K. Harada and S. W. Fox (1964) Stereospecific Synthesis of an Optically Active α-Amino Acid. Naturwissenschaften, 51, 106.

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