SECOND ANNUAL REPORT

30 September 1966

OUTLINE

PERIOD OF THE REPORT

This annual report covers the period 1 June 1965 through 30 September 1966. Four months were added because of an alteration in the fiscal year. The 16-month period will be referred to in this report as the year.

PERSONNEL OF THE INSTITUTE OF MOLECULAR EVOLUTION

Faculty

George Brown, Ph.D., Assistant Professor[a/]

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

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

George Mueller, Ph.D., Professor

Kaoru Harada, Ph.D., Assistant Professor and Senior Research Scientist[d/]

M. Michael Siegel, Associate Member of the Institute, Professor of Microbiology

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

David Durant, Ph.D., University of Leicester, 1963, Research Scientist[f/]

Heinz Hardebeck, Ph.D., University of Bonn, 1964, Research Scientist

Gertrude Hinsch, Ph.D., Iowa State University, 1957, Visiting Research Scientist[g/]

[a/] Same appointment in the Biology Department of the University of Miami

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

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

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

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

[f/] Now at University of Exeter, England

[g/] National Science Foundation Faculty Fellow

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

Thomas Waehneldt, Ph.D. (Dr. Nat.), University of Göttingen, 1963, Research Scientist

Angus Wood, Ph.D., University of Wales, 1965, Research Scientist

Research Associates

Kazuo Matsumoto, M.S.

Jun-ichi Oh-hashi, M.S.

Charles Ray Windsor, B.S.

Graduate Students

George Brown[a/]

John Chamberlain

Kenneth Gregg[b/]

David Joseph

Catherine Kirby[c/]

Jack Ryan[d/]

Samuel Stern[e/]

Fusae Suzuki[f/]

Pamela Tyler

[a/] Ph.D., University of Miami, 1966

[b/] Public Health Service Predoctoral Fellow

[c/] University Fellow

[d/] Interdisciplinary Graduate Fellow (National Science Foundation funds)

[e/] Ph.D., 1966; now at Johns Hopkins University

[f/] M.S., 1966; returned to National Polymer Laboratory, Tokyo

Research Assistants

Judith Anika, B.S.

Teresa Ferrer

Robert McCauley

Margret Soppelt[a/]

Pamela Thompson

Ching-Tso Wang, M.S.

Leonie Wulf[a/]

Undergraduate Assistants

Margaret Barilotti, Arthur Holden, Ania Mejido, Noelia Munoz, Beatriz Pujals, Richard Roberts, Delores Senior, Bruce Gordon[b/]

Office Personnel

Maynard E. Dockendorf, Administrative Officer

Dorothy Butterbrodt, Secretary

Shannon Maxwell, Secretary

Susan Riggs, Secretary

[a/] University of Bonn

[b/] National Institutes of Health Summer Research Scholar from Pomona College

PRINCIPAL ADVANCES DURING YEAR OF THIS REPORT

As presented in more detail in later sections, the principal advances during 1965-1966 are judged to be the following:

The reports from several laboratories of catalytic activities in thermal proteinoids.

A demonstration of heterotrophic proliferation of proteinoid particles. This suggests how whole primitive systems could begin, through simple natural experiments, the process of replication.

The finding that proteinoids containing protein-like proportions of all proteinogenous amino acids (including simultaneously low aspartic acid % and low lysine %) can be produced.

An amassing of evidence (on top of that accumulated before) that thermal proteinoids tend to be approximately as heterogeneous as unfractionated serum proteins. The degree of heterogeneity found is closely comparable to that of a biological assembly--for example, the unfractionated serum proteins of the turtle or of the human. This result throws new light on the basic relationship of entropy and evolution and on questions of the evolutionary development of code-related biomacromolecules.

The finding of stoichiometric relationships between thermal histone-like polymers and RNA and the finding of other properties. Some of this study has been accomplished in collaboration with the Molecular Biology and Virus Laboratory of the University of California at Berkeley.

The finding of hormonal activity in thermal polyamino acids, in assays by Dr. C. H. Li of the University of California at San Francisco.

A demonstration, using univalent antibodies, that the sea urchin egg lacks "fertilization antigens" at the egg surface. The effects on eggs reported from earlier multivalent antibody studies are now attributed to secondary action, namely, cross-linking of neighboring egg antigens by multivalent antibody.

A clear demonstration that conversion of multivalent to univalent fertilizin involves fragmentation of multivalent fertilizin into at least four large subunits.

Completion of an ultrastructural study of fish sperms, confirming by thin sectioning that most species examined lack acrosomes.

Completion of a comparative, ultrastructural study of crustacean sperms including details from two recently discovered, primitive groups.

PROGRESS IN RESEARCH AND INTERPRETATIONS

Rates of hydrolysis of proteinoids and proteins (Fox, Windsor)

Examination by remote monitoring of analyses of hydrolyzates of polyamino acids on Mars may have available only a few hours of hydrolysis instead of the 12 hours or more typically used in laboratories. Under the usual conditions, hydrolysis is incomplete in 4 hours. Studies of this process have shown that hydrolysis may be 25% complete in 3 hours at 110° with 6 N HCl. The rate of liberation of individual amino acids however varies with the amino acid. Glutamic acid, for example, is liberated more rapidly than is aspartic acid. Correction factors can be calculated, however, so that one might approximate a picture of a total hydrolyzate from the data obtained from a partial hydrolyzate. The profile for a total hydrolyzate might be of value in characterizing the nature of amino acid polymerization on Mars, whether it was geological or biological, and in which temperature range if the former, etc. For this, the backlog of data on thousands of syntheses and analyses in this laboratory are pertinent.

An hydrolysis carried out in the presence of four parts by weight of crushed pumice gave, however, 92% recovery after only four hours of hydrolysis. The analytical data obtained were normal for a 48 hour hydrolysis. This result indicates a) that hydrolysis can be carried out in the presence of some kinds of geological material without isolation of the polymer and b) some kinds of added substance can accelerate the usual process of hydrolysis.

Analysis of pumice from amino acids and polyamino acids (Fox, Windsor)

Samples of pumice from the eruption of Kilauea Volcano on 5 March 1965 were collected by Dr. Dallas Peck of the U.S. Geological Survey and sent to this laboratory for analysis. The samples proved to be contaminated, probably with Mucor, which was however not rigorously identified. Amino acid analyses of hydrolyzates did not distinguish from terrestrial microbes. The continuing search of amino acid profiles of hydrolyzates of volcanic samples has revealed some suggestive patterns, but none such is evident in this case.

Extraterrestrial macromolecular analyzer (Durrum, Fox)

In collaboration with Dr. Emmett Durrum, development of a device for analyzing Martian soil has continued. Determination of the power requirements of a 4 hour hydrolysis with 6 N HCl has been made. Thin layer chromatography and paper electrophoresis have been examined for their applicability to the problems of extraterrestrial analysis.

A new ion exchange method developed by Dr. E. Hare of the Geophysical Laboratory of the Carnegie Institution uses compressed nitrogen, obviating bulky pumps and yielding high sensitivity. This method is under investigation.

Laboratory work examining methods of obtaining reliable results in a 4 hour period is described elsewhere in this report.

Oligomerization of hydrogen cyanide (Harada)

The mechanism of amino acid formation by oligomerization of hydrogen cyanide was studied. Pyrolysis of ammonium formate or of formamide were employed, in which reaction hydrogen cyanide was produced as a pyrolysis product. Upon hydrolysis of the pyrolysis product, ninhydrin-positive material including glycine, alanine, aspartic acid, and α,β-diamino propionic acid was observed. The first three amino acids were identified by the use of two-dimensional paper chromatography, the automatic amino acid analyzer, and column chromatography of DNP-amino acids and the u.v. absorption spectra of these DNP derivatives. On the basis of these results, a pathway of amino acid formation by oligomerization of hydrogen cyanide has been proposed in a typescript in preparation.

Speculation on the formation of organic compounds during the formation of planetary bodies (Harada)

Many studies of the synthesis of organic compounds under possible primitive terrestrial conditions have been reported. However, these studies have been based on the assumption that the chemical reactions took place after the crust of the Earth was already formed.

Chemical reactions of primitive gases which composed the proto-Earth could have taken place before the Earth's crust was formed by the use of thermal energies due to the contraction process of the proto-Earth. Possibilities of the formation of several organic compounds from primitive gases

have been considered. These discussions have been applied to the origin of organic compounds in the carbonaceous chondrites, and on the Moon and Mars. A typescript is in preparation.

Stereochemistry of glycidic acid (Harada)

β-Phenylglycidic acid prepared by Darzens' method was resolved. Its absolute configuration was determined as follows: (-) isomer (2R), (3S); (+) isomer, (2S), (3R). During the course of the study, optically active (+) and (-) erythro-phenylisoserine were prepared and the configurations were determined as follows: (+) isomer, (2R), (3R); (-) isomer, (2S), (3S). Ethyl β-phenylglycidate, prepared by Darzens' method, was confirmed as being mostly or entirely composed of the trans isomer.

Optical resolution and configuration of trans-2,3-epoxybutyric acid (Harada, Oh-hashi)

(+)-trans-2,3-Epoxybutyric acid was resolved into its optically active (-) enantiomer. By the amination of (-) isomer with benzylamine and subsequent hydrogenolysis, (+) allothreonine was obtained. By the use of these results, the absolute configuration of (-)-2,3-epoxybutyric acid was determined.

Resolution of trans-2,3-epoxysuccinic acid and preparation of optically pure D-(-)-erythro-β-hydroxyaspartic acid (Oh-hashi, Harada)

trans-L (+)-2,3-Epoxysuccinic acid was prepared by resolution with ephedrine. The specific rotation of the (+)-epoxy acid is +117.8° which is the same in absolute value as that prepared by the fermentative method. Therefore, it was confirmed that the (-) epoxy acid prepared by the fermentative method ($[\alpha]_D$ = -118°) is optically pure and is a mirror image to that prepared by resolution.

By aminolysis with benzylamine and subsequent hydrogenolysis, (+)-epoxy acid gave (-)-erythro-β-hydroxyaspartic acid ($[\alpha]$ = -59°). The reported specific rotations were not consistent in a range of 49°-54°. The synthesized D (-)-erythro-β-hydroxyaspartic acid seems to be a pure compound optically and structurally. This suggests that the β-hydroxyaspartic acid prepared by transamination ($[\alpha]_D$ = 51°) was not pure structurally or optically.

Synthesis, resolution, and configuration of β-hydroxyvaline (Oh-hashi, Harada)

DL-β-Hydroxyvaline was prepared and resolved by the use of (-)-α-methylbenzylamine. The resolved (-)-β-hydroxyvaline was then converted to D (-) valine. Therefore the configuration of (-)-β-hydroxyvaline was determined as the D configuration.

Stereoselective syntheses of optically active amino acids from menthyl esters of α-keto acids (Matsumoto, Harada)

Menthyl esters of pyruvic acid, α-ketobutyric acid, and phenylglyoxylic acid were converted to their oximes and Schiff bases of benzylamine. These were hydrogenated catalytically. Optically active D-alanine (optical purity 16-25%), D-α-aminobutyric acid (8-21%), and D-phenylalanine (44-49%) were obtained.

Stereoselective synthesis of optically active aspartic acid from derivatives of fumaric acid and maleic acid (Harada, Matsumoto)

Optically active aspartic acid (5-15% pure) was synthesized by the amination of the derivatives of fumaric acid and maleic acid with (-) and (+) α-methylbenzylamine. Tnree kinds of reactions were carried out: (A) reaction of (-) and (+) α-methylbenzylamine with N,N'-di(S) and (R)-α-methylbenzyl fumaramide; (B) reaction of (-) and (+) α-methylbenzylamine with diethyl maleate; (C) reaction of (-) and (+) amine with diethyl fumarate. In each case, the reaction intermediates were isolated.

Syntheses of optically active α-amino acids from α-keto acids by hydrogenolytic asymmetric transamination (Harada)

During the course of study of hydrogenolysis, it was found that sodium phenylglycinate in aqueous solution was hydrogenolyzed easily to ammonia and phenylacetic acid. By the use of this finding, new asymmetric syntheses of various α-amino acids from their corresponding α-keto acids and optically active D- and L-phenylglycine in aqueous solution were investigated. By this synthetic method, 40-60% optically active α-amino acids (alanine, α-NH_2-n-butyric acids and glutamic acid) were usually obtained. The steric course of the asymmetric synthesis was studied.

Syntheses of optically active α-amino acids from α-keto acids by reductive amination (Harada, Matsumoto)

The object of this study is to elucidate the mechanism of Hiskey-type reaction (hydrogenolytic asymmetric transamination) between (+) or (-) α-methylbenzylamine and α-keto acids or its derivatives.

a) In this study, the possible steric course of the reaction between α-keto acids and (+) or (-) α-methylbenzylamine as carried out by Hiskey was studied. The steric course of the reaction as proposed by Kanai and Mitsui was found to be contradictory with our results and a new steric course was proposed.

b) Hiskey and Northrop reported D-alanyl-L-alanine formation from the benzylamine Schiff base of pyruvyl-L-alanine, a result which is contradictory to the rule proposed by Prelog. In this study of mechanism, it was proposed that substrate molecules could be adsorbed on the catalyst surface to form rigid ring-like structures. The molecule could then take a cisoidal conformation. As a result, the product has the opposite configuration to that expected by the Prelog rule.

Sterically controlled syntheses of optically active alanine from oxaloacetic acid — a β-decarboxylase model (Matsumoto, Harada)

Reductive amination of a mixture of oxaloacetic acid, (+), (-)-α-methylbenzylamine, and of (+), (-)-α-ethylbenzylamine resulted in optically active alanine. The expected optically active aspartic acid was not found in the product. It is assumed that the β-carboxylic group was lost by decarboxylation during or after the Schiff base formation. The reaction may be considered a β-decarboxylase model.

A similar reaction was carried out with oxaloacetic acid and (+), (-) phenylglycine in aqueous alkaline solution. The products were found to be a mixture of optically active alanine and aspartic acid. In this reaction, β-decarboxylation is slower than that described earlier.

Formation of β-hydroxy-α-amino acids from copper (II) complex of N-salicylideniminoacetic acid (Harada, Oh-hashi)

The complex reacts easily (at room temperature, neutral, or weakly alkaline solution, pH 8) with aldehyde (CH_3CHO, Ph-CHO) to form threonine and phenylserine. Reaction conditions, yield, threo-erythro ratio, etc. were studied. Formaldehyde gave rather poor yield, and glyoxylic acid gave β-hydroxyaspartic acid.

The mechanism of the reaction might be considered similar (in principle) to those of the β-hydroxy-α-amino acid formation from glycine catalyzed by pyridoxal

Thermal cocondensation of lysine (Fox, Suzuki, Joseph)

New developments in studies of binding of lysine-rich proteinoids with RNA and DNA, and in investigation of catalytic activity have focussed attention on thermal condensation of lysine. The most active polyanhydroamino acid catalysts have been some which were rich in lysine. Varying results have however been obtained in such preparations, some being inactive. Accordingly, closer study of this type of reaction has been carried out both at the Ames Research Center and in these laboratories. Another impetus is the attempt to understand the variation in binding potential for polynucleotides.

The resultant systematic study of the thermal condensation of lysine with dicarboxylic amino acids has revealed some quite specific influences. For instance, free lysine condenses thermally with glutamic acid to yield substantial amounts of water-soluble copolymer. Further, the polymer is readily hydrolyzed to return the amino acids in high yield. If, however, the hydrochloride of lysine is used as a comonomer, the polymer obtained is entirely water-insoluble. One cannot by unqualified extrapolation of these results make water-soluble proteinoids by using free lysine only. With aspartic acid, the results obtained are in part opposite to those from glutamic acid. Free lysine cocondenses with aspartic acid to yield only water-insoluble polymer. On the other hand, lysine hydrochloride does yield with aspartic acid water-soluble polymer. In this case a considerable proportion of nonhydrolyzable linkages must be formed, as judged by the fact that only 45-60% of the original amino acids are recovered following the usual period of hydrolysis.

The relationship between the two dicarboxylic amino acids and lysine is thus specific and subtle, and various

combinations and conditions merit extended study, especially since lysine plays a central role in various activities of a biological sort.

Degrees of heterogeneity, mean molecular weights, and isoelectric points have been tabulated for many of these polymers.

Internal order in proteinoids (Fox, Nakashima, Wang, Windsor)

The report of the previous year presented data showing heterogeneity in a proteinoid preparation. Many additional data have been accumulated during this year, and the new findings are reviewed here with the older data. First, a graphical description of what is meant by limited heterogeneity is presented by comparing the elution pattern of proteinoidamide from DEAE-cellulose using tris buffer with serum proteins from turtle serum (Figs. 1, 2). What is found is clearly not a random distribution of polymers. The degree of heterogeneity, as judged by number of fractions, is equal to or less than that of serum protein of the turtle (Figs. 1, 2). The individual peaks are not as sharp, but they are more symmetrical. The degree of heterogeneity is not comparable to, on one hand, that of a random population of molecules or, on the other hand, of a homogeneous polymer. The analogy to the array of proteins in a biological source may hold unique evolutionary significance. Other tests of randomness and of heterogeneity are described below.

2. Composition of feed and of polymer — The composition of any proteinoid differs from that of the reaction mixture. This alone establishes nonrandomness in the polymer, consistent with the statement of F. W. Billmeyer in his Textbook of Polymer Science, 1962, p. 332, "Since the reactivities of all functional groups in simple bifunctional stepwise polymerization are essentially identical, irrespective of the length of the molecule to which they are attached, comonomers in such systems are randomly distributed along the chains in amounts proportional to their concentrations in the feed - -". Amino acids are not distributed along the chains in amounts proportional to their concentrations in the feed; the distribution thus cannot be random.

3. Relatively constant amino acid composition on fractionation from water — Two fractionations from water yielded preparations which, with the crude, were almost identical in contents of the fifteen amino acids assayed. Had the total preparation been moderately heterogeneous, purification from water should statistically have yielded

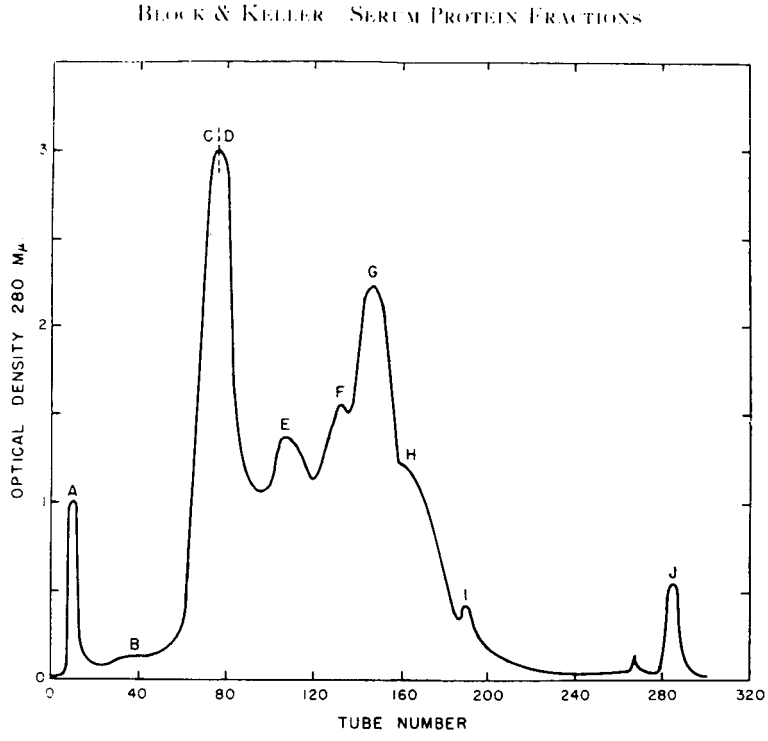


Fig. 1. Distribution of turtle serum proteins on elution from a DEAE-cellulose column by sodium phosphate buffer.

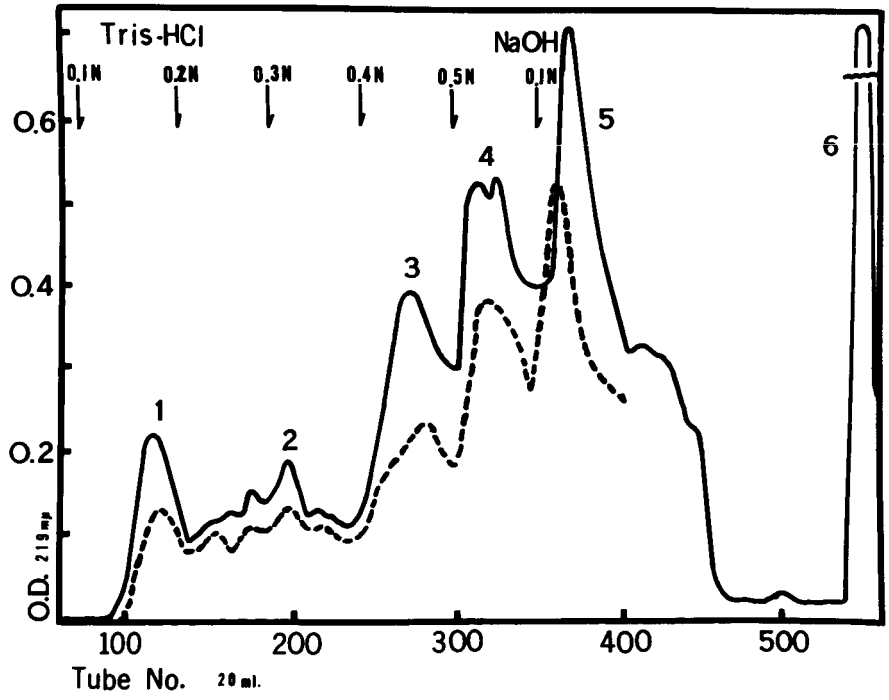


Fig. 2. Distribution of 1:1:1-proteinoidamide on elution from a DEAE-cellulose column by tris-HCl buffer.

fractions with different solubilities which in turn should have been reflected in differing amino acid contents.

4. Relatively constant amino acid composition in fractions of total hydrolyzates from DEAE-cellulose — This method, different than fractionation from water, gives however a result similar to the other. This result was reported last year.

5. Almost identical fingerprint patterns from partial hydrolyzates of fractions distributed on DEAE-cellulose — This result was reported last year. It suggests a highly uniform sequence throughout the entire polymer.

6. Discordance of total analyses and terminal analyses — In analyses reported during the last several years, many polymers have shown total analyses, NH_2-terminal analyses, and COOH-terminal analyses which differ. If the eighteen kinds of amino acid were randomly distributed throughout the chains, the analysis in any one position (such as a terminal locus) should be the same as in the total. Since it is not, the distribution is nonrandom. This result is also in agreement with a limited fingerprint pattern for the partial hydrolyzate of the fractions of the total polymer.

7. Ultracentrifugal and other types of homogeneity of individual fractions of proteinoidamide — In Fig. 3 are found the schlieren patterns of unfractionated proteinoidamide and of fractions 3, 4, and 5, as observed in the analytical ultracentrifuge. The pattern of the crude shows that heterogeneity can be identified. The patterns of the individual fractions have the appearance of homogeneity. Each fraction, however, is probably microheterogeneous. Some protein chemists, e.g. Dr. Joseph Foster, impute microheterogeneity to purified proteins. The individual fractions are however ultracentrifugally homogeneous.

8. A limited degree of heterogeneity of each individual fraction has been established also by high voltage electrophoresis and in other ways. Determination of molecular weights (4000-6000) by weight average molecular weight methods and by number average molecular weight methods for the proteinoidamide also indicate sharply limited heterogeneity, since the values tend to be concordant.

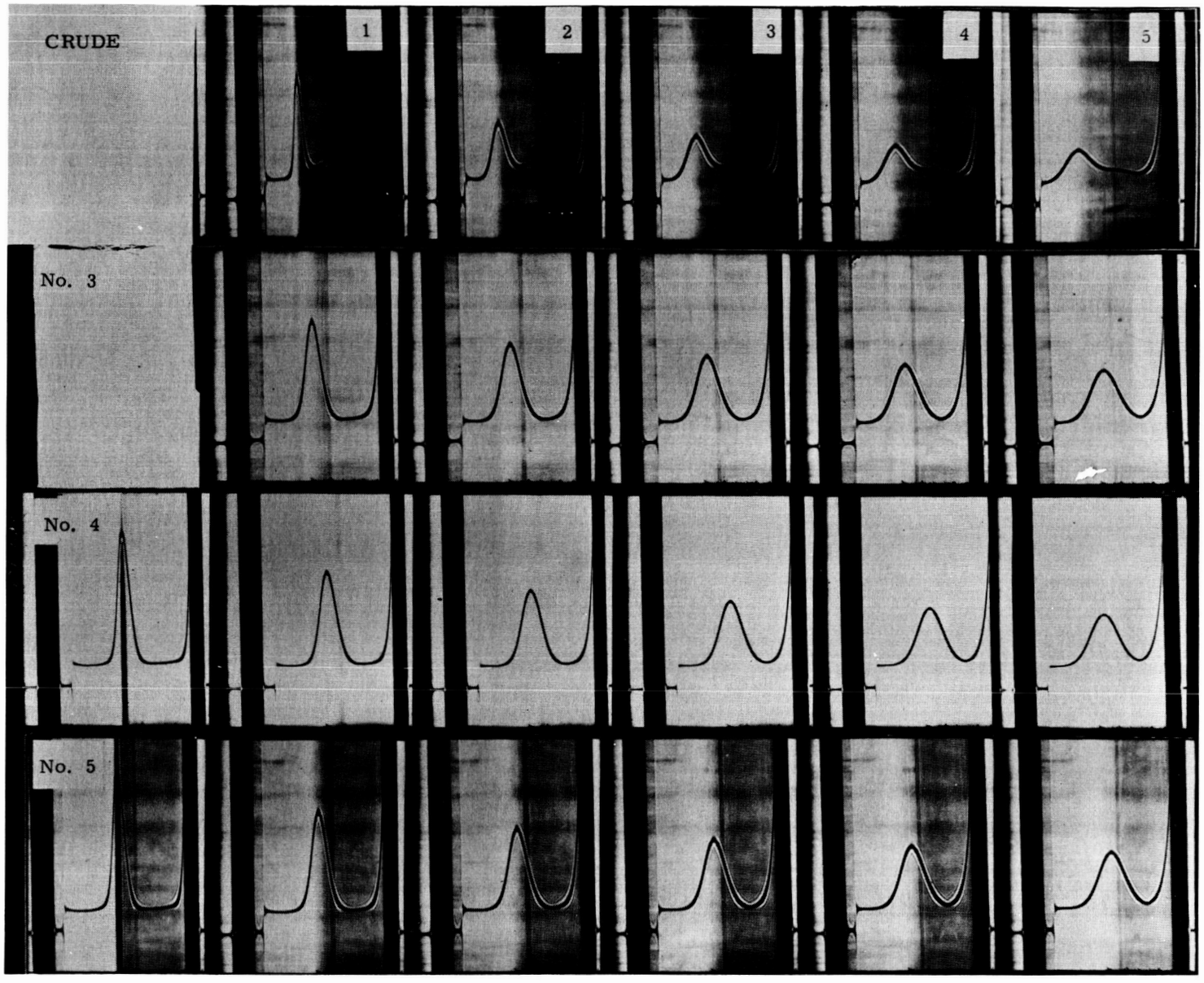


Fig. 3. Schlieren patterns of crude polymer and fractions 3, 4, and 5 of 1:1:1-proteinoidamide in the ultracentrifuge. Frames are 16 minutes apart.

Derived Inferences

The problem of the relationship of entropy to evolution is possibly more fundamental than that of the origin of life. The accumulated evidence, all of which points to limited heterogeneity in a model of preprotein, suggests an evolution from highly ordered protein to the less ordered protein of contemporary organisms.

Consistent with theoretical discussions from Pattee's laboratory and from this laboratory, the data available increasingly support the hypothesis that primitive protein would contain much of its own information, derived from the reactant amino acids rather than from the microenvironment or any component thereof.

The evolution of sharply formed proteinoid microspheres to biocells, which are not so neatly formed (and which are more reactive to their environment) can be regarded as the result of an evolution prescribed by the Second Law.

Synthesis of radioproteinoid (Fox, McCauley, Durant)

For purposes of studying budding of microspheres and other phenomena, a 2:2:1-proteinoid (159 g.) was synthesized from U-C^{14}-alanine, U-C^{14}-aspartic acid, U-C^{14}-lysine and fifteen other amino acids in nonradioactive form. The resultant polymer had an activity of 1100 ± 50 d.p.m./mg. Various fractions obtained in purifying this proteinoid have been conserved inasmuch as they may prove in the future to be useful in establishing mechanism of polymerization.

The radiopolymer has been uniquely useful in a study of "budding".

New compositions of proteinoids (Fox, Waehneldt, Windsor, Ryan)

The first characteristics of proteinoids which stamped them as being protein-like were their relatively high molecular weights and their qualitative content of all of the amino acids commonly found in protein. Quantitatively, the proteinoids have had high contents of either aspartic acid or lysine. These high contents are a reflection of a high proportion of either amino acid in the reaction. This has been regarded as necessary, being an outgrowth of the theoretical reasoning which led to the synthesis of the first proteinoids. This year an attempt to use lesser, or

intermediate, proportions of both lysine and aspartic acid was carried out. When this was successful, further study demonstrated that proper heating of equimolar proportions of all amino acids gave protein-like quantitative contents of all amino acids (except for serine and threonine, which can be especially protected as described in earlier reports). Analysis of a hydrolyzate of a neutral proteinoid is presented in Table I. Yields of such proteinoids are typically 2-6%. The aspartic acid and lysine contents are to be contrasted with previous typical figures of 60% or 50% respectively.

Table I

Amino Acid Analyses of Hydrolyzates of Neutral Proteinoids (Mole %)

Amino Acid	Insoluble Fraction	Soluble Fraction
Lysine	5.6	7.1
Histidine	1.3	2.8
Arginine	2.8	3.3
Ammonia	21	15
Aspartic acid	2.8	3.9
Glutamic acid	5.9	8.7
Threonine	0.3	0.3
Serine	0.1	0.2
Proline	4.1	2.3
Glycine	7.2	7.1
Alanine	8.3	10.3
Valine	4.6	8.2
Methionine	5.7	5.8
Isoleucine	3.4	4.0
Leucine	5.5	5.1
Alloisoleucine	2.4	3.1
Cystine	3.7	4.4
Tyrosine	6.7	4.3
Phenylalanine	8.3	3.9

At about the same time this study was being completed, Dr. Duane L. Rohlfing of the Ames Research Center was also employing intermediate proportions of lysine and of aspartic acid. His results have been reported at the September, 1966 meeting of the American Chemical Society.

The results indicate that a compositionally wide range of proteinoids can be produced. The opportunities for

formation of proteinoid, or preprotein on the primitive Earth, already very considerable, are by these data significantly enhanced. Similarly, since the neutral proteinoids yield microspheres which are not easily dissolved at neutrality, the conceptual possibilities in precursors of cells are also greatly enhanced. Furthermore, these polymers are found to bind polynucleotides to yield complexes of lesser solubility than has been true previously.

Catalytic activity of proteinoids

During the year, reports of catalytic activity in proteinoids have appeared from several laboratories. These laboratories include those of Dr. Gottfried Krampitz of the University of Bonn, Drs. Mitz and Usdin of Melpar, Dr. D. L. Rohlfing of the Ames Research Center, and the Institute of Molecular Evolution at the University of Miami.

1. Decarboxylation of pyruvate, glyoxalate, and other acids (Krampitz, Hardebeck) — The catalytic activity on pyruvic acid at pH 8.0 has been reported previously. During the year the first publication of this work appeared [Krampitz and Hardebeck, Naturwiss. 53, 81 (1966)]. In an attempt to locate the active site, a number of simple copolymers have been tested. Most active in this group has been polyanhydro (glutamic acid, threonine). C^{14}-Glyoxylate was also decarboxylated, but the pH optimum for this reaction is 6.0. Many other acids were tested for their susceptibility to decarboxylation catalyzed by proteinoids. α-Ketoglutaric acid was decarboxylated, but no such effect was observed with propionic acid, butyric acid, malic acid, malonic acid, succinic acid, fumaric acid, lactic acid, oxalic acid, citric acid, nor tartaric acid.

2. Decarboxylation of pyruvic acid (Durant, Fox) — The decarboxylation of C^{14}-pyruvic acid has been verified in Miami with proteinoids prepared under novel aseptic conditions. Studies of the kinetics yield intercepts in Lineweaver-Burk plots, whereas the proteinoid controls yield linear plots passing through the origin. These plots indicate, consistent with standard theory of enzyme kinetics, an interaction of proteinoid and substrate (Fig. 4).

Many kinds of proteinoids have been compared in this study. Those rich in lysine and lacking sulfur-containing amino acids have been most active. Fractions of proteinoid-amide made available by Dr. Nakashima have been tested for catalytic activity. The individual fractions show essentially the same low level of activity. Accordingly, the activities observed suggest that primitive protein possessed a fairly

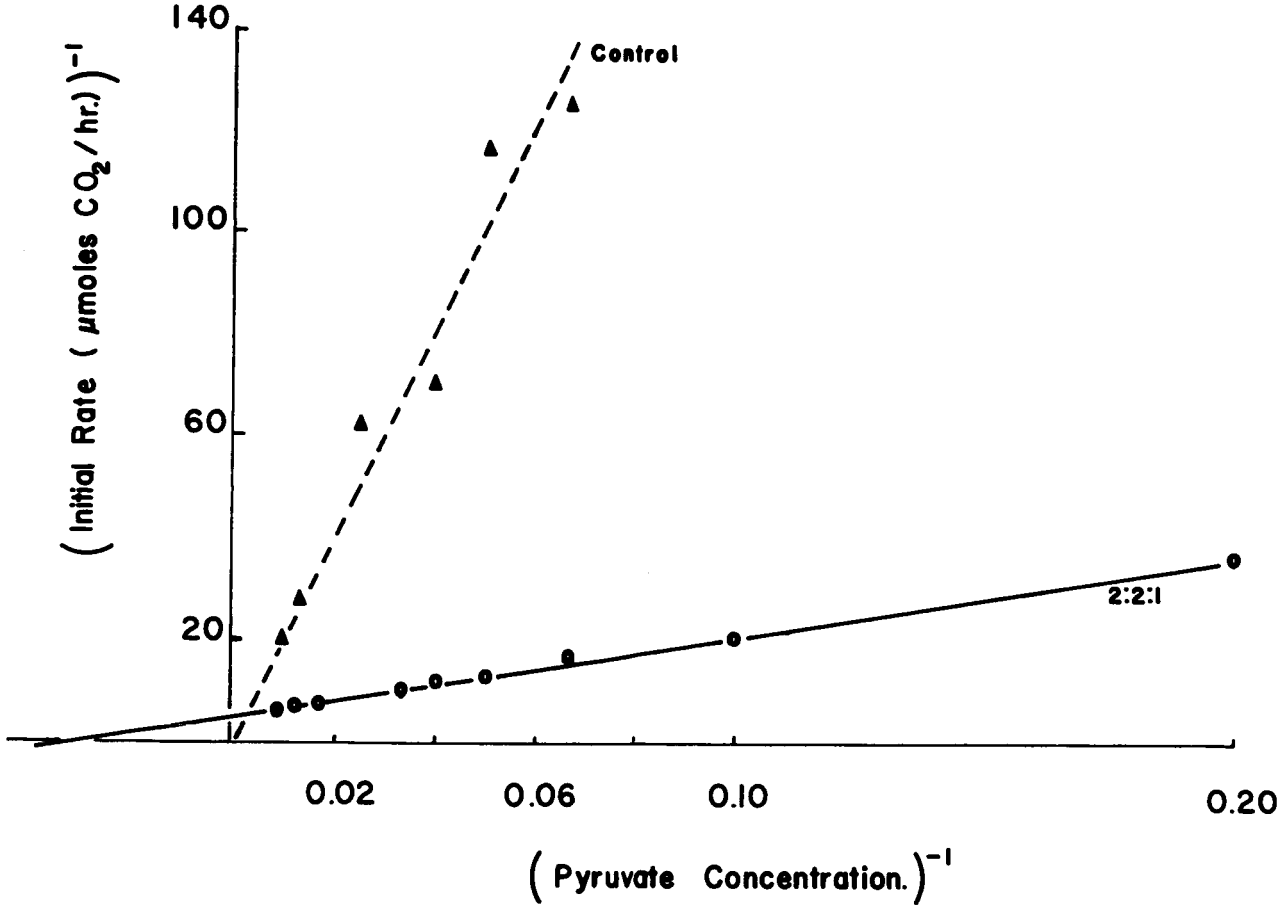


Fig. 4. Lineweaver-Burk plot for decarboxylation of C^{14}-pyruvic acid in presence of 2:2:1-proteinoid in aqueous solution.

wide array of weak catalytic activities which became enriched and specialized during organismic evolution. This is consistent with theoretical inferences derived in other ways in this laboratory.

3. Catalyzed conversion of glucose to carbon dioxide through glucuronic acid (Hardebeck, Fox) — The earlier reported conversion of glucose to carbon dioxide has been examined for the component reactions. The glucuronic acid formed can evidently be produced in small yield in the absence of proteinoid, a chemical reaction which evidently has not been previously identified. The decarboxylation of glucuronic acid is then catalyzed by proteinoid, although this reaction also has an appreciable rate in the absence of proteinoid.

In pH optima and in nature of most active polymers, the difference between glucuronic acid and α-ketoglutaric acid (Krampitz) and oxaloacetic acid (Rohlfing) shows a number of specificities. The pH optimum for decarboxylation of oxaloacetic acid, for example, is 5 whereas it is 8 for the other two acids. Basic lysine-rich polymers are active on oxaloacetic acid whereas the acid type of polymer lacks such activity. On the other two substrates, both types of polymer are active.

4. Transaminase activity in proteinoids (Krampitz) — The reaction:

$$\text{α-ketoglutaric acid} + \text{ornithine} \xrightarrow[\text{lysine-rich proteinoid}]{CuSO_4} \text{glutamic acid} + \text{α-keto analog of ornithine}$$

has been studied. This catalyzed reaction yields 3.0-3.5 mg. of glutamic acid from 10 mg. of keto analog within 2 hours.

Preliminary indications of pyruvic acid + alanine → phenylpyruvic acid + phenylalanine have also been obtained. Hydrolyzates of proteinoid are inactive and Cu^{++} alone or proteinoid without Cu^{++} are inactive in any of these reactions.

Proteinoids are thus demonstrated to catalyze some hydrolyses, several decarboxylations, and some instances of transamination.

Hormonal activity in thermal polyanhydro-α-amino acids (Fox, Wang, Nakashima)

Samples of thermal polymers containing the amino acids found in the active site of MSH (melanophore stimulating

hormone) have been submitted to Dr. C. H. Li for assay. Some activity has been reported in each of these (Table II). These activities are less than, but comparable to, the activities found in synthetic peptides prepared by traditional stepwise syntheses. Both the synthetic peptides and the polymers are constructed about the known active site of MSH which contains histidine, arginine, phenylalanine, tryptophan, and glycine.

Polymers from which individual amino acids have been systematically omitted are being tested. Since histidine and tryptophan are known to be in the active site, a lack of activity in pentatonic polymers excluding each of those will have confirmatory value for the activities already recorded.

The hexatonic polymer C has been separated on Dowex 50X2 into eight major fractions. Sequences of amino acid residues in each fraction will now be studied, and compared with the sequences in the stepwise-synthesized peptides.

Search for antigenicity in proteinoids (Fox, Ferrer, Joseph)

Although not present to the same degree as in proteins, proteinoids have been shown to have enzyme-like activity, to be susceptible to proteolytic enzymes, to have nutritive quality for several species, and they have recently been found to have hormonal (MSH) activity. One biological activity which they have not been shown to possess is antigenicity. Proteinoids and other thermal polyanhydro-α-amino acids have been sent to Dr. L. H. Frommhagen and to Dr. P. Maurer for testing as antigens. None of the polymers yet sent and tested have been found to have antigenicity.

In considering the absence of this quality, one should bear in mind that some proteins are claimed to be devoid of antigenicity. Some of the conceivable reasons for this absence include:

1. The molecular weights are too low. Effective antigens are often above 10,000 in molecular weight. The possibility of increase in molecular weight for this purpose is being studied.

2. Insufficient proportions of key amino acids are present. Tyrosine has often been implicated in antigenicity, but some of the polymers contain several % of tyrosine.

3. Active sites are covered by nonprotein linkages. This possibility is being investigated. After polymers of

Table II

MSH Activities of Some Synthetic Peptides and of Some Thermal Copolyanhydro-α-Amino Acids

Material Tested	Source	Reported Activity units/gm.	Activity Reported by
Poly (L-His, L-Arg, L-Glu, L-Phe, L-Try, Gly) Prep'n 1	IME	$3.3 \cdot 10^3$	Li's lab'y
Ditto, Prep'n 1	IME	$3.3 \cdot 10^3$	Ditto
Ditto, Prep'n 2	IME	$0.6 \cdot 10^4$	Ditto
Ditto, Prep'n 3	IME	$1.0 \cdot 10^4$	Ditto
Poly (L-Met, L-His, L-Arg, L-Glu, L-Phe, L-Try, Gly) Prep'n A	IME	$0.5 \cdot 10^4$	Ditto
Ditto, Prep'n B	IME	$0.8 \cdot 10^4$	Ditto
L-His-L-Phe-L-Arg-L-Try	H. Otsuka and K. Onouye, Bull. Chem. Soc. Japan 37, 289 (1964)	$3.6 \cdot 10^4$	Otsuka's lab'y
Ditto	H. Yajima and K. Kubo, Biochim. Biophys. Acta 87, 596 (1965)	$1.5 \cdot 10^4$	Yajima's lab'y
α_sACTH (s=sheep)	Dr. Li's lab'y	$1.5 \cdot 10^8$	Li's lab'y

much higher molecular weight are tested, this possibility will be more intensively investigated.

4. The content of D amino acids interferes with antigenicity. The amino acids are however more than 50% L.

5. As a primitive type of protein, proteinoid cannot be expected to be antigenic. Opinions on answers 2, 3, 4, and 5 should be deferred until more tests have been completed and particularly until after molecular weights of 20,000 or more are achieved.

Although nonantigenicity was reported in the first long (1960) paper on proteinoids as a proteinogenous property which almost alone had not been found, little effort has been expended in this direction since 1960. The reason for this is that biologically significant properties such as catalytic activity, specific catalytic activity, hormonal activity, etc. have commanded prior attention.

Binding of basic proteinoid with RNA and DNA (Waehneldt, Fox, Ryan, Ferrer)

Proteinoids having varying contents of basic amino acids resembling those of histones have been synthesized. The ability of these to bind with highly polymerized yeast RNA and with calf thymus DNA has been studied. The extent of binding to form insoluble particles has been found to be a function of lysine content, and does not occur below a minimum value. This minimum value varies from one series of proteinoids of various lysine contents to another. This variation is being studied. Evidence has been accumulated that, over a range, the binding between one lysine-rich proteinoid and yeast RNA is stoichiometrically constant.

The RNAs used, the DNA, and the lysine-rich proteinoid are each soluble in water. The combinations are however relatively insoluble, and characteristic morphologically. The combination with DNA is fibrous (Fig. 5). With RNA, very small microspheres (0.1-0.5 μ in diameter) are formed. Microspheres from neutral proteinoid are shown in Fig. 6. They can also bind RNA, and offer special investigative utilities because they are not readily soluble at pH 5 and lower.

These results have been adapted to the RNA of TMV by Dr. C. A. Knight of the Molecular Biology and Virus Laboratory of the University of California at Berkeley. With his permission is reported the fact that lysine-rich proteinoid complexes with TMV (Tobacco Mosaic Virus)-RNA to yield small microspheres. This combination is noninfective, but infective RNA can be recovered from the combination, and the complex is attacked directly by ribonuclease.

The studies of these combinations are designed to discipline thoughts on the origin of polynucleotide-polyamino acid complexes or of their evolutionary precursors as related to nucleoproteins, ribosomal particles, and viral particles. Many of the effects of RNA in these complexes are imitated by thermal polynucleotides.

Attempts to accrete proteinoid microspheres about "histonoid"-RNA complexes as models of nucleated cells have been carried out. Many of these were unsuccessful, probably due to the effect of acid proteinoid disrupting the RNA-histonoid combination. However, RNA and neutral proteinoid form microparticles and accretion from neutral proteinoid is being studied.

Experiments designed to contemporize the proteinoid microsphere model of the cell (Waehneldt, Ryan, Fox)

The local disciplining of evolutionary theory emphasizes that experiments themselves be allowed to suggest which properties of the cell preceded others in evolution. The attempt to adhere to this philosophy in research has led to the demonstration that microscopic particles organized on contact with water from thermal polyanhydro-α-amino acids have an astonishing array of the properties of the contemporary cell. These properties are thus quite direct manifestations of a genus of polymer. Not necessarily, but conceivably, the first cells might have arisen only in a sequence in which came first a highly organized unit, with such properties as have been demonstrated, and before contemporary genic mechanisms arose. Should this subsequently prove to be so, a willingness to allow the experiments to take precedence over the knowledge of the contemporary system will have proved to be crucial.

Two salient discernible differences between the unqualified proteinoid microsphere as a model of a primitive cell and, on the other hand, the contemporary cell are a) a highly ramified metabolic network (the proteinoid has however some catalytic powers) and b) the absence of nucleoprotein structures.

The nucleoprotein of particular interest is nucleohistone. For this reason, proteinoids resembling histones in composition have been synthesized (see above). Synthetic polymers prepared by thermal condensation of mononucleotides have been pioneered and are available in this laboratory. The control of their syntheses is not complete, however, and the similarity to nucleic acids is believed to be not as close as the similarity of proteinoids to proteins. While the polynucleotides are thus available for experimentation, and one is led to think also of microspherically produced polynucleotides (yet to come) also,

experiments aimed at bridging the gap between the primitive and the contemporary, in a model, have been performed with bionucleic acids, or with thermal polynucleotides and with proteinoids.

The performance of these experiments deserves some further explanation of the philosophy involved in their design. These experiments are partly synthetic and partly what we would refer to as reconstitutive. Knowledge of contemporary living systems owes much to work by leaders such as Fraenkel-Conrat, Kornberg, Spiegelman, etc. who have taken apart the highly evolved contemporary systems and then reassembled, reconstituted some of, or individually studied these parts in meaningful ways. The knowledge thus obtained does not inform us as to the sequence of evolutionary events leading to the most primitive system, and may even becloud our thinking about that earlier, and presumably simpler, antecedent development. Experiments using synthetic proteinoids and, in part, bionucleic acids can however be of instructive value. Proteinoids of various types have therefore been allowed to react with DNA or RNA. Even so, the intellectual capital gained by using contemporary RNA is only borrowed. A knowledge of how that RNA first arose will have to be paid for later.

The more advanced contemporary cell contains nucleohistone particles whereas presumably primitive organisms such as Bacillus megaterium (Bonner and Tso: The Nucleohistones, pp. 42-43) lack histones. Many attempts to produce proteinoid microspheres containing as "nuclei" RNA-"histonoid" complexes have failed. Presumably, when attempts to develop by accretion of acid proteinoid or of neutral proteinoid around RNA-"histonoid" particles of various kinds have failed, they failed because the complex was unstable in the presence of acid proteinoid or of neutral proteinoid. One experiment, which has yielded a new model, however, is that in which yeast RNA, without a basic protein, was allowed to act as a center of accretion of neutral proteinoid. The resultant organized particle, like some bacteria, lacks a nucleus but contains nucleic acid in less organized form, approximately 5-30% by weight.

Speculation on the course of man-made experiments in the laboratory and on natural experiments can be exercised. One might speculate that natural experiments, like laboratory experiments, would lead in the direction of evolutionary development through the phenomena that occurred most easily. In this context, the fact that primitive organisms, such as some bacteria, have chromatin instead of well-formed nuclei and that also the laboratory experiments lead easily to RNA-containing particles lacking basic polymer is a suggestive one.

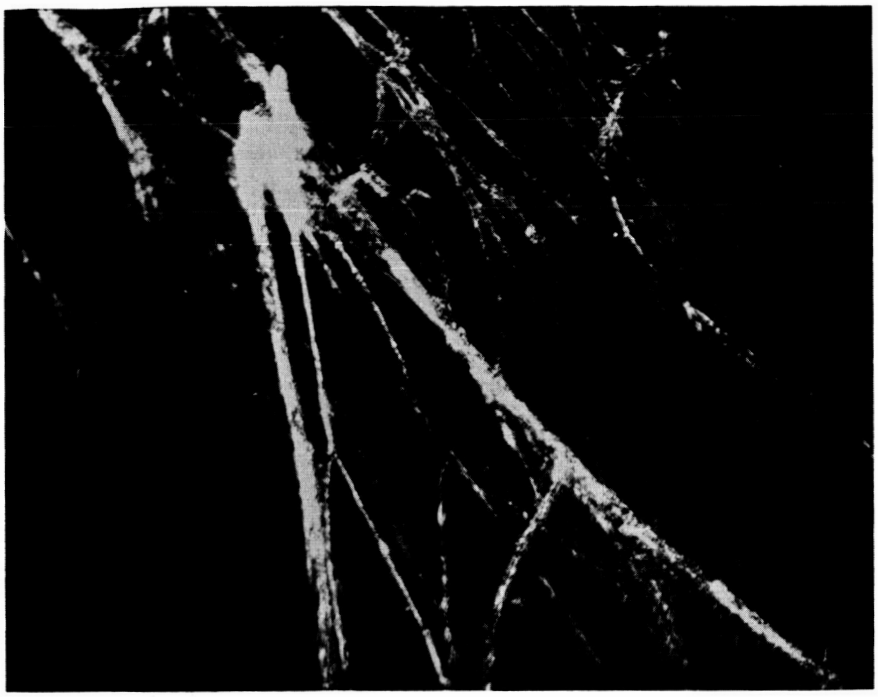


Fig. 5. Photomicrograph of fibrous complex of calf thymus DNA and lysine-rich proteinoid.

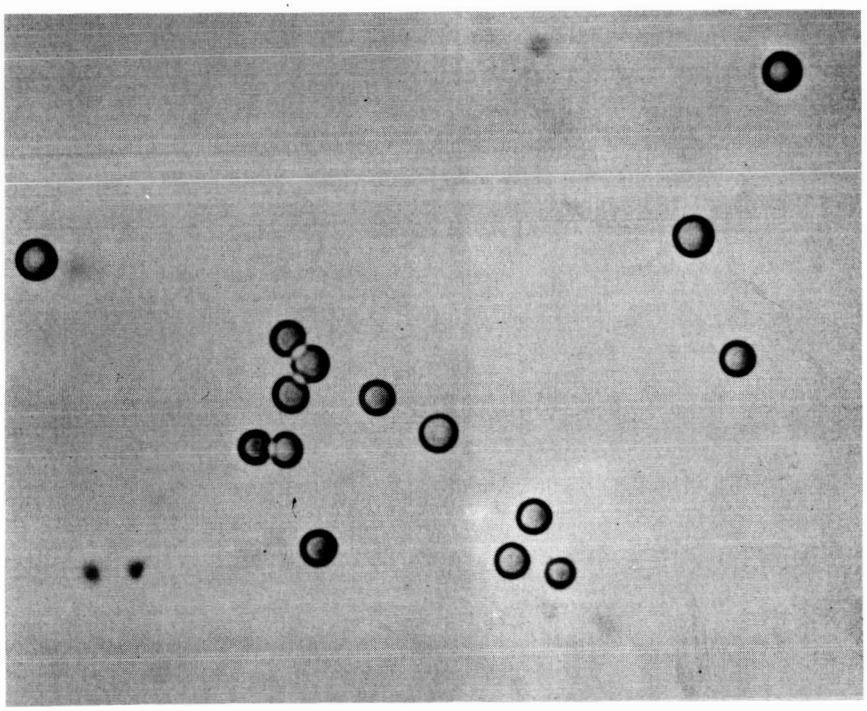


Fig. 6. Microspheres of neutral proteinoid.

Polynucleotide synthesis (Kornberg) with proteinoid
(Fox, Waehneldt, Ferrer)

The Kornberg system for synthesis of polynucleotides dAT and dGdC was studied. Controls containing the Kornberg polymerase were active. Attempts to replace polymerase by proteinoid have not been successful with the samples of proteinoid so far tested.

However, basic lysine-rich proteinoids have been found to inhibit the normal enzymic synthesis completely. In view of the ease with which such polymers can be altered, this finding opens new doors to controlled inhibition of polynucleotide synthesis in enzyme systems. The search for a synthetic polymerase continues.

Heterotrophic proliferation of proteinoid particles
(Fox, McCauley, Wood)

A demonstration of how proliferation or replication might have appeared in primitive proteinaceous particles has been needed to advance fundamental understanding of the origin of life.

Proliferation, or increase in number, can be conceptualized as a process less intricate than replication which connotes increase in number plus exact, or almost exact, copying of the parent material and morphology. In the most advanced, contemporary, forms of replication, a complex system involving nucleic acids and proteins in a coded relationship, and an intricate network of biosyntheses are found. Evolutionary principles lead to the premise that this complex contemporary system has however evolved from simpler systems of proliferation or replication. The idea that a contemporary genic type of replication came after the protocell first appeared has been held open in one way or another by various experts (e.g. E. L. Tatum, J. Lederberg, K. V. Thimann, F. Lipmann, M. Calvin, J. M. Buchanan, S. Granick). Inasmuch as the processes of division and of growth (in size) of proteinoid particles has been reported earlier, attempts to demonstrate proliferative or replicative phenomena in a cyclical fashion have been made.

That contemporary metabolism was not needed in the first self-reproducing system has been stated in various ways. At the extreme, a self-replicating system could have been a "complete heterotroph" as first indicated in the Oparin-Holdane hypothesis. Biosynthesis could have come later as stated by C. B. Van Niel.

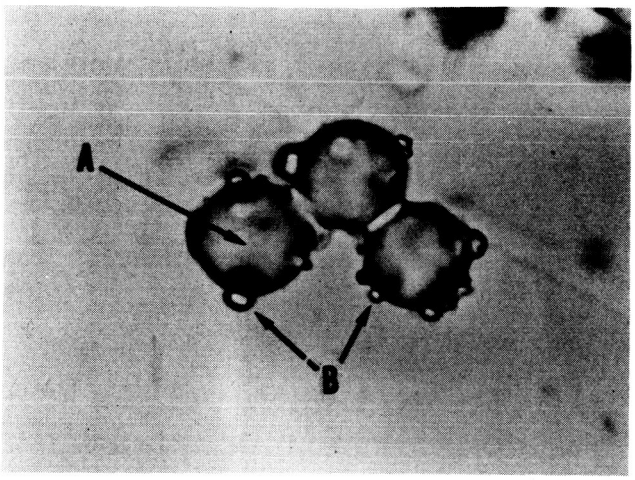


Fig. 7. "Budded" proteinoid microsphere

A. microsphere
B. "bud"

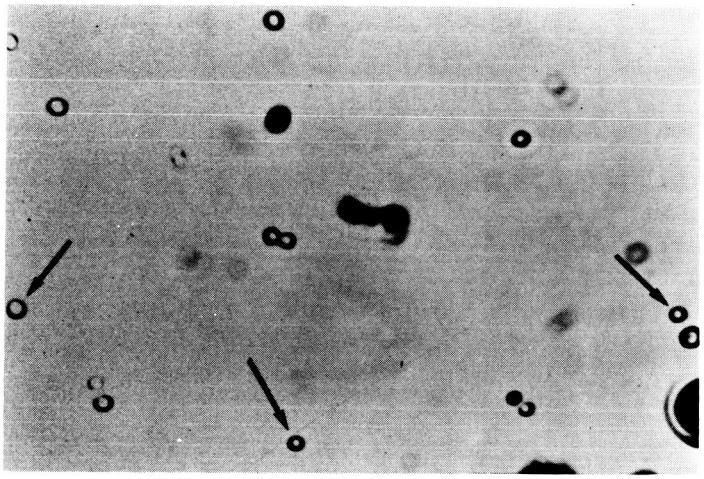


Fig. 8. Separated "buds"

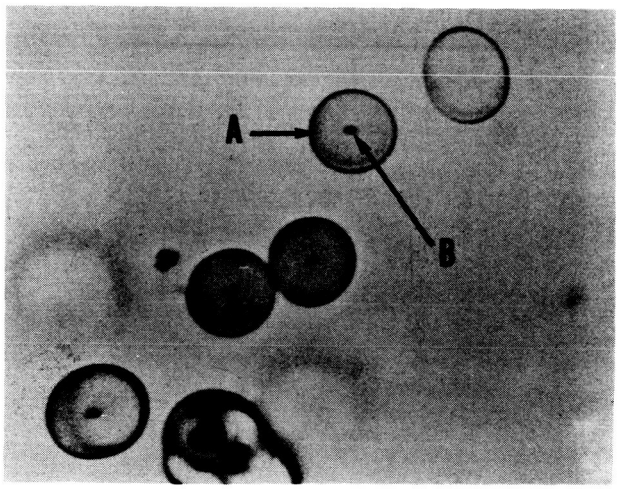


Fig. 9. Accretion of proteinoid particle around Crystal Violet-stained "buds"

A. proteinoid particle
B. stained center

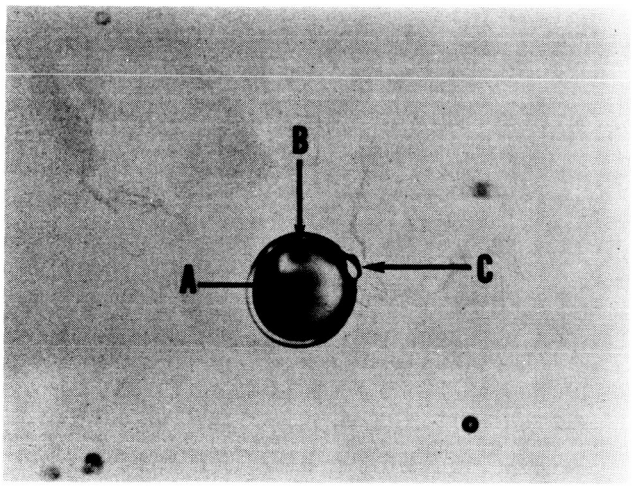


Fig. 10. "Daughter bud" on particle of Fig. 6

A. stained center
B. microsphere by accretion
C. "daughter bud"

"Acceptance of the postulate that chemical evolution preceded biopoesis further suggests that the organized structures representing primitive life were capable of self-reproduction before they acquired mechanisms whereby they could chemically transform the components of their environment."

The concept of an initial heterotroph or near-heterotroph permits the initial absence of a coded sequence-controlling mechanism if the material used structurally for descendant cells was somehow kept within necessary limits by internal factors operating in that material. Such internal control is manifest in thermal proteinoid (see earlier pages). Proliferation can be regarded as essentially equivalent to self-replication under these circumstances.

The demonstration of proliferation of a model of primitive protein (proteinoid) particles is shown in Figs. 7-10.

In Fig. 7 is illustrated the way in which bud-like appendages appear on proteinoid microspheres which have been allowed to stand for 1-2 weeks in the liquid from which they were formed.

Fig. 8 shows "buds" removed from "mother" particles. This removal can be accomplished by mechanical, thermal, or electrical shock. In this experiment, a Tesla coil was used.

For Fig. 9 the "buds" were stained with Crystal Violet so that they could be pictured differentially. When the stained "buds" were transferred to proteinoid solution saturated at 37° and allowed to cool to 25°, they developed by accretion, to the size shown, in 30-60 minutes.

Fig. 10 shows that "buds" have appeared on this full-grown particle. The necessary process was again one of standing for 1-2 weeks.

The "bud" of Fig. 10 thus represents a product of a complete cycle from a "bud" of Fig. 7.

The natural experiments leading to this kind of proliferation could, or must, have occurred innumerable times on the surface of the Earth during its long history.

Once a dynamic organized structure of this nature appeared, other natural experiments could conceptually have introduced other phenomena leading to complex, contemporary cells. Evolution could, in fact, have proceeded more rapidly than before.

The discernible differences between this type of unit and a contemporary type are the presence of RNA or DNA-RNA and a complex system of biosynthesis.

While this system does not imitate the contemporary organism, for the reasons given, it can be studied as a model of organized structure (a la Van Niel), or organism, of a primitive type with many of the properties of contemporary cells (cf. antecedent reports for the numerous properties). Of particular significance is the fact that the polymer, in which the demonstrated properties are intrinsic, is not obtained from a contemporary biological system, but is of purely synthetic origin, by processes which can be imputed to planetary surfaces in a spontaneous mode.

The concept that replication is the property of a whole system has of course been expressed before (Oparin).

New light on stability of proteinoids, proteinoid microspheres and its theoretical geophysical significance (Fox)

Two of the fundamental contributions from this laboratory to the theory of abiogenesis have been identification of two processes that could, or must, have occurred innumerable times on the Earth during its long history: 1) the polymerization condensation of amino acids in dry, warm or hot locales, and 2) the ease with which such polymers form on contact with water subtly organized microstructures with many of the properties of contemporary cells. Most astonishing is the roster of properties intrinsic to the wide variety of individual poly-anhydro-α-amino acids which can result. The probabilities of occurrence of the sequence of two processes listed above is conceptually enhanced if the polymer of step 1) is understood as being quite stable. If it is, step 2) would not need to follow immediately. The probability of frequent occurrence of sequence is regarded as very high anyhow, but with a stable dry proteinoid much higher probability can be visualized. A high degree of stability of proteinoid in the dry state has long been recognized in this laboratory and in the laboratory of Dr. Richard S. Young. During the report period new experimental and theoretical advances have been brought to bear on this question.

The stability referred to has also been noted in studies by A. E. Smith and F. T. Bellware in Science 152, 362 (1966), and is consistent with the observations of Young (unpublished) and of Fox (published). In addition, Fox pointed out in a paper on Radiation and the First Biopolymers at the Third International Congress of Radiation Research, 28 June 1966 other

laboratory information which indicates the stability of amino acid polymers on the primitive Earth. One aspect of this stability is the fact, published in 1950 by Proctor and Bhatia [Food Technol. 4, 357] that dry protein is highly resistant chemically to high energy radiation whereas protein in aqueous solution is known to be quite unstable. Once the dry preprotein was brought into contact with water on the primitive Earth it would form microspheres and, under many sets of conditions, would sink to below the surface of water deeply enough to be protected from further heat and various forms of radiation.

An independent evaluation of the above mentioned roster of properties of proteinoid microspheres is also found in a comment by John Keosian ,author of the book, The Origin of Life (Reinhold, 1964). This comment could have been made only recently due to the fact that only since 1965 have the properties of proteinoid microspheres been described in any comprehensive way in a scientific paper. In a book review under the title An Explanation of Life Based on the Laws of Physics in Science 152, 1496 (1966), Keosian states,

"A wide gap exists between coacervates and the most primitive living thing - - -. This is a critical part of the story. - - -. One can make other premises based on other models - - - and can take his departure and press his own case. This departure could have been delayed, I believe, had Woolridge chosen Fox's proteinoid microspheres for his precellular models, in view of the recent advances reported in the construction, properties, and maintenance of microspheres."

Also, relevant to proteinoid microspheres, a documented statement of A. I. Oparin is presented in BioScience 16, 480 (1966) to the effect "that the use of polymers synthesized under terrestrial conditions was superior to the use of biopolymers in modeling the origin of the first cell." Oparin's statement was made during discussion at a 1965 colloquium in Paris on Elementary Biological Systems and Abiogenesis.

In addition to the several kinds of geophysical conditions which have earlier been identified as appropriate to processes 1) and 2), a newly identified set arising from the inferences of the volcanologist Rittmann about the cooling of the Earth can now be cited. A. Rittmann (Volcanoes and Their Activity, Interscience, New York, 1962, p. 274) visualizes what happened when the temperature of the Earth's surface first fell below 374° C, the critical temperature of

water, as follows, "An intense rain of hydrothermal solutions showered down upon the still hot crust of the earth, where they were first volatilized again, and then recondensed, so that a very vigorous circulation was initiated." In this kind of dynamic cycle one can visualize that amino acids could polymerize in hot dry regions, and the polymers would then organize into spherules when the water recondensed and cooled. The opportunity thus provided for reactions as described in the laboratory must have been almost limitless.

New evaluations of the likelihood of geological occurrence of the proteinoid→microsphere sequence (Fox)

The likelihood of geological repetitions of the proteinoid→microsphere sequence on the surface of the Earth has earlier been analyzed as innumerable. During the year, a number of experiments and theoretical analyses have led to a judgment of totally greater likelihood. None of the factors analyzed suggest a decreased likelihood relative to earlier evaluations. Those factors analyzed follow:

1. The possibility of a geophysical sequence of hot nonaqueous conditions (for polymerization) followed by water (spherulization) in innumerable repetitions is indicated by a paragraph describing the refluxing of the primitive ocean on the Earth while it was cooling through the critical temperature of water (A. Rittmann, Volcanoes and Their Activity, Interscience, New York, 1962, p. 274).

2. The finding in this laboratory of the ease with which aspartic acid or lysine can be thermally copolymerized with other amino acids at very low proportions of aspartic acid or lysine. The necessary reaction mixture is thus very much wider in scope than was previously recognized. Moreover, such reaction mixtures yield polymers having quantitatively protein-like proportions of the key amino acids, aspartic acid and lysine, in the same polymers.

3. The stability of proteinoids has been reassessed in a theoretical treatment for the Third International Congress of Radiation Research. While many of the amino acids in protein in aqueous solution are known to be unstable to high energy radiation, Proctor and Bhatia had earlier shown that protein in the dry state is highly resistant. Accordingly, when protein or preprotein was synthesized in a dry locale, it would continue under these conditions to be stable to ambient radiation and thermodynamically stable in the absence of water, as well. The need for conversion to microspheres

under protecting water would not be immediate and the polymer could simply remain as such for lengthy periods, until spherule formation occurred in the presence of water.

4. Recent studies of the scope of polymerization condensation of amino acids in terms of temperature and time reveal that polymers covering a wide range of molecular weights readily form organized microstructures. As judged by Bio-Gel fractionation, the smallest polymers which yield microstructures are in the range of tripeptides to octapeptides. The range of protocell formation is thus conceptually enhanced.

5. Earlier discussions have pointed out that microspheres would be protected from radiative decomposition by the fact that they would form under layers of water. During 1966, workers at Sir George Williams University have demonstrated a high degree of stability of proteinoid microspheres [A. E. Smith and F. T. Bellware, Science 152, 326 (1966)].

6. Some of the cell-like properties of the pre-cell, modeled by proteinoid microspheres, would theoretically contribute to the protection of the latter. One such property could be motility, demonstrated earlier, which might lead to selection of units which moved from the site of deleterious phenomena. Another would be proliferation, described in this report. The beginning of the adaptive versatility of contemporary unicellular organisms can thus be imputed to some of the properties which may have appeared in the course of precellular evolution.

The synthesis of living systems

Due especially to President C. C. Price of the American Chemical Society, new attention has been focussed recently on the goal of synthesis of living systems. This focus is on contemporary systems and, as such, has some analyzable relationships to attempts to synthesize "primitive" living systems.

The work which has been carried out on synthesis of prebiological molecules and systems can be said to encourage and to have influenced thinking in the direction of synthesis of contemporary systems. The studies performed earlier and reported from this laboratory have helped to make possible serious thought about synthesis of contemporary systems. In particular, the striking nature of the self-assembling properties of appropriate macromolecules (proteinoids) permit one to entertain the thought that if most or all of the

proteins, lipids, etc. of a contemporary cell are synthesized they will spontaneously assemble themselves into a cell-like structure of a contemporary type.

A crucial difference between this newer approach by traditional chemical synthesis and the "origin-of-life" approach is that only one of these can be expected to tell us how life was first synthesized spontaneously, i.e. how it came into existence without a chemist to perform the detailed operations. The chemist-less processes are of course the ones that can provide understanding that has had and will undoubtedly continue to have direct relevance in a space context.

Another way in which the contemporary should be distinguished from the primitive type is in those systems which are not fully synthetic in a chemical sense. The recombination or hybridization of TMV-RNA, and TMV-protein, the enzymic synthesis of polynucleotides, and the replication of RNA with an enzymic replicase are not truly syntheses in the rigorous sense. They are to a large degree reconstitutions of highly evolved molecular mechanisms.

A synthesis of the contemporary organism can be approached through traditional studies of biological and organic chemistry. The more fundamental problem of the primordial organism requires application of knowledge and understanding from geology and from the principles of evolution. When these principles are properly applied, many inferences drawn about the emergence of the primordial organism gain much in rigor that cannot be appreciated in the absence of the geological and evolutionary perspectives.

Direction of Studies on Physiology of Reproduction (Metz)

Research and instruction in reproductive physiology continued on the ultrastructural, biochemical, and immunochemical fronts. Marine invertebrates constituted the organisms employed in most of the studies, but one new investigation was initiated, namely, immunochemical studies on rabbit semen.

Ultrastructural Studies

Comparative ultrastructure of crustacean spermatozoa (Brown, Metz)

The detailed study of sperm ultrastructure, the acrosomal reaction, and sperm-egg interaction in the edible crab,

Callinectes sapidus, cited and illustrated in the previous annual report, has been completed and published (G. G. Brown, J. Ultrastructural Research 14, 425-440). Comparative studies on crustacean sperm morphology have been continued with an examination of representatives of most of the major subclasses. Details of many of these, including both flagellated and aflagellated spermatozoa have been worked out. One currently accepted phylogenetic classification (Dahl, 1963) places four of the crustacean subclasses in the cohort Maxillopoda. Spermatozoa of this group have conventional morphology for the most part. They possess an acrosome, mitochondria, and a flagellum with the typical 9 doublet + 2 microtubular substructure. The spermatozoa of the other crustacean subclasses are very atypical and lack flagella.

Two of the subclasses studied are of particular interest. These are the Mystacocarida and the Cephalocarida. These groups are very primitive crustaceans that have been known for only a few years. One representative of each was examined with the electron microscope. The cephalocarid, Hutchinsoniella macracantha, was found to have a most unusual sperm (Fig. 11). It is seen to possess an acrosome with interesting substructure, a large nucleus, and a posterior spike-like appendage. This spike does not have the characteristics of a flagellum and the sperms dissected into sea water were not motile. A canal passes through the nucleus and contains a forward projection of the posterior spike.

Sperms of the mystacocarid, Derocheilocaris typicus, are more conventional and consisted of an acrosome, nucleus, mitochondria, and flagellum. The difference in the sperm morphology between the Cephalocarida and Mystacocarida is consistent with assumed evolutionary relationships and tends to confirm that the cephalocarids have undergone considerable evolution from a presumed ancestral group with flagellated spermatozoa. The mystacocarids proved to have an unusual reproductive feature (see Fig. 12). The mature spermatozoa from the vas deferens are contained in tubular spermatophores. Each spermatophore contains two spermatozoa. These are arranged with the acrosomal regions of the spermatozoa at opposite ends of the spermatophore such that the flagella of the two spermatozoa overlap in the central half of the spermatophore. The spermatozoa are arranged in the spermatophores very precisely, so precisely, in fact, that the central microtubules of the two flagella are all in register, e.g., all fall on the same plane (Fig. 12a). The spermatophore itself has interesting substructure which has not yet been examined in detail. No hint as to how the spermatophores are formed or how the spermatozoa are oriented has yet been

obtained. This is a most remarkable evolutionary adaptation and especially so, considerint the fact that the mature organisms are only 0.3-0.4 mm. long and 0.1 mm. wide. These studies on crustacean spermatozoa and sperm-egg interaction constitute the Ph.D. dissertation of George Gordon Brown.

One additional observation of considerable interest is a confirmation of the report of Pochon-Masson, 1965, that spermatozoa of some malacostracans contain bundles of microtubules. In some cases these extend from near the acrosomal region, through the nuclear region, and out into the arm-like structures characteristic of this group. These are preliminary observations. Dr. Brown and Dr. Gertrude Hinsch, who joined the group in September, 1966, plan a more thorough study of the relationships and origin of these microtubules. Results of rather general interest may be expected from this since microtubules appear in several fundamental subcellular structures, including flagella, cilia, mitotic spindles, and centrioles.

Ultrastructure of fish spermatozoa (Tyler, Metz)

The evolution of bony fishes is reasonably well understood. Most reports in the literature indicate that the sperms of the higher bony fish (teleosts) lack an acrosome. Unfortunately, the published accounts do not contain supporting electron micrographs of thin sections. Therefore, it seemed of interest to undertake a comparative study of bony fish sperms at the electron microscope level for its evolutionary interest as well as providing information of interest in relation to fertilization. Electron microscope sections of most teleosts (toadfish, Opsanus beta, spiny boxfish, Chilomycterus schoepfi) examined had no trace of an acrosomal structure. One genus, Gambusia, is reported from light optical studies to have an acrosomal organelle. This was confirmed in the present investigation of Gambusia affinis to the extent that this sperm had an acrosome like projection extending forward from the nucleus. Interestingly, two primitive bony fish sperms, namely the bowfin, Amia calva, and the gar, Lepisosteus platyrhincus (Fig. 13), also lacked recognizable conventional acrosomes. This study constituted the Master's thesis of Miss Pamela Tyler.

Chemical and immunological studies on fertilizin, the sperm isoagglutinin from eggs (Stern, Metz)

The details of most of this study were reported in the previous annual report. Therefore, they will be summarized briefly here. The jelly surrounding sea urchin eggs slowly

dissolves in sea water. The dissolved material has a striking, species specific, agglutinating action on spermatozoa. This, and other factors, indicate that the jelly material has an important, if not essential, role in fertilization. The jelly material (called fertilizin) is a sulfato rich glycoprotein or mucopolysaccharide. Accordingly, studies on fertilizin are of inherent interest in relation to the structure of large molecules, in addition to significance for the role in fertilization.

One unusual property of sea urchin fertilizin is its capacity to change from "multivalent" to a "univalent," non-agglutinating form. Hathaway, working in this laboratory in 1961, showed indirectly that the change results in the release of an "inert" fraction which fails to combine with sperm. The present study was designed to investigate the nature of Lytechinus variegatus fertilizin and its conversion to the non-agglutinating, "univalent" form.

Lytechinus fertilizin was found to contain a broad spectrum of amino acids and a single monosaccharide, namely fucose. In addition, small amounts of amino sugars were also detected.

Physical analysis of multivalent and univalent fertilizins showed that conversion to the univalent form by H_2O_2 treatment results in decreases in viscosity and sedimentation rate, indicating fragmentation or depolymerization of the original material. This fragmentation does not involve splitting off of low molecular weight fragments but consists in an ordered, relatively non-random fragmentation or depolymerization into relatively large subunits. Evidence for four such subunits have been obtained by cellulose acetate electrophoresis. At least two of these are inert with respect to sperm combining capacity. These results on fertilizin structure provide an interesting parallel with antibody structure. The work constitutes the Ph.D. dissertation of Samuel Stern.

The effect of antibodies on fertilization of sea urchin eggs
(Metz, Thompson)

Studies from several laboratories extending over the past fifteen years show that treatment with antibodies prepared in the rabbit has marked effects on the morphology, cleavage, and fertilizing capacity of sea urchin eggs. These studies have led to the view that the sea urchin egg surface contains a constellation of antigens, at least some of which perform essential roles in fertilization, presumably by functioning in sperm attachment, fertilization specificity or sperm-egg membrane fusion. These studies, then, have contributed

considerably to current thought on the subject and form a model system for consideration of cell surface interactions in general. However, the earlier work involved the use of normal, multivalent antibody. Such antibody agglutinates cells and precipitates antigen in solution by formation of cross-linked lattices. This fact confuses interpretation of the action of antibody on the eggs because the effects could result from the secondary action of lattice formation such as precipitation, steric masking of essential cell components, changes in surface tension, etc., rather than a direct blocking of essential antigens.

Recent advances in immunochemistry have provided techniques for fragmenting the antibody molecule into univalent subunits. Such univalent fragments possess only one combining site for the appropriate antigen. Therefore, they can combine with and block the specific antigen. However, they do not agglutinate cells, precipitate dissolved antigen, or otherwise cross-link antigens in the form of a lattice. Such univalent antibody was prepared by the papain digestion-reduction method of R. R. Porter (1959) and tested for effects on the morphology, cleavage, and fertilizing capacity of Lytechinus variegatus eggs.

Anti-jellyless Lytechinus egg rabbit antisera were employed in most of the experiments. The univalent form of this material failed to produce the agglutination and wrinkling effects of the parent multivalent antibody. However, such effects were obtained when univalent antibody pretreated eggs were subsequently exposed to multivalent anti-rabbit gamma globulin sheep serum (antiglobulin or Coombs test) as seen in Fig. 14. It is evident from these experiments that the morphological effects of the anti-egg homogenate antibody result secondarily from cross-linking of antigens.

Likewise, univalent antibody failed to inhibit the fertilizing capacity of Lytechinus eggs. This was true even of eggs from which the vitelline membrane had been removed by pretreatment with proteolytic enzymes ("protease," a mixture of trypsin and chymotrypsin). Again, restoration of cross-linking in the antiglobulin test (pretreatment of eggs with univalent anti-egg homogenate rabbit antibody followed by exposure to anti-rabbit gamma globulin sheep serum) resulted in inhibition of fertilization. Data from a typical experiment are given in Fig. 15.

These results show that inhibition of fertilization, at least with the antibody employed, again results from cross-linking of antigens, not from blocking of specific antigens

that perform an essential role in fertilization. These experiments have been repeated and confirmed by Graziano (trainee in the Fertilization and Gamete Physiology Training Program at the Marine Biological Laboratory, Woods Hole, Massachusetts) and C. B. Metz, using a second species of sea urchin, Arbacia punctulata.

In another series of experiments, reversal of the multivalent antibody inhibition of fertilization was attempted. Multivalent antibody pretreated eggs were exposed to protease and subsequently assayed for fertilizing capacity. In Lytechinus variegatus, protease treatment restored fertilizing capacity of multivalent antibody pretreated eggs nearly to control levels. Comparable results were obtained in Arbacia punctulata with one of the two anti-sera employed. However, marked reversal of fertilization inhibition was not obtained in similar experiments employing antiserum from a second rabbit. The difference between the two antibody preparations is presumably related to the fact that the second antibody produced more precipitin bands in Ouchterlony agar gel precipitin tests than the first serum.

The mechanism of reversal of fertilization inhibition by protease is not clear as yet. Protease could fragment the antibody or digest the antigen-antibody complex from the egg surface, permitting interaction of the sperm with the egg. Further study will be required to clarify this point.

Inhibition of cleavage (Metz, Thompson)

The univalent antibody study was extended to the inhibition of cleavage reported by Tyler and Brookbank (1956). Multivalent anti-egg jelly sera strongly inhibit cleavage of Lytechinus variegatus eggs. Much, but not all, of this inhibiting activity is lost, following digestion of the antibody to the univalent form as seen in Fig. 16. Comparable results were obtained in Arbacia punctulata by Cocanour (trainee in the Fertilization and Gamete Physiology Training Program at the Marine Biological Laboratory, Woods Hole, Massachusetts) and Metz.

A full investigation of this has yet to be made. The data available could be explained by quantitative differences in active antibody combining sites in the digested and undigested antibody. However, the possibility remains that two different classes of egg antigens exist, one of which results in cleavage inhibition only when combined with multivalent antibody, whereas the other yields inhibition with either univalent or multivalent antibody.

Fig. 11. a. Mature sperm of Hutchinsoniella macracantha. Shown are the phase contrast of the sperm showing the acrosome, the nucleus, and the posterior projection. No fixation. X 2,900.

b. Longitudinal section (slightly oblique) of the mature H. macracantha sperm. The three major regions are the acrosome (A), the nucleus (N), and the posterior projection (PP). Several membranes are partially resolved. These are a membrane (m) around the acrosome, a plasma membrane (PM), and a nuclear membrane (NM). Karnovsky's glutaraldehyde. X 42,900.

c. H. macracantha. Relationship of the acrosome (A) to the origin of the posterior projection. The nuclear membrane (NM) and the intracanal membrane (IM) are resolved. Karnovsky's glutaraldehyde. X 39,000.


a
PM
m
PP
PP
c
b

Fig. 12. Spermatophores of Derocheilocaris typicus, longitudinal section.

a. Ends of two spermatophores showing trough-like acrosome (A), nucleus (N), and flagellum (F). Note the overlapping of the above organelles (arrow), the flagellum is capped by an electron dense cone. Some details of the complex spermatophore cap are also visible. Glutaraldehyde. X 31,700.

b. Cross sections of spermatophores. Sections through ends of spermatophores showing the trough-like acrosomes (A) are seen at the top of the figure. Sections in the middle of the figure are through the nucleus (N) of one of the two sperms. Sections at the bottom of the figure are through the middle region of the spermatophore and show the flagella (F) of the two sperms in the spermatophore. Glutaraldehyde-osmium. X 29,000.

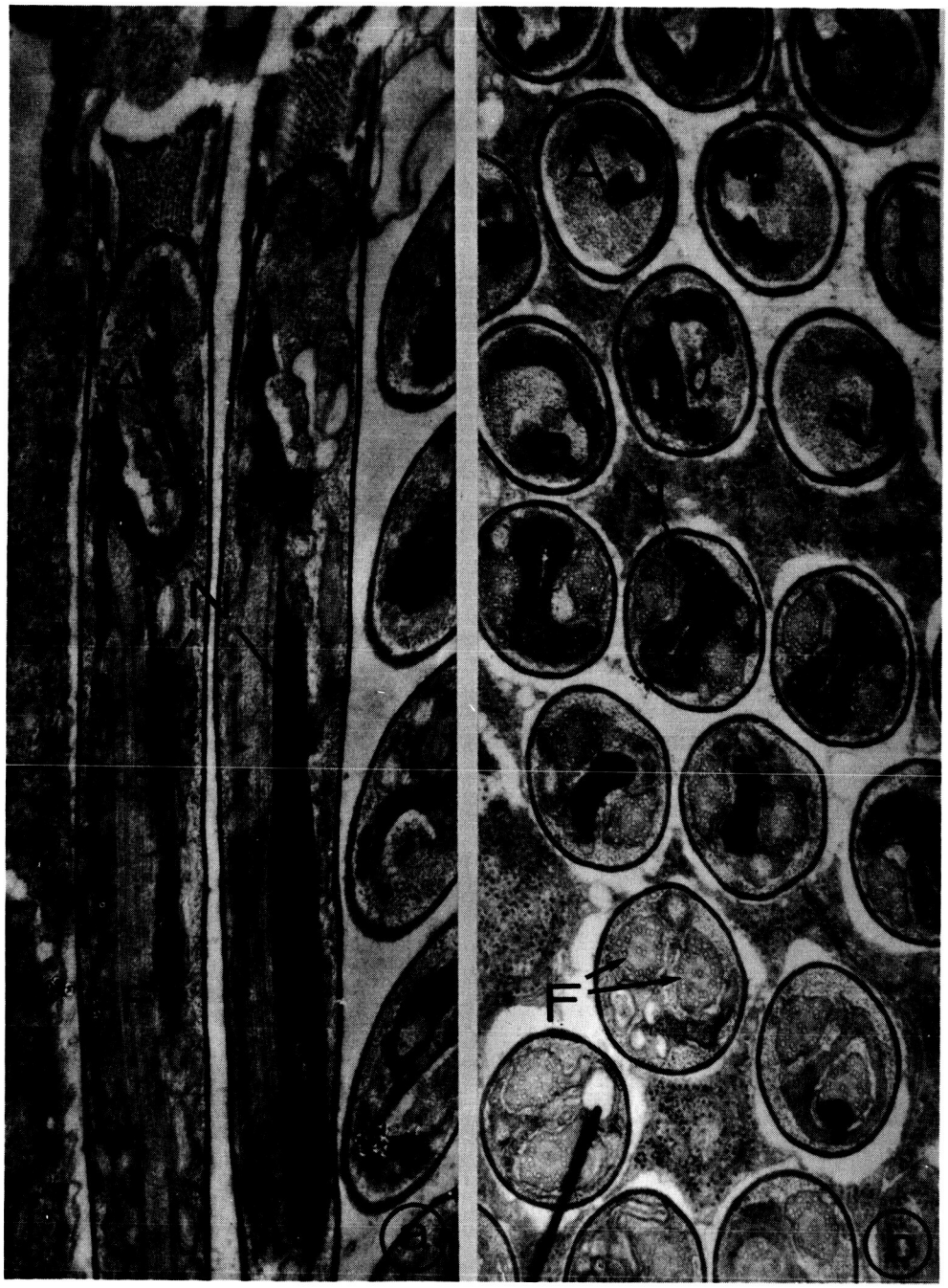

F

Fig. 13. Gar fish spermatozoon, longitudinal section. The figure includes cross sections of many spermatozoan flagella. Karnovsky's fixative, X 49,500.

Fig. 14. Egg wrinkling and agglutination action of anti-Lytechinus jellyless egg rabbit γ-globulin on Lytechinus jellyless eggs followed by Coombs' test for presence of univalent antibody.

a. Acid dejellied Lytechinus eggs + papain digested anti-Lytechinus jellyless egg γ-globulin 4 hours after mixing.

b. Acid dejellied Lytechinus eggs + papain digested control γ-globulin after 4 hours treatment.

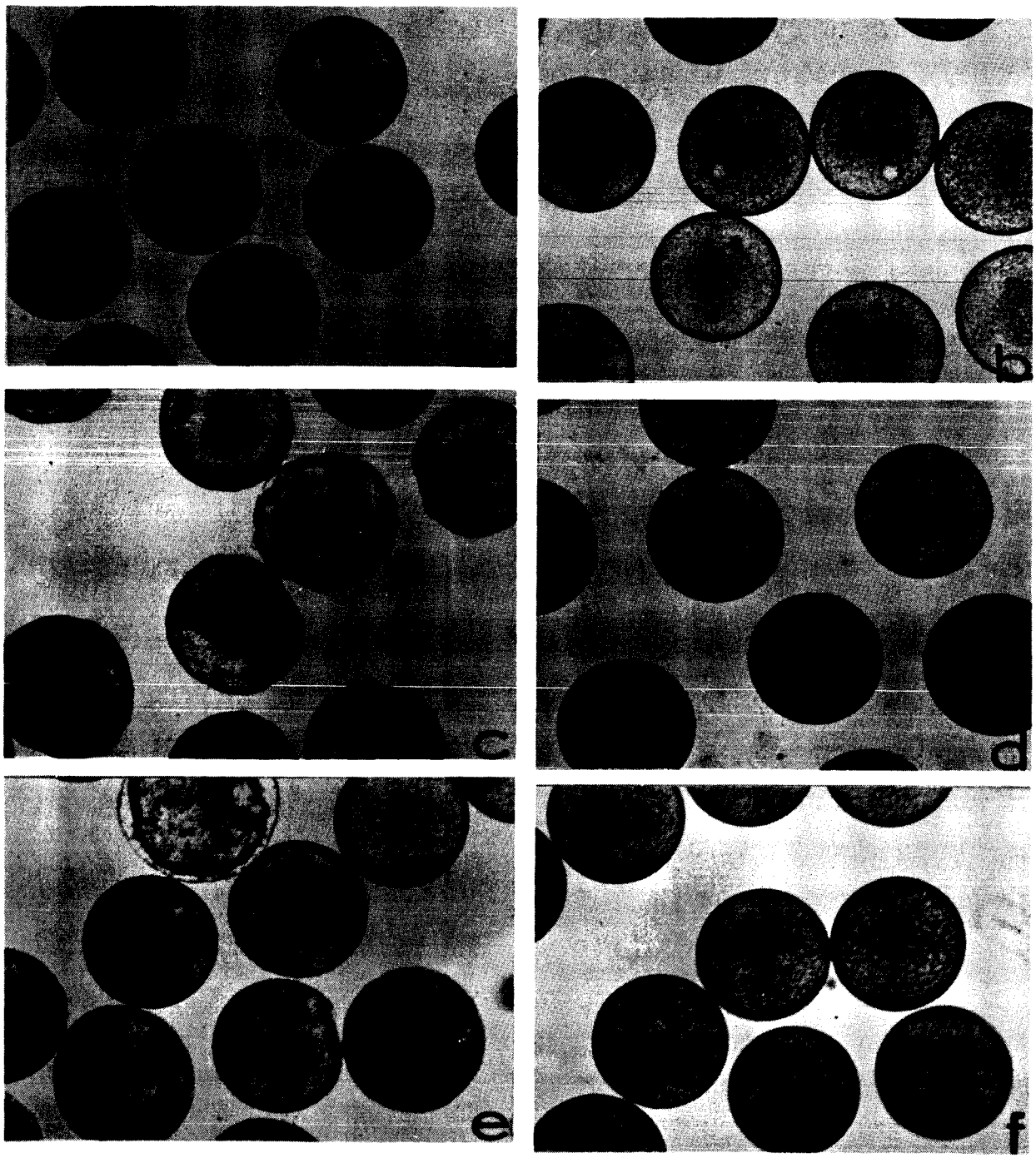
c. Acid dejellied Lytechinus eggs + undigested anti-Lytechinus jellyless egg γ-globulin after 4 hours treatment.

d. Acid dejellied Lytechinus eggs + undigested control γ-globulin after treatment for 4 hours.

e. Digested anti-Lytechinus jellyless egg rabbit γ-globulin pretreated eggs from a washed after 50 minutes treatment and transferred to sheep anti-rabbit globulin. Picture taken after 3 hours treatment with sheep γ-globulin.

f. Digested control γ-globulin pretreated eggs from b washed after 30 minutes treatment and transferred to sheep anti-rabbit globulin. Picture taken after 3 hours treatment with sheep γ-globulin.

NOTE: Washings from a and b were also added to sea water as controls at same time as addition to sheep γ-globulin. These controls appeared as a and b above.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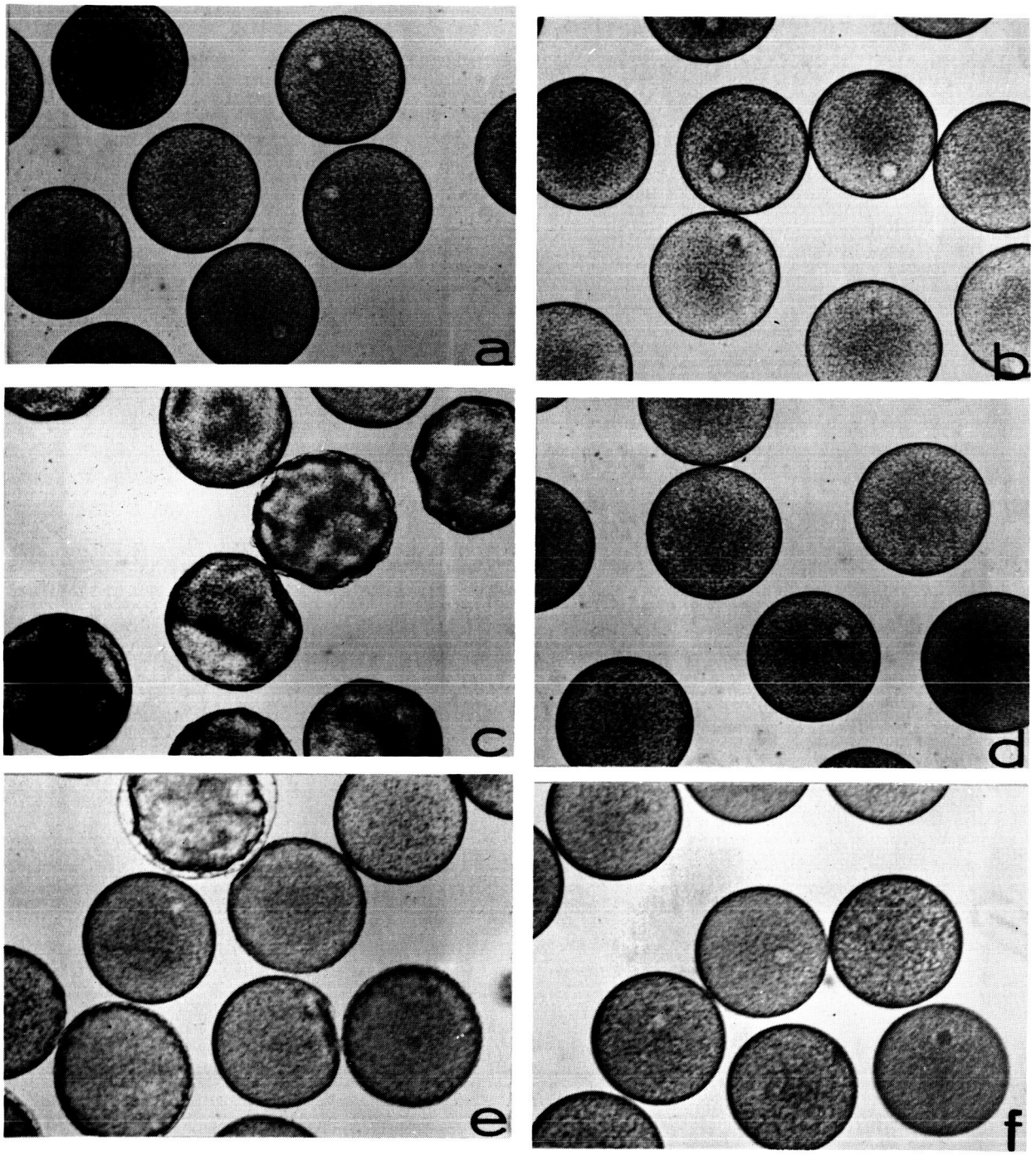
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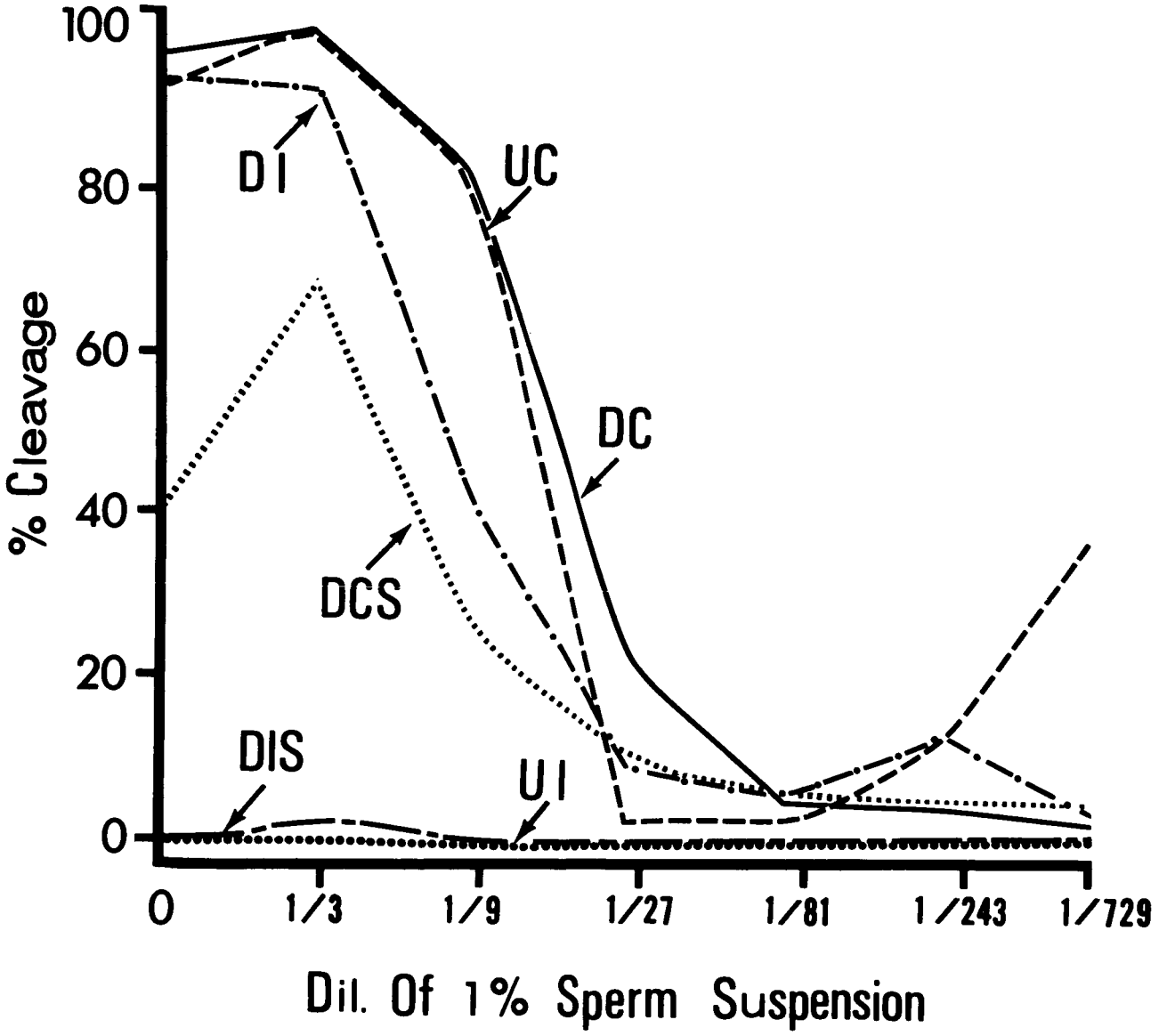
Fig. 15. Fertilization capacity of Lytechinus jellyless eggs after pretreatment with digested and undigested anti-Lytechinus jellyless egg rabbit γ-globulin followed by subsequent treatment with anti-rabbit sheep γ-globulin. Sixteen drops of acid dejellied Lytechinus eggs were mixed with 32 drops of rabbit γ-globulin and allowed to stand 30 minutes. All eggs were then washed twice in 10 ml. sea water and 8 drops of the pretreated digested immune and digested control eggs were then transferred to an equal volume of sheep anti-rabbit γ-globulin or sea water. These eggs were allowed to stand 30 minutes and were then flooded with 5 ml. sea water. Four drop samples of the washed, treated eggs were then added to 5 ml. sperm suspensions. Percent fertilization was then based on counts of percent cleavage read 1.5 to 2.5 hours after insemination. D.I., Digested immune + sea water treated eggs; D.I.S., digested immune + sheep anti-rabbit treated eggs; D.C., digested control + sea water treated eggs; D.C.S., digested control + sheep anti-rabbit treated eggs; U.I., undigested immune + sea water treated eggs; U.C., undigested control + sea water treated eggs. Immune rabbit No. 6514 and control rabbit No. 6604 were used in this experiment.


100
80
60
40
20
0
% Cleavage
DI
UC
DC
DCS
DIS
UI
0
1/3
1/9
1/27
1/81
1/243
1/729
Dil. Of 1% Sperm Suspension

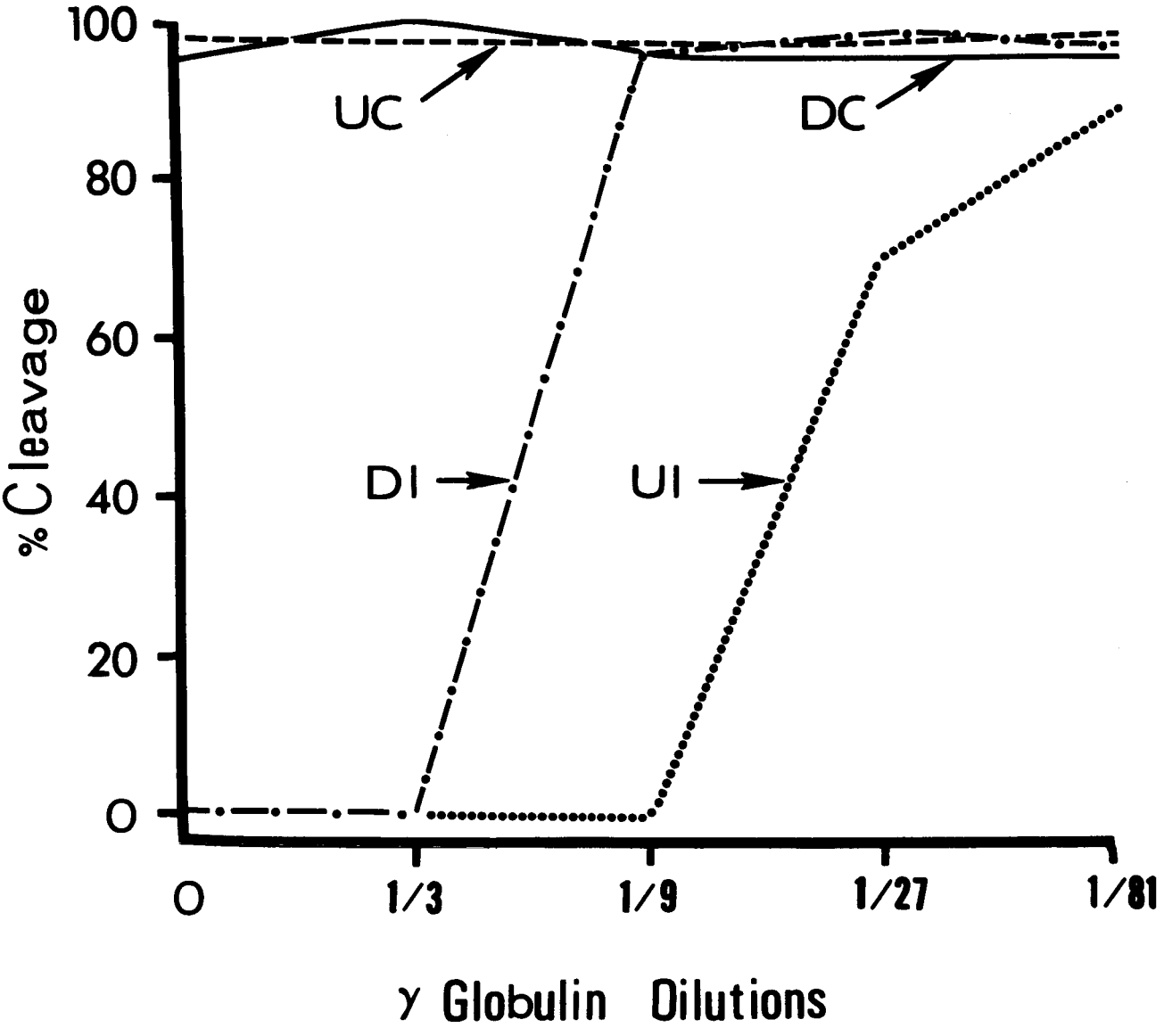


Fig. 16. Effect of anti-Lytechinus univalent fertilizin on the cleavage of fertilized, demembranated Lytechinus eggs. Two drops of fertilized, demembranated eggs were added to 4 drops of 3 fold dilutions of the γ-globulin preparations. The above results were read 1.5 hours later. D.I., Digested immune γ-globulin treated eggs; D.C., digested control γ-globulin treated eggs; U.I., undigested immune γ-globulin treated eggs; U.C., undigested control γ-globulin treated eggs. No. 6509 immune and No. 6606 control γ-globulin were used in this experiment.

NEW PROGRAMS AND NEW FOCI OF ATTACK

New programs which have grown out of research in the Institute of Molecular Evolution and which are emerging from or have progressed beyond the planning stage are described:

New Programs

Studies of lunar samples (Fox, Harada, Mueller et al.)

The syntheses performed in these laboratories, the analyses of volcanic and other geochemical samples, and questions arising from general and theoretical geochemical, prebiochemical, and postbiochemical questions make the examination of lunar samples very timely. The detailed presentation of these problems has been documented elsewhere. Examination of lunar samples will provide an opportunity to obtain answers to questions of extraterrestrial organic chemistry, will provide a framework for evaluating previous laboratory studies, and will serve as orientation for chemical studies of other extraterrestrial bodies.

Initiation of cosmochemical and organic geochemical analyses of Earth and Moon (Mueller)

Professor George Mueller has joined the Institute and has outlined the extension of his past research in other documents. This program will be in essence a comprehensive comparative study of meteorites, lunar samples, and appropriate terrestrial samples. While this work will of course constitute an independent program within the institute, collaborations with the programs of Professor Harada and Professor Fox are planned in addition. The logical relatedness of these various programs is illustrated by the first two chapters of I. Breger's Organic Geochemistry.

The affiliation of Professor Mueller with the Institute permits the first steps to be taken to teach at the graduate (and senior) level a unifying course in science. This course will be developed on the evolutionary origins of the Cosmos → the elements → the Solar System → the Earth → evolution of the inanimate world to life → behavior → .

Preparation for studies on space platforms (Fox, McCauley)

The problem of the effect of weightlessness on the organization of cells has been discussed recently in the pages of the Journal of Theoretical Biology by Professors E. C. Pollard and A. Tyler. The discussion concerns the question of whether differences in behavior can be discerned between 1 G and zero G. The problem also centers around the question of whether cellular particulates or the organization of the whole cell is the appropriate context.

The variety of polyamino acids already on hand, and the knowledge available for making others, offer a rich background from which tests of difference between zero G and 1 G can be performed on organized microstructures composed of the same general kind of material from which biocells are produced. The advantage in this case is that the molecular nature of the protein-like material can be narrowly controlled and widely varied. Furthermore, the addition of other substances, e.g. lipids, alters the stability of the microparticle. In these circumstances, a variety of activities which microspheres share, and others which they perhaps share, with biocells can be tested at zero G. One of these would be chemotaxis. Another would be "budding", to help decide whether "buds" arise from within or without the microsphere. Morphological variety, fissionability, motility, and many other properties could be scrutinized at zero G and compared with their manifestations at 1 G. Some orientation in the subject matter may be expected by comparing effects at 1 G and at higher Gs; these studies can be initiated prior to the availability of a space platform.

New Foci of Attack

Studies of sequence and other structure in thermal polyanhydro-α-amino acids (Nakashima, Wang, Fox)

Now that an extensive evaluation of the intrinsic heterogeneity of thermal polymers has been performed, other studies, such as are classical for proteins, can be repeated. Since the patterns of heterogeneity obtained are comparable in extent to those of the proteins of blood serum, the attack appears promising. Studies of the degree of branching and other structural features have been carried out, but in the future this may be done more satisfactorily on relatively homogeneous fractions.

A particularly interesting application of this sort will be to the polyanhydro (histidine, tryptophan, glutamic acid, glycine, arginine, phenylalanine) which has weak MSH hormonal activity. The full sequence of MSH is known, and the activity of various sequences which are fragments of MSH has also been

tabulated. After the activity of the polymer is fully verified, the sequences may be identified and compared with those of MSH from organisms.

Maintenance of a line of proliferating microspheres (Fox, McCauley, Ferrer)

Now that a (heterotrophically) proliferating organized structure has been synthesized, the line is being maintained. In the course of the "subculturing" of each generation, proliferating units can be placed in modified environments and subjected to circumstances which may lead to further evolution. Such experiments are planned. In particular will be studied ATP-dependent polymerization and the effects of polynucleotides bound in the microparticles.

Entropy and evolution (Fox)

Basic questions of entropy and evolution can be attacked with data now being accumulated on heterogeneity of proteinoids. These data can be obtained for degree of heterogeneity of polymers, degree of nonrandomness in distribution of amino acid residues in polymers, variation of composition in molecular types within such polymers, and variation of sequences in such preparations, etc. Such data can be compared with analogous data from contemporary proteins, of which several lines of study originated in this group of investigators. Perhaps the most astonishing inference to emerge from the studies so far is the finding of a high degree of order in the model of primitive protein, the only experimental model which is available. Accordingly, that model merits intensive investigation for what light it can throw on the entropic context of evolution.

Metabolic pathways in proteinoid microspheres (Krampitz, Hardebeck, Ryan, Fox)

A major discernible difference between the proteinoid microsphere and the contemporary cell appears to be the complex metabolic pathways that are found in the latter. However, metabolic activities including weak decarboxylase, esterase, and phosphatase catalyses have been identified. Further work will consist of increasing the strength of these, searching for other pathways in microspheres, and in particular attempting to incorporate pathways of biopolymerization. A first step of this latter sort has been identified in zinc-containing microspheres which split ATP.

Binding of proteinoids and polynucleotides (Waehneldt, Ryan, Fox)

A number of new lines of investigation are possible because of the finding that appropriate proteinoids simulate histones in their binding with nucleic acids. One of the

applications of these findings is the study of the effects of bound or partly bound polynucleotides on the various cell-like properties of proteinoid microspheres. Another set of studies involves the effect of variations in proteinoid composition on nucleic acids. How, for example, will variations in composition affect a) binding and b) activity or a) and b) simultaneously?

Studies of Kornberg polymerase systems (Waehneldt, Ferrer, Fox)

The fact that "histonoids" inhibit the Kornberg system opens the possibility that various types of inhibitor can be produced and identified. Such results would be a simulation of control of polynucleotide synthesis by histones, such as described by Hurwitz and others.

Models of primitive behavior (Fox, McCauley)

Following recognition of the fact that motility is simulated by zinc-containing microspheres in solutions of ATP, other experiments imitating primitive behavior are planned. Some of these experiments searching for chemotactic and phototactic responses have been performed. Future experiments with Dr. Gordon Tollin's phototaxigraph are planned. The fact that dyes such as Crystal Violet are attracted to microspheres suggests that the inverse effect of attraction of microspheres to appropriate substances may be demonstrable. Rigorous proof that any such demonstration is the result of nonrandom, rather than random, motion is required.

Nutritive quality in proteinoids (Lewis, Nakashima, Fox)

Nutritive quality has been demonstrated in three species. In some studies, the nutritive quality of the proteinoid appears to be less than that expected on the basis of amino acid composition alone. Accordingly, systematic studies on the digestibility by proteases of simple thermal polyanhydro-α-amino acids has begun, to parallel the feeding experiments.

Steric course of asymmetric synthesis (Harada, Matsumoto)

The asymmetric syntheses of amino acids will be further studied. In particular, the mechanism of the Strecker synthesis will be examined.

Studies of oligomerization of hydrogen cyanide (Harada, Oh-hashi)

Because of the increasing evidence for the occurrence of hydrogen cyanide as a primordial compound, other conversion products will be studied. These will include particularly pyrimidines, purines, and some amino acids. The latter will be scrutinized in the context of original optical activity.

Studies of resolution by seeding (Harada)

Inasmuch as this method is believed to have unique significance in the prebiological context, and because of astonishingly effective resolution, this procedure will be further explored.

Immunochemical studies on mammalian fertilization (Metz, Anika)

Immunological studies on sea urchin fertilization have progressed to the point where some of the information and techniques can be extended to mammals. During the year studies were begun on the relation of seminal antigens to the capacitation phenomena in the rabbit. The studies will be extended to include the effect of univalent antisperm antibody on rabbit fertilization.

Microtubules in crustacean spermatozoa (Hinsch, Brown, Metz)

Recently it has been found that certain crustacean spermatozoa contain extensive bundles of microtubules. This provides an unusual opportunity to study the structure, function, and genesis of these subcellular organelles. Since microtubules are essential components of cilia and the mitotic apparatus and occur in a variety of cells, information of general significance to cell as well as reproductive biology may result from the study.

Nucleic acid and protein synthesis in sea urchin development (Chamberlain, Metz)

Results from several laboratories have shown that dramatic changes in protein synthesis occur at fertilization in the sea urchin egg. Studies on some aspects of protein and nucleic acid synthesis before and after fertilization

are in progress. Particular attention is being given to RNA and protein synthesis in non-nucleated egg fragments.

Mucopolysaccharides and sea urchin fertilization (Gregg, Metz)

Classical studies have shown that the egg cortex is the site of most of the morphogenetically important organization of the egg. The most striking change in the egg cortex at fertilization is the discharge of cortical granules. The mucopolysaccharide components of these granules are under study.

EDUCATIONAL PROGRAM

The professors of the Institute have participated in formal graduate courses, particularly in the Cellular and Molecular Biology program, with lectures on amino acids, peptides, proteins, molecular evolution, reproductive physiology, and embryology.

Lectures have been delivered to undergraduate courses (natural science classes, biology, etc.) on the current concepts of the origin of life. Special lectures have also been given to high school science clubs in the Coral Gables-Miami area.

Graduate students have also been taught, supervision of M.S. theses and Ph.D. dissertations having been completed and also being under way.

A committee composed of Professors Metz, Harada, and Fox has organized a campus lecture series entitled The Evolutionary Sequence from Matter to Man. At the time this report was prepared, the first four scheduled lecturers and their subjects were:

William Fowler:	The Universe and the Origin of Matter
Edward Anders:	The Origin of the Solar System
Brian Mason?:	The Prebiological History of the Earth
Cyril Ponnamperuma:	Abiological Organic Synthesis on the Primitive Earth

A course on the same plan, for beginning science graduate and select senior students is being organized. With the affiliation of Professor George Mueller the minimum number and types of professors to deliver the subject matter on a rigorous scientific basis, in an evolutionary continuum, has been assembled.

In line with educational objectives, institute faculty has served on academic committees to: search for a chairman for the biochemistry department and to advise on reorganization of the School of Environmental and Planetary Sciences. Faculty members have served during the year also on the Graduate Council and on the Research Council, on the Cellular

and Molecular Biology Committee, on a Committee for a Center of Excellence, on the President's Committee for a Center for the Study of Aging, on the University Space Research Committee, and on the Scientific Council of the Center for Theoretical Studies.

During the summer, Professor Metz has served as program director in Fertilization and Gamete Physiology research training at the Woods Hole Marine Biological Laboratory.

ADMINISTRATIVE FUNCTIONS

(Dockendorf, Fox)

The maintenance, and new activities, of the Institute has been made possible through financing by the University of Miami, by several federal agencies, and by the administrative activities of the director's office. The financing from all sources has consisted of buildings, building maintenance, research support, fellowship support, fees for lectures, etc. The extramural funds have been provided by the National Aeronautics and Space Administration, The National Science Foundation, and the Public Health Service. Financial reports on these have been submitted elsewhere.

The number of individual grants has been increasing and the amount of administration has increased, although not correspondingly. The average amount of support per individual principal investigator has decreased while the total funding has not varied much during the two plus years.

ACTIVITIES AND RECOGNITIONS OF FACULTY OF INSTITUTE

DURING PERIOD OF 1 JUNE 1965-30 SEPTEMBER 1966

S. W. Fox

Banquet lecture at national meeting of Tissue Culture Association of America, 1 June 1965, on a Model of Cellular Origins.

Four lectures in summer institute on Molecular Biology, University of California at Berkeley.

Member NASA Manned Lunar Science Working Group, Falmouth, Massachusetts.

Participation in meetings of NASA Bioscience subcommittee in Boston, Atlanta, and Pasadena.

Invited speaker at Colloquium on Elementary Biological Systems and Abiogenesis in Paris, 23-25 November. Appointed to Comitee d' Honeur.

Invited speaker in Synthesis, Structure, and Function of Macromolecules, Miami, 3-4 February 1966. Title of paper: "Some Properties of Thermal Polyanhydroamino Acids."

Invited lectures at Southwest Graduate Research Center, Pomona College, Upjohn Co., and the University of Münich (Gesellschaft für Ernahrungsbiologie).

Invited speaker on a Prospectus for Synthetic Proteinaceous Foodstuffs at conference at Ames Research Center, 15 April 1966.

Invited participant in Wistar Institute conference on Mathematical Challenges to the Neo-Darwinian Interpretation of Evolution.

Invited to organize with Dr. Cornelius A. Tobias and participate in a symposium on Radiation and the Origin of Life, at Cortina D'Ampezzo for the Third International Congress of Radiation Research. Title of paper: Radiation and the First Biopolymers.

Lecture to an NSF-supported institute on the popularization of science, at Iowa City, 26 September 1966.

Preparation of tapes for RIAS for foreign broadcast and for American Chemical Society's series on Molecules and Men.

Appointment to the editorial advisory board of the new journal, Cell Biology Communications. This journal will cover such subject matter as the origin of the cell, and the origin of life.

C. B. Metz

General Biology and Genetics Fellowship panel, National Institutes of Health.

Science Faculty Fellowship panel, National Science Foundation.

Research Grant Committee, Florida Division, American Cancer Society.

Special lectures at Rensselaer Polytechnic Institute and William and Mary College.

Meetings attended: American Association for the Advancement of Science, Berkeley; Board of Trustees of Marine Biological Laboratory of Woods Hole; Society of General Physiologists, Woods Hole; and Developmental Biology Conference, New Orleans.

Other Meetings Attended

American Institute of Biological Sciences, papers delivered by George G. Brown and Samuel Stern; American Society of Biological Chemists, paper (Selected) delivered by Dr. David Durant.

SOME SCIENTISTS VISITING DURING THE YEAR FOR SCIENTIFIC DISCUSSION OR EXPERIMENT

Dr. Carolyn J. Burdick	Rockefeller University
Dr. E. L. Durrum	Durrum Instruments Corporation, Palo Alto, California
Dr. Gordon Tollin	University of Arizona
Dr. Charles M. Ise	Bristol Laboratories
Mr. W. F. Wilhite	Jet Propulsion Laboratory
Dr. Klaus Hofmann	University of Pittsburgh
Dr. Feodor Lynen	Max Planck Institute for Cell Chemistry, Munich
Dr. Victor Weisskopf	Massachusetts Institute of Technology
Dr. Jerome J. Wolken	Carnegie Institute of Technology
Dr. Frank D. Drake	Cornell University
Dr. Wendell M. Stanley	University of California at Berkeley
Dr. Richard Donovick	Squibb Institute for Medical Research
Dr. Guy T. Barry	Squibb Institute for Medical Research
Dr. Lois Tiffany	University of Michigan
Dr. Philip H. Abelson	Geophysical Laboratory, Carnegie Institution of Washington
Dr. Sturla Fridriksson	University of Reykjavick
Dr. A. Pacault	Institut de magnetochemie, Bordeaux
Dr. Herbert Riehl	Colorado State University

Dr. W. S. Vincent	University of Pittsburgh
Dr. George Mueller	University of London and University of Concepcion
Dr. Earl R. Stadtman	National Institutes of Health
Dr. Thressa Stadtman	National Institutes of Health
Dr. W. Whelan	University of London
Dr. C. O. Clagett	Pennsylvania State University
Dr. T. C. Helvey	University of South Florida
Dr. J. Lawrence Fox	Rockefeller University
Dr. H. Linskens	University of Nijmegan
Dr. William Fowler	California Institute of Technology
Dr. Gertrude Hinsch	Mt. Union College
Dr. Jane Baxandal	Wenner Gren Institute

PUBLICATIONS FROM THE INSTITUTE

Books

S. W. Fox, ed. (1965) "The Origins of Prebiological Systems," Academic Press, 1-xx + 1-482.

C. B. Metz and A. Monroy, eds. (1966) "Fertilization, Comparative Morphology, Biochemistry, and Immunology. Vol. I." Academic Press. In press.

Scientific Articles (*indicates invited)

K. Harada and S. W. Fox (1962) A Total Resolution of Aspartic Acid Copper Complex by Inoculation. Nature 194, 768.

C. A. Shivers and C. B. Metz (1962) Inhibition of Fertilization in Frog Eggs by Univalent Fraction of Rabbit Antibody. Proc. Soc. Exptl. Biol. Med. 110, 385-387.

*S. W. Fox and K. Harada (1963) Experiments Related to the Chemical Origins of Protein in G. H. Bourne, ed., "Space Flight," Academic Press, 261-270.

*S. W. Fox and S. Yuyama (1963) Abiotic Production of Primitive Protein and Formed Microparticles. Ann. N.Y. Acad. Sci. 108, 487-494.

C. B. Metz (1963) Immunochemical Studies on Fertilization Mechanisms. Proc. Conf. on Immuno-Reproduction, La Jolla, Calif., 107-115.

*S. W. Fox (1963) The Outlook for Synthetic Foods. Food Technology 22, 388-392.

S. W. Fox and S. Yuyama (1963) Effects of the Gram Stain on Microspheres from Thermal Polyamino Acids. J. Bacteriol. 85, 279-283.

S. W. Fox, K. Harada, K. R. Woods, and C. R. Windsor (1963) Amino Acid Compositions of Proteinoids. Arch. Biochem. Biophys. 102, 439-445.

S. W. Fox, T. Hayakawa, and K. Harada (1963) The Synthesis of α-L-Aspartyl-L-Serylglycine. Bull. Chem. Soc. Japan 36, 1050-1051.

*S. W. Fox (1963) Experiments Suggesting Origins of Amino Acids and Proteins in J. Kastelic, H. H. Draper, and H. P. Broquist, eds., "Protein Nutrition and Metabolism," University of Illinois College of Agriculture Special Publication No. 4, 141-154.

T. Mann (1963) 5-Hydroxytryptamine in the Spermatophoric Sac of the Octopus. Nature 199, 1066-1067.

K. Harada (1963) Asymmetric Synthesis of α-Amino Acids by the Strecker Synthesis. Nature 200, 1201.

C. R. Austin (1963) Gametogenesis and Fertilization in the Mesozoon Dicyema Aegira. Parasitol. 54, 597-600.

T. Mann and C. L. Mann (1963) Comparative Biochemical Aspects of Animal Reproduction. Bull. Acad. Royale de Med. de Belgique 3, 563-593.

*S. W. Fox and T. Fukushima (1964) Electron Micrographs of Microspheres from Thermal Proteinoid in V. L. Kretovich, T. E. Pavlovskaya, and G. A. Deborin, eds., Problems of Evolutionary and Industrial Biochemistry, U.S.S.R. Publishing House, 93-100.

S. W. Fox and S. Yuyama (1964) Dynamic Phenomena in Microspheres from Thermal Proteinoid. Comp. Biochem. Physiol. 11, 317-321.

C. R. Austin and C. L. Baker (1964) Spermatozoon of Pseudobranchus striatus axanthus. J. Reprod. and Fertility 7, 123.

K. Harada and T. Hayakawa (1964) Syntheses of α-Amino Acid Menthyl Esters. Bull. Chem. Soc. Japan 37, 191-194.

K. Harada and S. W. Fox (1964) Thermal Synthesis of Natural Amino-Acids from a Postulated Primitive Terrestrial Atmosphere. Nature 201, 335-336.

S. W. Fox (1964) Thermal Polymerization of Amino-Acids and Production of Formed Microparticles on Lava. Nature 201, 336-337.

C. B. Metz, C. A. Shivers, and C. L. Mann (1964) Some Properties of Pig Follicular Fluid. J. Reprod. and Fertility 8, 115-120.

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

C. B. Metz, H. Schuel, and E. R. Bischoff (1964) Inhibition of the Fertilizing Capacity of Sea Urchin Sperm by Papain Digested Non-Agglutinating Antibody. J. Exptl. Zool. 155, 261-272.

K. Harada (1964) The Direct Resolution of DL-Aspartic Acid by the Use of an Optically Active Amine. Bull. Chem. Soc. Japan 37, 1383-1384.

A. Schwartz and S. W. Fox (1964) Thermal Synthesis of Internucleotide Phosphodiester Linkages. Biochim. Biophys. Acta 87, 696-698.

*S. W. Fox, K. Harada, G. Krampitz, T. Hayakawa, and C. R. Windsor (1964) Chemical Synthesis of Proteinoids in Conference on Nutrition in Space and Related Waste Problems. NASA SP-70, 331-337.

S. W. Fox and G. Krampitz (1964) The Catalytic Decomposition of Glucose in Aqueous Solution by Thermal Proteinoids. Nature 203, 1362-1364.

*S. W. Fox (1964, Dec.) Experiments in Molecular Evolution and Criteria of Extraterrestrial Life. BioScience 14, 13-21.

*S. W. Fox (1964) A Model of Abiogenesis and the Origin of Memory at the Molecular Level in "RNA and Brain Function, Memory, and Learning," University of California Press, 21-26.

K. Harada and S. W. Fox (1965) The Thermal Synthesis of Amino Acids from a Hypothetically Primitive Terrestrial Atmosphere in S. W. Fox, ed., "The Origins of Prebiological Systems," Academic Press, 187-201.

A. W. Schwartz, E. Bradley, and S. W. Fox (1965) Thermal Condensation of Cytidylic Acid in the Presence of Polyphosphoric Acid in S. W. Fox, ed., "The Origins of Prebiological Systems," Academic Press, 317-326.

S. W. Fox (1965) Simulated Natural Experiments in Spontaneous Organization of Morphological Units from Proteinoid in S. W. Fox, ed., "The Origins of Prebiological Systems," Academic Press, 361-382.

K. Harada and S. W. Fox (1965) Thermal Polycondensation of Free Amino Acids with Polyphosphoric Acid in S. W. Fox, ed., "The Origins of Prebiological Systems," Academic Press, 289-298.

L. E. Franklin (1965) Morphology of Gamete Membrane Fusion and of Sperm Entry into Oocytes of the Sea Urchin. J. Cell Biol. 25, 81-100.

K. Harada and S. W. Fox (1965) Characterization of Thermal Polymers of Neutral α-Amino Acids with Dicarboxylic Amino Acids or Lysine. Arch. Biochem. Biophys. 109, 49-56.

K. Harada (1965) Optical Resolution of a DL-Amino-Acid by a Stereoselective Ligand Exchange Reaction. Nature 205, 590-591.

C. R. Austin (1965) Fine Structure of the Snake Sperm Tail. J. Ultrastructure Res. 12, 3-4.

M. Y. Menzel (1965) Preferential Chromosome Pairing in Allotetraploid Lycopersicon esculentum-Solanum lycopersicoides. Genetics 50, 855-862.

K. Harada (1965) Optical Resolution of Free α-Amino Acids by the Inoculation Method. Nature 206, 1354-1355.

T. Hayakawa and K. Harada (1965) The Synthesis of α-Amino Acid Menthyl Esters. II. Bull. Chem. Soc. Japan 38, 1354-1358.

K. Harada (1965) Optical Resolution of DL-Aspartic Acid, DL-Glutamic Acid, DL-Asparagine, and DL-Glutamine by Preferential Crystallization. Bull. Chem. Soc. Japan 38, 1552-1555.

S. W. Fox (1965) A Theory of Macromolecular and Cellular Origins. Nature 205, 328-340.

*S. W. Fox (1965) Experiments Suggesting Evolution to Protein in V. Bryson and H. J. Vogel, eds., "Evolving Genes and Proteins," Academic Press, 359-369.

T. Hayakawa, K. Harada, and S. W. Fox (1966) The Synthesis of Some Peptides Related to the Active Site of Enzymes. Bull. Chem. Soc. Japan 39, 391-395.

K. Harada (1966) Optical Resolution and Absolute Configuration of trans-β-Phenylglycidic Acid. J. Org. Chem. 31, 1407-1410.

K. Matsumoto and K. Harada (1966) Stereoselective Syntheses of Optically Active Amino Acids from Menthyl Esters of α-Keto Acids. J. Org. Chem. 31, 1956-1958.

G. G. Brown (1966) Ultrastructural Studies of Sperm Morphology and Sperm-Egg Interaction in the Decapod Callinectes sapidus. J. Ultrastruct. Res. 14, 425-440.

S. W. Fox, D. Joseph, R. J. McCauley, C. R. Windsor, and S. Yuyama (1966) Simulation of Organismic Morphology and Behavior by Synthetic Poly-α-Amino Acids in A. H. Brown and M. Florkin, eds., "Life Sciences and Space Research, IV," Spartan Books, 111-120.

T. Hayakawa, K. Harada, and S. W. Fox (1966) The Synthesis of Some Peptides Related to the Active Site of Enzymes. Bull. Chem. Soc. Japan 39, 391-395.

*S. W. Fox and K. Harada (1966) Thermal Polycondensation of α-Amino Acids in P. Alexander and H. P. Lundgren, eds., "A Laboratory Manual of Analytical Methods of Protein Chemistry, Vol. IV," Pergamon Press, 127-151.

*S. W. Fox (1966) Experiments with a Precellular Model in Proc. Conf. Theoretical Biol., NASA SP-104, 121-138.

*S. W. Fox (1966) Development of Rigorous Tests for Extra-terrestrial Life in C. Pittendrigh, W. Vishniac, and J. P. T. Pearman, eds., "Biology and the Exploration of Mars," NAS-NRC Publication 1296.

A. W. Schwartz and S. W. Fox (in press) The Polymerization of Cytidylic Acid with Polyphosphoric Acid. Biochim. Biophys. Acta.

K. Harada and J. Oh-hashi (in press) Optical Resolution and Configuration of trans-2,3-Epoxybutyric Acid. Bull. Chem. Soc. Japan.

J. Oh-hashi and K. Harada (in press) The Synthesis, Resolution and Configuration of β-Hydroxyvaline. Bull. Chem. Soc. Japan.

K. Harada and K. Matsumoto (in press) Stereoselective Synthesis of Optically Active Aspartic Acid from Derivatives of Fumaric Acid and Maleic Acid. J. Org. Chem.

*S. W. Fox (in press) The Terrestrial Origins of Macro-molecules and of Cells in P. Gavaudan, "Colloquium on Elementary Biological Systems and Abiogenesis" (in French).

*S. W. Fox (in press) Prospectus for Chemical Synthesis of Proteinaceous Foodstuffs (in a conference at the Ames Research Center).

*S. W. Fox (in press) Radiation and the First Biopolymers in G. Silini, ed., Third International Congress of Radiation Research, Cortina D'Ampezzo, Italy.

D. L. Rohlfing and S. W. Fox (in press) The Catalytic Activity of Thermal Polyanhydro-α-Amino Acids for the Hydrolysis of p-Nitrophenyl Acetate. Arch. Biochem. Biophys.

T. Hayakawa, C. R. Windsor, and S. W. Fox (in press) Copolymerization of the Leuchs Anhydrides of the Eighteen Amino Acids Common to Protein. Arch. Biochem. Biophys.

T. V. Waehneldt and S. W. Fox (in press) Phosphorylation of Nucleosides with Polyphosphoric Acid. Biochim. Biophys. Acta.

M. Y. Menzel and J. M. Price (in press) Fine Structure of Synapsed Chromosomes in F_1 Lycopersicon esculentum Solanum-lycopersicoides and its Parents. Amer. J. Bot.

Book Reviews

*S. W. Fox (1962) Life: Its Origin, Nature, and Development, by A. I. Oparin. Science 136, 309.

*S. W. Fox (1965) The Chemical Origin of Life, by A. I. Oparin. J. Chem. Education 42, A220.

*S. W. Fox (1965) Comparative Biochemistry. Vol. VII, by M. Florkin and H. L. Mason ,eds. J. Amer. Chem. Soc. 87, 3789.

*S. W. Fox (1966) Evolving Genes and Proteins, by V. Bryson and H. J. Vogel, eds. J. Chem. Education 43, A544.

Articles Reprinted in Anthologies by Special Invitation and Arrangement

*S. W. Fox (original in Science, 1960; reprint 1966) How Did Life Begin? C. Pittendrigh, W. Vishniac, and J. P. T. Pearman, "Extraterrestrial Life," NAS_NRC Publication 1296A, 48-64.

*S. W. Fox (original in BioScience 1964; excerpted 1966) Experiments in Molecular Evolution and Criteria of Extraterrestrial Life in E. J. Kormondy, ed., "General Biology II," Wm. C. Brown Co., Inc., 148-150.

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