FOURTH ANNUAL REPORT

INSTITUTE OF MOLECULAR EVOLUTION

UNIVERSITY OF MIAMI
CORAL GABLES, FLORIDA

FOURTH ANNUAL REPORT

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*Since this report is prepared primarily for scientists in the offices of the National Aeronautics and Space Administration, data on Professor Charles B. Metz and his group, supported by other agencies, are not included in this report.

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PRINCIPAL UNPUBLISHED ADVANCES DURING THE YEAR

Dr. Fox

Discovery of the utility of various ammonium phosphates as terrestrial catalysts for reactions at local low pH (with Dr. Klaus Dose).

Establishment of α-glutamyl linkages in a dominant hexapeptide isolated from a thermal copolymer of glutamic acid, glycine, and tyrosine (with Dr. Nakashima).

Discovery of ability of mixed amino acid adenylates to couple with thermal proteinoid to yield larger polymers (with Dr. Krampitz).

Discovery of a new kind of microsphere arising spontaneously from adenylate-modified thermal proteinoid and having stability at pH 1-10 (with Dr. Krampitz).

Discovery of a model of binary fission in particles synthesizing peptide bonds internally (with Mr. Brooke and Dr. Krampitz).

Establishment of binding of hematin by proteinoid of sufficient histidine content (with Mr. Weber).

Discovery of selectivity in interactions of polynucleotides and polyamino acids as demonstrated by omission of individual "neutral" amino acids (with Dr. Yuki).

Dr. Harada

The observation of spontaneous resolution of DL-aspartic acid copper complex. This observation was confirmed by two other independent laboratories. These phenomena are of interest in connection with the optical origin of bioorganic molecules in general. The results suggest a fundamental bias toward L amino acids and D aldoses in nature. Sure avoidance of contamination is however difficult to attain.

Establishment of chelation hypotheses in catalytic hydrogenation. The extension of the chelation hypothesis to the field of asymmetric syntheses has been in progress. By the use of chelation hypothesis, several stereochemical courses of asymmetric syntheses and the mechanisms of other stereospecific reactions have been clarified.

The formation of phenylalanine in the thermal syntheses of amino acids recorded earlier. The new observation that phenylacetylene is a possible intermediate suggests that acetylenic compounds are relevant to prebiological chemistry. The conversion of acetylenic compounds to carbonyl compounds is now under investigation as an area of prebiological chemistry.

Dr. Mueller

Establishment of a process of gradual devolatilization and graphitization of the carbonaceous complex in chondrites, with the loss of inorganic volatiles. Discovery of interrelations between reduced Fe/Ox, Fe ratio, and S/C ratio in chondritic matter.

Observation of complex biomorph structures and four distinct types of motility of microspheres within inclusions of differentiated carbonaceous complexes in quartz crystals from the PreCambrian of several localities of S. W. Africa.

Discovery of differentiation into condensed aromatic, simple aromatic, and aliphatic phases of bitumens associated with hydrothermal veins within the Abbott mercury mine and other locations of California.

Microscopical observation of differentiation into two immiscible liquids of bitumen inclusions within quartz crystals from Herkimer County, New York.

Establishment of close and detailed similarities between the size distribution diagrams of a) microchondrules and chondrules in chondrites; b) fog droplets and rain drops in clouds; c) products of unusually high temperature and extensive ash eruptions from the Quaternary of the San Pedro de Atacama District, Chile.

PRINCIPAL ADVANCES REPRESENTED IN PUBLICATIONS AND ORAL PRESENTATIONS DURING THE YEAR

Dr. Fox

Discovery of the ability to produce proteinoids from equimolar mixtures of all amino acids common to protein. These polymers produced have low proportions of aspartic acid, less than 5% in some cases.

Discovery of hormonal (melanocyte-stimulating) activity in thermal polymers of amino acids.

Binding of polynucleotides by lysine-rich (histone-like) proteinoids. Relationship of this ability to the exact content of basic amino acid.

Finding of formation of nucleoproteinoid particulates as models of organelles within cells.

General from Several Laboratories

Increased number of reports on catalytic activities in thermal proteinoids.

Oral Presentations

New perspectives on geological conditions preceding the formation of living systems. This includes the need for open systems, and the relevance of irreversible thermodynamics.

Establishment of the concept of self assembly as relevant to the formation of the first organisms in a manner analogous to the formation of structures in contemporary organisms.

Explanation of the evolutionary meaning of 18-20 diverse amino acids. This includes a description of how the information in the first proteins could arise from the reactant monomers instead of from a coded macromolecule.

Review of criteria for distinguishing, in terrestrial volcanic samples, amino acids and peptides which have arisen a) as a result of chemical synthesis from those which appear b) as the consequence of organismic decomposition. β-Alanine and high glycine contents have been shown to be ambiguous.

Establishment of a definite hexapeptide sequence as a dominant structure in the thermal copolymer of glutamic acid, glycine, and tyrosine.

Dr. Harada

Establishment of chelation hypothesis in the catalytic hydrogenation reaction. By the use of the hypothesis, sterically controlled synthesis of dipeptides was reported in the First American peptide symposium.

Optical resolution of DL-aspartic acid copper complex by the use of biopolymers was reported.

Dr. Mueller

Discovery of microspherical morphology in organic inclusions in quartz crystals from the PreCambrian of the Gaius 6 Farm, Klein Karas District, S. W. Africa.

Recognition of close similarity of redistribution ratios of volatile elements between a) carbonaceous chondrites/mean ordinary chondrites; b) hydrothermal deposits/mean Earth's crust.

Reconstruction of a process of gradual sintering and coalescence of microchondrules in the course of the cosmological history of chondritic meteorites.

Quantitative chemical and geological evidences in support of a new theory of genesis of Chilean nitrate deposits through capillary concentration.

Reconstruction of the assemblage of mineral deposits of the Moon, through interpretation of rocket photographs.

The first outlines of a new theory of evolution.

CONCISE ASSESSMENT OF THE STATE OF THE INVESTIGATION OF THE MODEL OF THE ORIGIN OF LIVING SYSTEMS

Research in the program of the institute has demonstrated how a protocell could have come into existence spontaneously on the primitive Earth. The attendant research has answered in principle such questions as:

1. How did enzymes arise in the absence of enzymes to make them?

   The concise, oversimplified answer is that what was required was the presence of mixtures of diverse amino acids (including sufficient aspartic acid, glutamic acid, or lysine) and appropriate geophysical conditions (temperatures or other conditions which would remove water).

2. How did cells arise in the absence of cells to father them?

   The concise answer is that they result from self-assembly of appropriate macromolecules, which could have been (according to experiment) a rather wide range of polyamino acids.

3. How could a first membrane appear in the absence of parental membrane?

   Phenomenologically, assembly of proteinoid into spherules has been shown to yield selective boundaries. The presumably necessary lipid is found in the hydrocarbon quality of the side-chains of many of the amino acid residues.

4. How did "information" in proteins come into existence in the absence of a complex DNA-RNA-protein system?

   Internal constraints have been shown to be the consequence of reactions of diverse monomeric amino acids, making coding macromolecules at first unnecessary. This realization leads to understanding how information would be transferred from environment to individual. It should also lay to rest uncertainties about postulated discontinuity between prelife and life.

5. How could self-replication arise?

   This is shown also to be an intrinsic property of particulate proteinoid microsystems, composed of budding (perhaps binary fission) and growth by accretion to a limit.

The five answers above are not independent. They are interrelated and associated, and their roots are found in the same polyamino acid material.

Other questions are also answered in principle, notably the origin of optical activity. Prebiochemical occurrences which could lead to optical activity without life have been demonstrated in a number of ways by the Harada group.

The research is now emphasizing how a more contemporary type of peptide bond synthesis might have come into existence through amino acid adenylates, these being universally or almost universally intermediates in the biosynthesis of protein. Little investigation of the chemistry of amino acid adenylates has been carried out earlier; for instance, no copolymerization has been found recorded in the literature. In the research performed here, copolymerization of eighteen to twenty amino acid adenylates in the presence of preformed polyamino acid has shown striking results. Coupled with studies of selective reaction of polyamino acids and polynucleotides, this work is now providing a basis for construction of a theory of the origin of the code.

The adenylate model is promising not only for contemporization of the model of a primitive cell, but for illuminating directly the biosynthesis of contemporary protein. As one example of the parallelism, formylmethionine contributes both to protein biosynthesis and to adenylate-type proteinoid synthesis.

ADVANCES IN LABORATORY AND FIELD RESEARCH PROGRAMS[*]

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[*] Beginning with this report, no attempt to distinguish between laboratory studies supported by funds for laboratory work, terrestrial analyses, lunar studies, or Martian studies is attempted. The rationale of the commingling, of course, is that studies in all of these four realms have the same sets of objectives and are highly related one to the other.

A Search for Criteria to Distinguish Abiotically Synthesized Polyamino Acids from Peptides Obtained by Decomposition of Terrestrial Organisms (Fox, Windsor)

Samples from many volcanic regions including Stromboli, the Island of Hawaii, St. Lucia in the Antilles, and Iceland have been analyzed in the effort to determine if polyamino acids found in such samples were of chemically synthetic origin. These results have been compared carefully with amino acid compositions obtained in experiments in model prebiotic synthesis of amino acids in other laboratories and in these laboratories. The analyses have been compared also with compositions in thermal polyamino acids, and with the compositions of organisms of proteins. Two criteria which might have distinguished geochemically synthesized amino acids from organismic amino acids have been examined. One of these is the presence of β-alanine, as suggested by its frequent appearance in amino acid synthesis experiments, and the other is a relatively high proportion of glycine (40-60%) in "prebiotic" amino acid mixtures and also in many volcanic samples.

The search for β-alanine in volcanic samples has been fruitless so far. β-Alanine has however been found as a result of hydrolysis of polymers in the case of chemical synthesis of amino acids. The fact that β-alanine appears to be absent from all volcanic samples argues against those volcanic samples being of synthetic origin. Some other explanation such as the possibility that a particular synthesis of a volcanic sort would not yield this non-α-amino acid is however reasonable.

A more promising criterion of distinction has been that of the relatively high glycine content that has been observed in many "prebiotic" amino acid syntheses. This invited further investigation because of the fact that many volcanic samples also show a relatively high glycine content. One reservation with respect to such a correlation is the knowledge that some organismic proteins, especially collagen, tend to run relatively high (30%) in organisms. A more serious reservation arises from the known fact that glycine is one of the most stable of amino acids. Accordingly, if the other amino acids were to decompose more rapidly than glycine either in polymers or as the free amino acid, the glycine content would be increased relatively. This effect conceivably could explain the fact that glycine is at times found to constitute 50% of the total amino acids of some volcanic samples.

Experiments were constructed to test these hypotheses. The kind of situation employed was particularly that of boiling for extended periods a protein, bovine serum albumin, in the water of the acid pool of St. Lucia. Most boiling mineral pools have a pH of about 7 but this one has a pH of 2-1/2. Insufficient water of this pH was collected at first. Accordingly, distilled water was adjusted to pH 2.6 with sulfuric acid since sulfate was shown to be present in abundance. Decomposition of amino acids of recrystallized bovine serum albumin was studied in this liquid refluxed for 24 days. While much decomposition of amino acids was not anticipated in boiling water from pools at pH 7.2, the breakdown of some amino acids was substantial at neutrality. This was true both for filtered water and for suspensions in which insoluble minerals were present.

The result of one set of analyses is presented in Table I. The detailed data are presented in order to present evidence on several points. One of these points deserving emphasis is the fact that calculating relative % figures from absolute % figures requires some caution in precise interpretation. Relative percent figures from large absolute % figures are, for example, internally restricted. The increase in NH_3% cannot, for instance, exceed 35 relative % because that would require $>$ 100 absolute % in the value at 24 days.

Table I shows that the two sulfur-containing amino acids, methionine and cystine, are totally decomposed. While this was true in St. Lucia mineralized water at 7.2, it was not true in distilled water at pH 2.6.

The changes of primary significance are the increases in relative percents of lysine, proline, glycine, and unknown decomposition products reacting with ninhydrin.

In Table II are presented all amino acids, plus ammonia, which show increases relative to other amino acids. The two amino acids which exhibit relative increase in both suspension and in filtered water are glycine and proline. Glycine is thus shown to increase in content under two sets of geological conditions. The likelihood of relatively high glycine content by decomposition of other amino acids in at least one kind of geological material is thus supported by experiment and analysis.

The validity of this explanation for high glycine values seems increased when one other likelihood is recalled. This is the probability that collagenous proteins are among the most likely to survive from the decomposition of organisms or other contaminating material. Material of this sort could be expected to contain 20-30% glycine. In order for analyses to show 50% glycine in amino acid assays, only a relative doubling is necessary.

One explanation of the finding of a single or a few amino acid(s) in a volcanic sample is the decomposition of contaminating proteins to the stage of a residue of one or a few amino acid(s). The basic difficulty in attempting to distinguish between synthesis and decomposition is that the stable amino acids are the same ones that are most likely, for thermodynamic reasons, to be formed in synthesis.

Table I

Amino Acid Assays of Hydrolyzates of Bovine Serum Albumin Refluxed in Filtered St. Lucia Water at pH 7.2

At Outset			After 24 Days of Boiling		
Amino Acid	Mole %	Mole % Calc'd without NH_3	Amino Acid	Mole %	Mole % Calc'd without NH_3
Lys	2.52	9.58	Lys	2.61	11.09
His	0.77	2.91	His	0.69	2.92
NH_3	73.67	0	NH_3	76.46	0
Arg	0.92	3.48	Arg	0.69	2.92
Asp	2.77	10.51	Asp	2.28	9.70
Thr	1.54	5.86	Thr	1.33	5.64
Ser	1.17	4.45	Ser	0.98	4.18
Glu	3.84	14.59	Glu	3.70	15.72
Pro	1.41	5.34	Pro	1.57	6.65
Gly	0.78	2.95	Gly	0.85	3.61
Ala	2.33	8.85	Ala	2.15	9.13
Cys/2	0.67	2.55	Cys/2	0	0
Val	1.76	6.67	Val	1.49	6.34
Met	0	0	Met	0.0	0.0
Ileu	0.63	2.38	Ileu	0.58	2.47
Leu	2.81	10.67	Leu	2.66	11.28
Tyr	0.77	2.91	Tyr	0.60	2.53
Phe	1.26	4.77	Phe	1.09	4.63
Allo Ileu	0.17	0.65	Allo Ileu	0	0
Unknowns	0.24	0.89	Unknowns	0.28	1.20

Table II

Relative Percent Increase in Individual Amino Acid Contents on 24 Days of Boiling Bovine Serum Albumin in St. Lucia Hot Pool Water at pH 7.2

Amino Acid or Ammonia	In Filtered St. Lucia Water	In St. Lucia Water Suspension
Ammonia	3.6	6.3
Lysine	11.3	-33[a]
Aspartic acid	-17.7[a]	10.1
Glutamic acid	-3.6[a]	2.0
Proline	11.3	6.5
Glycine	9.0	16.5
Unknowns	16.7	$200\left(\frac{.21}{.07} - 1\right)$

[a] Decrease

Models of Early Terrestrial Phosphates (Dose, Mejido, Fox)

Phosphates, pyrophosphates, and polyphosphates have been used in this laboratory as condensing agents in the preparation of anhydropolymers, heterocyclic compounds such as are numerous in organisms (e. g. pyrimidines), and in phosphorylation of nucleosides. Both in this laboratory and in similar experiments in others, acidic conditions have often proved most favorable. The occurrence of acidic conditions on the primitive Earth has however been disputed, despite the fact that acidic conditions can be found locally today, and despite the reasoning of Vinogradov, Rubey, etc. regarding an early acid ocean.

In studies carried out here, other possibilities have been demonstrated. Ammonium salts, such as would exist in equilibrium with the postulated ammonia-containing early atmospheres, are decomposed readily at temperatures below 300° C to yield highly acid phosphate products.

For example,

$$NaNH_4HPO_4 \xrightarrow{heat} NaH_2PO_4 + NH_3 \uparrow$$

and

$$(NH_4)_2HPO_4 \xrightarrow{heat} H_3PO_4 + 2\ NH_3 \uparrow$$

When such phosphates are mixed and heated, "Graham's Salt" results.

Amino acids have been successfully condensed in the presence of such phosphate mixtures. The behavior of such ammonium salts indicates how such substances, compatible with some inferences about an ammonia-rich primitive atmosphere, would nevertheless easily yield local acid conditions.

Photochemical Activity of Pigmented Proteinoid (Fox, Weber)

The pigment reported last year to have photochemical activity has been studied. Purification has been effected in a number of ways. The pigment has been found to undergo changes to other compounds during fractionation. The experiences suggest that the lability of the pigment is increased by separation from the polyamino acids with which it is formed.

Binding of Hemin by Proteinoid (Weber, Fox)

A study of the binding of hemin by proteinoids of varying histidine and lysine contents was carried out, histidine being a key residue in such bindings by proteins. Both histidine-rich proteinoids and microspheres therefrom were shown by spectrophotometry to bind hemin. The binding in the particles was also visible to the eye because of the color. The photochemical capacities of such systems is being studied.

Condensation of Amino Acid Adenylates (Fox, Krampitz)

As stated elsewhere in this report, experiments in this institute have demonstrated how the heating of amino acids could yield polymers which upon contact with water would assemble to form cell-like systems which answer in principle the following questions: how enzymes came into existence in the absence of enzymes to make them, how cells came into existence in the absence of cells to father them, how macromolecular information arose from amino acids instead of a coded macromolecule, how a first membrane appeared, and how the initial self-replication of a primitive living system could occur. This cell-like system is a model of a primitive system. It is also a heterotroph, requiring that its nourishment be obtained preformed from the environment. In being a heterotroph it differs from the vast majority of organisms which are found contemporaneously, although it differs only in part. In order for such a primitive organism to have evolved to a typical contemporary type of organism, the ability to synthesize macromolecules internally would have had to develop. The first investigations here of possible syntheses of this sort concerned amino acid adenylates.

Amino acid adenylates are known to function universally, or almost universally, in organisms as intermediates in the biosynthesis of protein. Despite wide acceptance of the significance of this intermediate biologically, remarkably few investigations on the chemistry of the amino acid adenylates have been reported. This is explainable in part by the relative instability of the amino acid adenylates; they are difficult to work with in the laboratory. This same instability, however, qualifies this and other compounds for function as rapidly active intermediates in significant reactions. Thanks to the work of Meister, and of Berg, methods for preparation of amino acid adenylates in the laboratory have been developed. The method published by Berg is especially useful. It employs the reaction of a free amino acid with adenosine monophosphate in the presence of DCCD (dicyclohexylcarbodiimide). The energy-rich adenylate derivative has been prepared with most of the amino acids of protein. No report found in the literature, however, deals with the polymerization of more than a single amino acid adenylate simultaneously. Most of the various polymerization studies in this laboratory have however been built on simultaneous copolymerization. This approach has yielded many results which could not have been anticipated from studies of homopolymerization. The first of these results was the polymerization of the eighteen amino acids common to protein by the simple process of heating them together whereas many of them decompose when heated alone. This experiment also recognized the fact that protein contains eighteen to twenty amino acids and was based on the premise that such a variety of monomers must have had great evolutionary advantage. A second type of panpolymerization was that of the Leuchs' anhydrides, published in 1965 and in 1967. By each of these two methods one may combine in a single polymer simultaneously some proportion of each of the amino acids common to protein. The adenylate approach now represents a third mode. Again, new results are being foun d and as in the case of the first, or thermal, mode these seem to have unique significance.

While all amino acid adenylates will condense to yield a polymer containing all of those amino acids, they do so with much greater facility in the presence of preformed (thermal) proteinoid.

An analysis of an unprimed adenylate condensation product is given in Table III. One may see that all of the amino acids are well represented; only tyrosine is present in relatively small proportion. One feature of this condensation is that although the reaction begins with equimolar amounts of 17 amino acids (cystine omitted), the proportions found in the product are strikingly like those that are observed in an hydrolyzate of a contemporary protein, contemporary proteins being much alike in their amino acid composition (Fox and Homeyer, 1955; M. H. Smith, 1966).

A remarkable feature of this analysis is the obvious similarity in composition to an average protein as computed by Vegotsky and Fox in 1962. Although one cannot assume that such compositions resulted from equimolar mixtures of amino acids in the primitive scene, the similarities are suggestive. They are especially eye-catching against the background of the fact that amino acid compositions of various proteins are much alike. The possibility of constrained compositions is accordingly being examined by varying compositions of mixtures.

At this time insufficient data are at hand to permit deciding whether adenylates will yield a second generation of answers to the fundamental questions of origins and primordial sequence. Were thermal proteinoids necessary or contributory at first? A full assessment of the meaning of the adenylates will require further characterization of the polymers and of the microparticles arising therefrom. Also required is a filling of the gaps in knowledge of how amino acid adenylates might have first come into existence until they could yield the enzymes necessary for continuation of the line (in a contemporary mode; e. g. phosphokinase, adenylate synthetase, and RNA polymerase).

Table III

Amino Acid Contents of Hydrolyzate of Unprimed Condensation Product of Seventeen[a] Adenylates

Amino Acid	Content	
	Adenylate Polymer	Ratios in Average Protein
Lysine	6. 5	5. 9
Histidine	2. 4	1. 8
Arginine	4. 2	4. 9
Aspartic Acid	10. 3	9. 7
Threonine	4. 9	4. 8
Serine	4. 2	6. 0
Glutamic Acid	9. 7	12. 7
Proline	5. 1	6. 2
Glycine	11. 1	12. 6
Alanine	14. 3	9. 6
Valine	7. 3	5. 9
Methionine	0. 7	1. 8
Isoleucine	4. 5	6. 0
Leucine	9. 6	6. 0
Tyrosine	0. 1	2. 3
Phenylalanine	4. 5	3. 7

[a] Tryptophan shown to be present by qualitative test only

Thermal Synthesis of Polymers having Melanocyte-Stimulating Activity (Fox, Wang)

The last annual report recorded the fact that thermal polymerization of six amino acids which are important in the active site of melanocyte-stimulating hormone can be condensed to a polymer having some of such activity. This work has been published [S. W. Fox and C.-T. Wang, Science 160, 547-548 (1968)]. While the finding of catalytic activities in thermal proteinoids is especially appropriate to explaining the origin of biochemical systems, the finding of hormonal activity has an added significance. The special inferences for the hormonal activity derived from the fact that hormones are more often thought of as occurring or as being useful later in evolution after the first organisms. In particular they serve to regulate actions at supracellular levels; especially would this be true for melanocyte extension. Accordingly the finding of active polymers suggests that the first cells had available to them hormonal compounds, or alternatively, these compounds were available in the plasm for use by biological forms which evolved subsequently. A third possibility is that active sequences or structures emerged prebiotically and then also emerged independently in biological forms which occurred later in evolution.

The finding of such activity has however made possible the production of "omission polymers," from which individual amino acids have been omitted systematically. Many of these polymers were then tested for their hormone-like activity. The results indicate a reaffirmation of the initial finding, and they also begin to provide data from which one may hope to identify the most active contributors to the active site. While these experiments have not been thoroughly analyzed, the initial indications are that the crucial amino acids in the synthetic polymers are not simply the same as those in the natural hormone, but that some complex relationship may apply. The studies coming from both directions may prove to be especially fruitful in identifying the locus of activity in the molecules.

Dr. Nakashima has fractionated such a polymeric preparation and is accumulating information on the sequence of amino acids found in this hexatonic polymer.

Sequences in a Thermal Polyamino Acid (Fox, Nakashima)

An initial report of a hexapeptide sequence appearing in a thermal copolymer of glutamic acid, glycine, and tyrosine was presented last year. The derivative of the hexapeptide is pyroglutamylglycyltyrosyl-α-glutaminyltyrosylglycine.

This hexapeptide has been isolated to the extent of 9% of the total polymer, and it is unquestionably represented more fully than to 9% in the original polymer because of inevitable losses in fractionation. On the basis of a priori assumptions of random distribution one should expect approximately 1/90 of the material to be the hexapeptide derivative indicated. When the assumptions include also the presence of a pyroglutamic acid residue, the derivatization of the other glutamic acid residue as an amide, and the fact that both α and γ linkages of glutamic acid might have appeared, the probability of finding what has been found is decreased probably by at least one order of magnitude. To find 9% when less than 0.1% can reasonably be anticipated is indication of considerable internal ordering. This kind of evidence is as rigorous as one could hope to obtain.

Evidence for the preference of the sequence identified is even greater than that indicated however. As much as 12% of a pentapeptide corresponding to the hexapeptide as indicated below is found.

Fraction	Proportion of polymer	Peptide
4-2	9%	pyroglutamylglycyl-tyrosyl-α-glutaminyl-tyrosylglycine
4-3	12%	glycyltyrosyl-α-glutaminyltyrosyl glycine

This proportion of pentapeptide indicates further the tendency to internal order. At the same time, evidence for some other linkages is found in fragments from other fractions of the polymer.

These results are consistent with at least seven other kinds of evidence which indicate that heated amino acids form polymers non-randomly. The fundamental inference derivable therefrom is that ordered primordial proteins could have come into existence in the absence of nucleic acids.

Proteinoid Microparticles Resembling Microfossils (Fox, McCauley)

Microfossils photographed and published by Barghoorn and his associates resemble microparticles which have been obtained inadvertently in this laboratory. Almost all types which have been published are imitated by the laboratory product. Three comparisons are presented in Fig. 1. The conditions under which the microparticles are obtained in the laboratory resemble very closely those

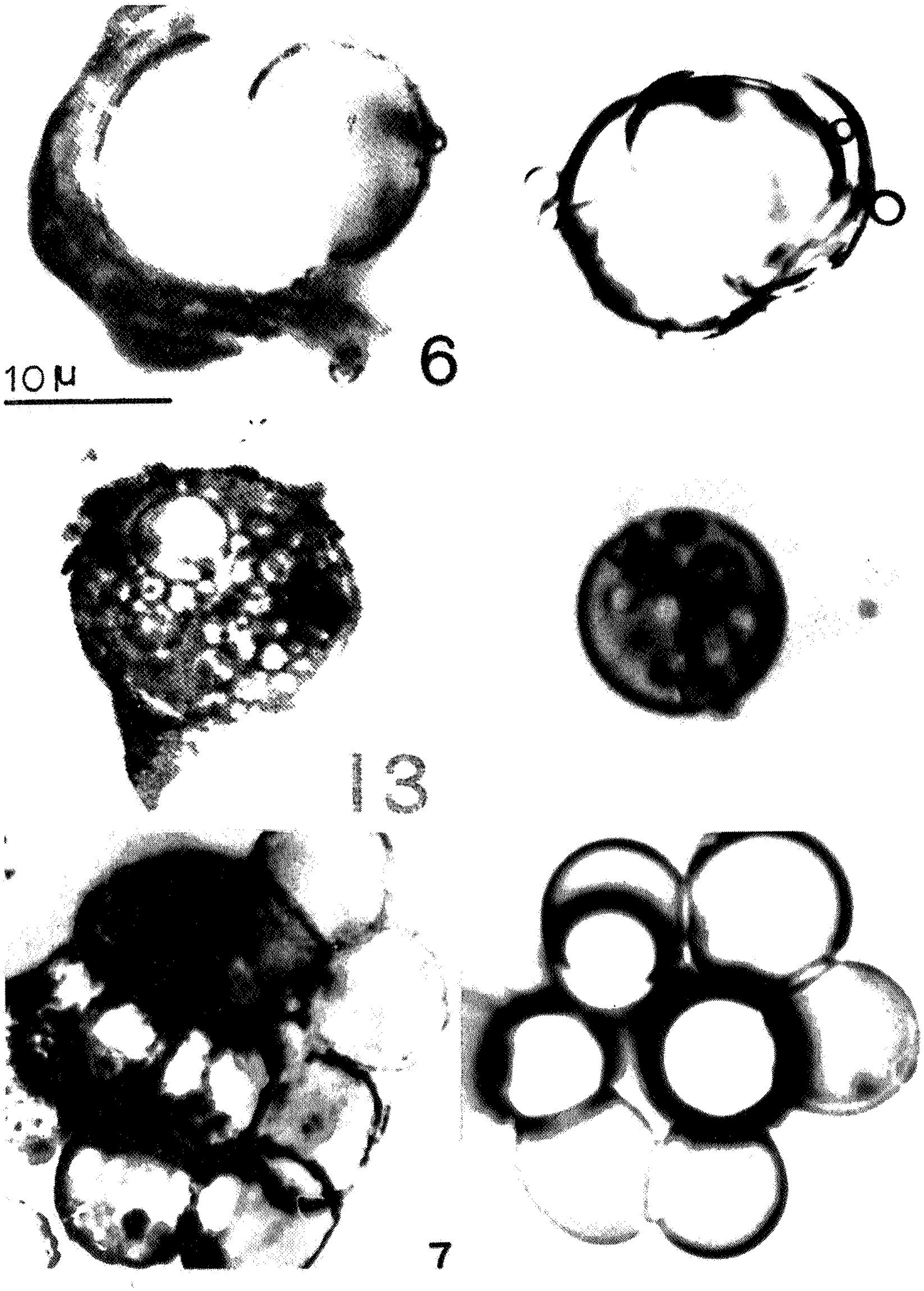


Fig. 1. "Microfossils" of Barghoorn on left. Proteinoid microparticles on right.

of the conditions which are believed to have been present with the microfossils. The geological strata would not have had to be subjected to temperatures such as those ordinarily used for condensation of amino acids in the laboratory--for one of two reasons. The temperatures often used in the laboratory, such as 130° C or 170° C, are not required for the condensation. These condensations occur in the laboratory at considerably lower temperatures in the presence of phosphates which in general are found relatively abundantly in the crust of the Earth. Secondly, the proteinoid yielding microparticles terrestrially could have come from elsewhere and then precipitated. The resultant structures would then have been captured as fossils. These could have undergone replacement by minerals. Of relevance is the finding by Barghoorn that amino acids have been found in the microfossils.

The consequence of these comparisons is that microfossils examined by Barghoorn might have been either organisms or prebiological systems which had not reached the stage of development of contemporary organisms which they resemble morphologically. If they are indeed prebiological systems they are then in some respects of considerably more significance than if they are organisms which have been captured by geological processes.

The microfossils, many of the organized elements of meteorites, and the proteinoid microparticle have much in common morphologically. The similarity to organized elements of the proteinoid microparticles was first depicted in the Second Annual Report, the Institute for Space Biosciences, Florida State University and in a publication [S. W. Fox and S. Yuyama, Ann. N. Y. Acad. Sci. 108, 487-494 (1963)].

Demonstration of Incorporation of Nucleic Acid in Proteinoid Microsphere (Fox, Ryan)

The inclusion of nucleic acids in proteinoid microspheres has been demonstrated in earlier reports. A rigorous new kind of evidence is that which has been obtained by producing neutral proteinoid microspheres in the presence of yeast RNA. When treated with Acridine Orange and examined under the fluorescing microscope (thanks to Dr. Charles B. Metz), fluorescence is marked in those units containing RNA and is absent in microspheres containing no RNA (Fig. 2).

Microparticles from Condensation of Amino Acid Adenylates (Fox, Brooke, Krampitz)

The reaction of the mixed 18 or 20 amino acid adenylates leads immediately to turbidity and particles. These tend to be spherical like the microspheres from thermal proteinoid. They are, however,

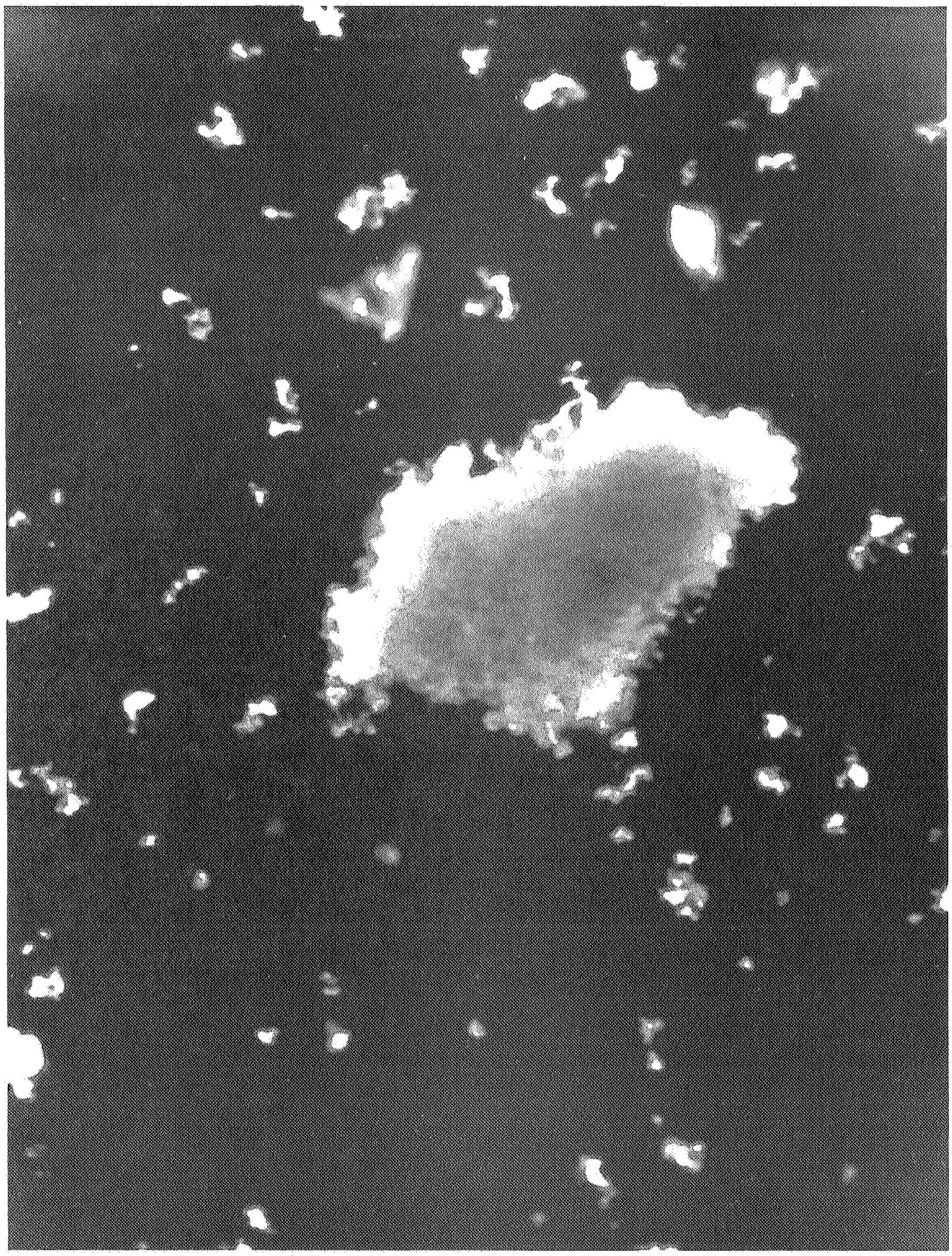


Fig. 2. Neutral proteinoid microspheres containing RNA, fluorescing with Acridine Orange.

simultaneously more stable and more dynamic. The microspheres obtained by such reaction with a mixture of basic proteinoid and acidic proteinoid do not dissolve in the pH range of 1-10. They easily form buds which separate spontaneously, and they undergo binary fission spontaneously.

In Fig. 3 is seen a budded microsphere of the kind described. In Fig. 4 are seen frames in a time-lapse series demonstrating before-and-after of spontaneous binary fission. Similar cleavage has been demonstrated in thermal proteinoid microspheres by slight increase in pH. The defect of that model of binary fission was the dissipation of the polymer and the particles due to increased pH. At the time, the overcoming of that defect was predicted to require internal synthesis of peptide bonds, possibly to lead to a swollen state ("twoness" as suggested by Mazia). The adenylate-derived structures appear to provide a first model of such internal synthesis. They also do not dissolve substantially as the pH is increased.

Feeding of Proteinoids to Rats (Fox, Mejido)

Ten feeding experiments have shown that adult rats survive on 50:50 mixtures of proteinoid and casein, although they cannot survive on proteinoid alone. In adult rats a period of adaptation seems to be necessary. After ten days of eating the proteinoid, the rats begin to gain weight rapidly and even surpassed the casein control rats in some cases.

One obstacle encountered was the rats' dislike for the bitter taste of the proteinoid. Taste has been improved by washing the proteinoid with ethanol; by treating the proteinoid with liquid ammonia; and by increasing the time of dialysis. All of these procedures decrease the yield considerably.

When weanling rats were fed 1% equimolar proteinoid and 9% casein they immediately gained more weight than the casein control rats. When the percentage of proteinoid was increased, however, they rejected the food, probably due to the taste.

The feeding experiments were performed by Mrs. Estelle Lench under the supervision of Dr. George Lewis.

Selective Interactions of Polynucleotides and Polyamino Acids (Fox, Yuki)

Numerous studies have been completed and the data are being digested. The extreme ease with which histone-like compositions can

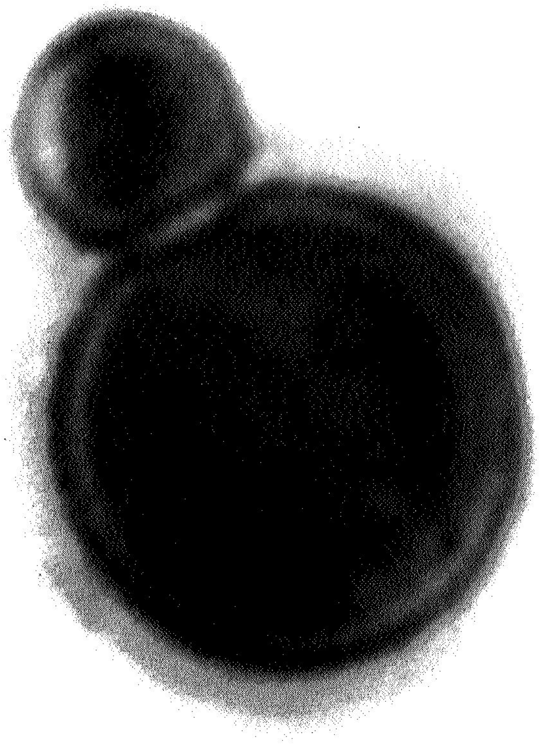


Fig. 3. A budded microsphere from reaction of 18 adenylates with a mixture of acidic and basic thermal proteinoid (4:1).

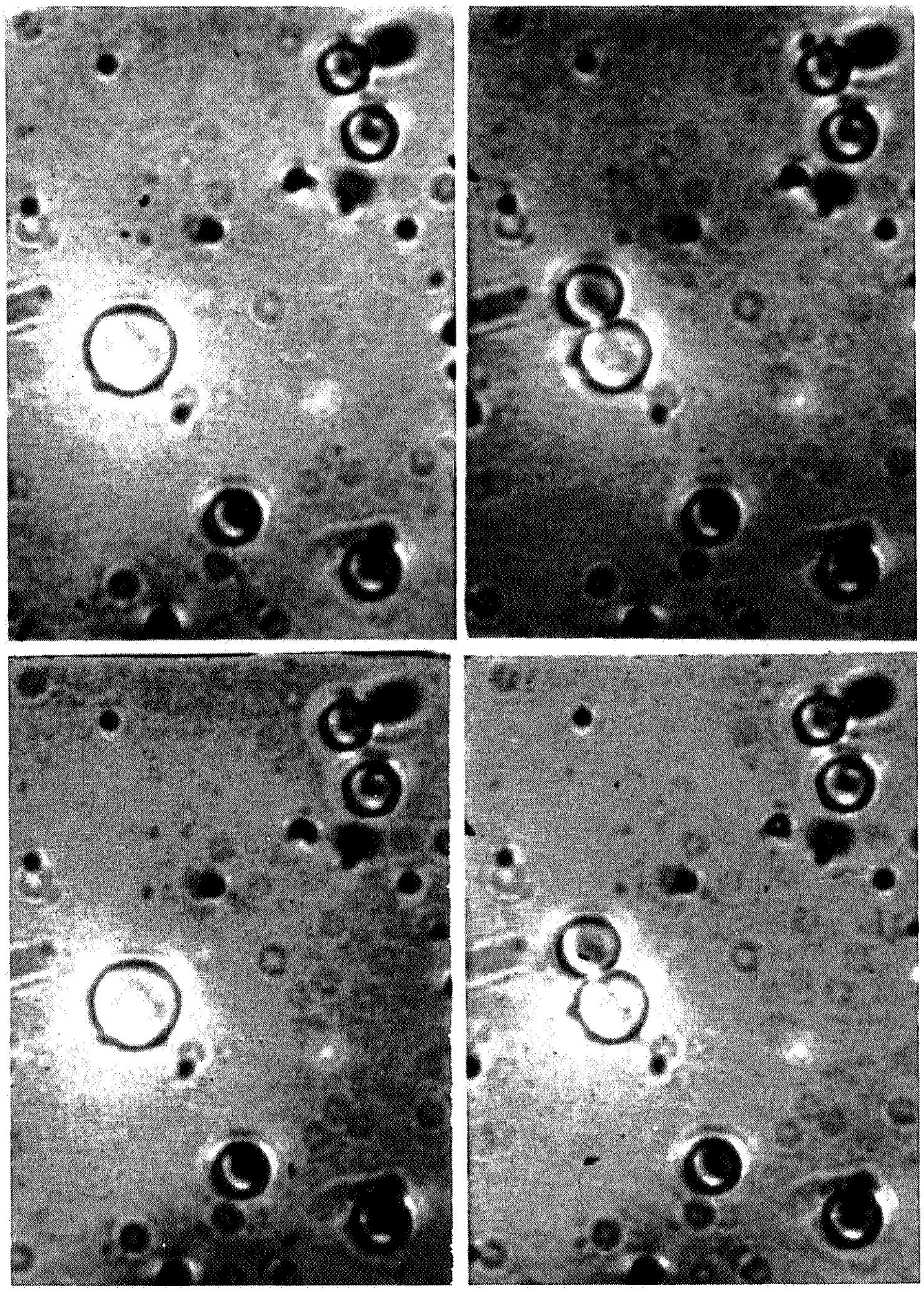


Fig. 4. Frames from a time-lapse sequence showing binary fission of microparticles resulting from reaction of 18 mixed amino acid adenylates with a clear solution of acidic-basic thermal proteinoid mixture (4:1). Time interval between photomicrographs 20 seconds. Separation of daughters has been observed by eye in other experiments.

be quickly and precisely varied and produced aids in the construction of these experiments.

Examples of results are given in Tables IV and V.

Table IV

Effect of Omissions on Reactions of Proteinoids and Polyadenylic Acid to Yield Minispheres

	Omission					
Lys % in Proteinoid (in mixture)	None	Glu	Arg	Hsd	Ile	Leu
60	0. 52	0. 46	0. 03	0. 28	0. 11	0. 40
40	0. 32	0. 65	0. 72	---	0. 63	0. 63
20	1. 04	1. 34	0. 00	0. 05	1. 44	0. 08

Figures are measures of turbidity at 600 m*u*. All proteinoids contain also asp, ala, gly, pro, val.

The percent of lys influences the result. Turbidity is maximal at the 20% level for 7 of the 11 proteinoids tested. For arg omission, maximum is at the 40% lys level (> at 30% and > at 50%). Hsd and leu are also largely necessary to particulate formation at the 20% level. The neutralization effects are, thus, specifically modified by a non-charged amino acid, e. g. leu. Other experiments under way indicate that combined omissions affect the results in unpredicted fashion.

Table V

Effect of Lysine Content of Proteinoids on Polynucleotide-Proteinoid Complex

Lys Content (%)	Poly A	Poly U	Poly C	Poly G	Poly I
60	0.52	0.47	0.40	0.32	0.51
50	0.13	0.31	0.51	0.03	0.09
40	0.32	0.44	0.40	0.11	0.34
30	0.97	0.89	0.72	0.80	0.96
20	1.04	0.97	0.76	0.89	1.04
10	0.01	0.00	0.00	0.01	0.03
0	0.01	0.00	0.00	0.03	0.01

Figures are measures of turbidity at 600 mμ.

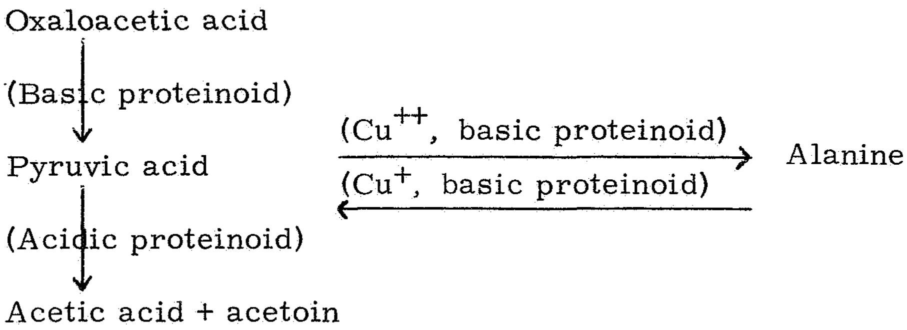
In Table V, effects of individual polynucleotides may be seen. A minimal lys content is essential to reaction by the proteinoid. Maximum effects are all at the 20%, minimal, level. Poly I and poly G are relatively unreactive at the 40% and 50% levels. Under test with "deletion proteinoids", new specific effects are also being observed.

Tables IV and V are samples. Specific effects in interaction are being observed in a number of ways. As the construction of the experiments indicates, one may search for the flow of information in each of two directions: polynucleotide → amino acid and polyamino acid → nucleotide. These studies can explain the chemistry involved in the origin of the code. Their application to studies of adenylate type synthesis of proteinoids is especially sought.

The Origins of Enzymes and Metabolism (Fox, Krampitz)

The list of catalytic activities found in thermal proteinoids has increased during the year. These activities have been reported from these laboratories: Krampitz and associates in Bonn, Noguchi and Saito in Hokkaido, Rohlfing at Ames Research Center, Oshima at Ames Research Center, and Usdin and Mitz at Melpar. The types of reaction include hydrolysis, decarboxylation, amination, and deamination. For each of these a number of substrates have proved to be active whereas other substrates were not. Specificities in reaction of proteinoids and substrates have been observed. Table VI lists the discoveries reported in full publication. Others have been found.

The integrated studies reveal how metabolic pathways might have originated:



This metabolic network has its contemporary counterpart in substrates. Whether the types of enzyme correspond to the types of proteinoid has not been established. Each kind of reaction is catalyzed, however, by a different kind of proteinoid.

Of much significance to the space program is the demonstrated ease with which geologically plausible thermal polyamino acids could arise to give positive tests for enzymes. It is now possible to state with assurance that neither enzyme-like activity nor optical activity is a sure sign of life as we know it. The finding of many catalytic powers of high specific activity associated with predominantly monoconfigurational monomers in polymers in organized units would however strongly suggest that organismic evolution had occurred.

Table VI

Catalytic Activities Identified in Proteinoids and Other Thermal Polyamino Acids

Substrate and Reaction	Authors	Year
Hydrolysis		
p-Nitrophenyl acetate	Fox, Harada, and Rohlfing	1962
	Rohlfing and Fox	1967
	Noguchi and Saito	1962
	Usdin, Mitz, and Killos	1967
p-Nitrophenyl phosphate	Oshima	1968
ATP (by Zn salt)	Fox	1965
Decarboxylation		
Glucuronic acid	Fox and Krampitz	1964
Pyruvic acid	Krampitz and Hardebeck	1966
	Hardebeck, Krampitz, and Wulf	1968
Oxaloacetic acid	Rohlfing	1967
Amination		
α-Ketoglutaric acid	Krampitz, Diehl, and Nakashima	1967
	Krampitz, Baars-Diehl, Haas, and Nakashima	1968
Deamination		
Glutamic acid	Krampitz, Baars-Diehl, Haas, and Nakashima	1968

Spontaneous Resolution of DL-Aspartic Acid Copper Complex (Harada)

DL-Aspartic acid copper complex was partially resolved by spontaneous crystallization from aqueous solution. Optical purity is in the range of 5-30%, and the configuration of aspartic acid crystallized is usually L. The results were confirmed by two other laboratories (Dr. Nakaminami of Osaka University and Dr. Hayakawa of Shinshu University). The reason for the crystallization of L-aspartic acid copper complex is not known yet. Two explanations are possible. One is by contamination by optically active seed from the air. The question of contamination certainly always applies in the experiments in this study because the whole world is contaminated with biological materials. However, as found in our laboratory, DL-aspartic acid copper complex resulted in D-aspartic acid when seeded with cotton or wool, which are typical biomolecules in plant and animal kingdoms [K. Harada, Nature 218, 119 (1968)]. Therefore, any seed for the spontaneous resolution of DL-aspartic acid copper complex could not be cotton or wool, and the seed must be distributed throughout the whole world. The seed could however be a common contaminant in the production process of DL-aspartic acid.

The second possible reason, rather more speculative, is that the optical activity may be related to parity non-conservation. Some physicists speculate that electromagnetic attraction could also be disymmetric, in addition to the weak interaction. It might be possible that the spontaneous resolution may be related with the disymmetry of electromagnetic attraction.

Optical Resolution of *trans*-DL-2, 3-Epoxysuccinic Acid and Preparation of D(-)-*erythro*-β-Hydroxyaspartic acid (Oh-hashi, Harada)

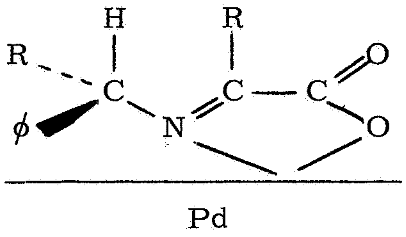
Trans-DL-2, 3-Epoxysuccinic acid was resolved and optically active *erythro*-β-hydroxyaspartic acid was prepared. These materials were compared with optically active 2, 3-epoxysuccinic acid obtained by fermentation and also with optically active *erythro*-β-hydroxyaspartic acid obtained by transamination.

Optical Resolution of DL-Aspartic Acid Copper Complex by the Use of Biopolymers (Harada)

DL-Aspartic acid copper complex in aqueous solution containing acetic acid was resolved by the use of cotton or wool. D-Aspartic acid copper complex was crystallized by use of these biopolymers. The resolution could be regarded as a combination of asymmetric adsorption of D-aspartic acid copper complex on the biopolymers and a preferential crystallization of optically active copper complex from the supersaturated solution.

Solvent Effect in a Sterically Controlled Synthesis of Optically Active α-Amino Acids from α-Keto Acids by Hydrogenolytic Asymmetric Transamination (Harada, Matsumoto)

In the earlier study [J. Org. Chem. 32, 1794 (1967)], a steric course of the Hiskey reaction [J. Am. Chem. Soc. 83, 4798 (1961)] was proposed. In this mechanism, the substrate formed a chelated structure with the catalyst. The chelation should be weak. (If the attraction between substrate and catalyst is strong, the substrate would act as a catalytic poison.) If this is the case, chelated intermediate would be strongly affected by the solvent effect. Therefore, several solvent effects were studied on the Hiskey reaction. The results were as expected, i. e. optical purity of the product was affected by the solvent used. It was found in the glutamic acid synthesis that the configuration of product was inverted depending on the solvent used.



Synthesis of Optically Active Alanine from Oxaloacetic Acid by Hydrogenolytic Asymmetric Transamination (Matsumoto, Harada)

In this investigation, reaction of oxaloacetic acid with optically active α-methylbenzylamine was studied in order to obtain optically active aspartic acid. However, the resulting amino acid was found to be only optically active alanine. Therefore, very fast decarboxylation of oxaloacetic acid during the reaction must take place. The mechanism of the decarboxylation was studied. The mechanism might be similar to that of the natural aspartic acid β-decarboxylase.

Optical Resolution of DL-Aspartic Acid by a Stereoselective Ligand Exchange Reaction (Harada, Tso)

Optical resolution of DL-aspartic acid copper complex by the use of stereoselective ligand exchange reactions has been reported from this laboratory [Nature 205, 590-591 (1965)]. A detailed study has been carried out in order to confirm the proposed mechanism. The stereoselective reaction is not only interesting as a new method of resolution of an α-amino acid, but also as an explanation of the development of optical activity of amino acids and other biomolecules in the prebiological world.

Mechanisms of the Thermal Synthesis of Phenylalanine from Methane, Ammonia, and Water (Harada)

Formation of phenylalanine was observed among the products of the thermal reaction of methane, ammonia, and water [K. Harada, S. W. Fox, Nature 201, 335-336 (1964)]. The mechanism of phenylalanine formation (nine carbon-molecule from one carbon methane) has been studied. Phenylacetylene was found to be the most likely intermediate which was converted to phenylalanine by the method employed in the thermal gas reaction.

Asymmetric Synthesis of α-Amino Acids by the Use of Asymmetric Prototropy

It was first found that the $>C=N-$ bond was reduced by heating with hydrogen donor (isopropyl alcohol or glucose) and catalyst (Raney Nickel) in a suitable solvent. By the use of this reaction, asymmetric syntheses of optically active α-amino acids are now under way.

Discovery of a New Factor in the Escape Mechanism of Volatile Elements from Celestial Bodies (Mueller)

It was established in the course of work during the previous year that a) the percentage of volatilizable C and S within the organic phase of chondritic meteorites decreases with the over-all percentage of constituents volatilizable at 1000° C, in N_2 atmosphere; b) as a first approximation, most of the relatively volatile elements decrease linearly with the total of volatilizable constituents. The percentages of C and S, however, reveal a considerable range of variation within types of meteorites which have approximately the same amount of total volatiles. The S/C ratio tends to increase with the reduced iron/oxidized iron ratio as summarized in Table VII.

The above interrelations can be explained through the anomalously high degree of change of fugacities of C and S with the redox potential. Under reducing conditions which are indicated with the Red. Fe/Ox. Fe values above unity (and presumably reducing atmosphere), the S is retained mainly as nonvolatile FeS, and the C tends to escape as saturated hydrocarbons. Under more oxidizing conditions, the S would tend to volatilize in the free form, and the C would remain in the parent body as unsaturated and highly polymerized organic substances, and as graphite.

The results outlined above indicate that as regards the origin of life, the range of redox potentials of the atmosphere of a given celestial body may prove to be an even more crucial factor than was hitherto generally believed. It is possible that on the relatively larger and cooler parent bodies of the enstatite chondrites, reducing transient atmospheres prevailed, and the chances of evolution of primitive organisms would be low, on account of the escape of the bulk of carbon as low molecular, saturated hydrocarbons, and the fixation of most of the sulfur as FeS. The mildly oxidizing atmosphere indicated for the type II and type III carbonaceous chondrites may represent optimum conditions, as the bulk of the carbon is retained in the high molecular weight organic substances, and free sulfur is available for reactions with the carbonaceous complex. The appearance of appreciable quantities of trivalent iron in the type I carbonaceous chondrites indicates pronouncedly oxidizing conditions, which explain the appearance of carbonate and sulfate, and the highly carbonized state of the organic complex in the meteorites in question; these features appear unfavorable for the genesis of the protoorganism.

The interrelations between the percentage and the distribution of N, P, etc., and the Red. Fe/Ox. Fe ratio of chondritic meteorites cannot be determined at present on account of insufficient chemical data. Work in the future may lead to the establishment of the following

Table VII

S/C Ratios in Meteorites

Type of Chondrite	Volatiles	Red. Fe/Ox. Fe	S%	C%	S/C
Olivine-bronzite	1%	2. 73	2. 20	0. 03	73. 4
Olivine-pidgeonite	1	0. 39	1. 94	0. 19	10. 2
Enstatite	4. 74	170	4. 34	0. 24	17. 5
Carbonaceous, Type III	5. 12	0. 10	2. 26	0. 87	2. 56

The table is based on 50 analyses from the literature and 14 analyses of the author.

data: a) optimal redox potential for the retention of each biologically significant element in the chondrites; b) optimal redox potential for the entry of the highest proportion of each of the elements into the carbonaceous complex; c) optimal redox potential for the highest value of the ratio carbonaceous complex/C+H+O+N+P+S.

Complex Structures in Organic Inclusions of Quartz Crystals from the Precambrian of S. W. Africa and Bechuanaland (Mueller)

Microspherical objects from a small quartz crystal of the Precambrian of S. W. Africa, which has been obtained through exchange with the museum of Johannesburgh, were described on p. 53 of the third annual report of the IME.

A field trip to S. W. Africa uncovered in four remote localities free bitumens or bitumen inclusions in quartz crystals. A fifth locality was discovered in the course of the field trip. The geological observations made in the course of the trip strongly support the theory that the bitumens within the early Precambrian granites, gneisses, and basalts are of juvenile magmatic origin.

Each of the five hitherto known localities proved to have different morphological and chemical types of inclusions, and the microstructures have been found to differ from each other within distinct morphological and chemical types of inclusions, and the microstructures have been found to differ from each other within distinct inclusions of the same quartz crystal. These observations seem to be indicative of the great variability of conditions which may occur within the hydrothermal sites of the Earth.

The morphologically more significant features of the structures are summarized below:

1. Budding. In most of the inclusions, the ratio between the diameter of the microsphere:diameter of the bud varies between the rather narrow limits of 5 to 12 (Figs. 5, 6, 8). In certain inclusions, budding appears only on those microspheres which have diameters superior to a well defined "budding" minimum (usually from 10 to 20 microns), and the number of "buds" per microsphere increases with increasing diameter (Fig. 5). A few of the microspheres reveal two generations of buds; the secondary buds are smoother and of lighter shade than the primary buds (Fig. 6).

2. Complex microspheres. In some microspheres a well defined central core of darker (Fig. 6b) or lighter (Fig. 7) material appears. Randomly distributed black or light hemispheres (Fig. 6a) and spheres (Fig. 6b) are also seen in some of the microspheres. These structures closely resemble the structures in certain proteinoid microspheres, coacervates, or higher cells.

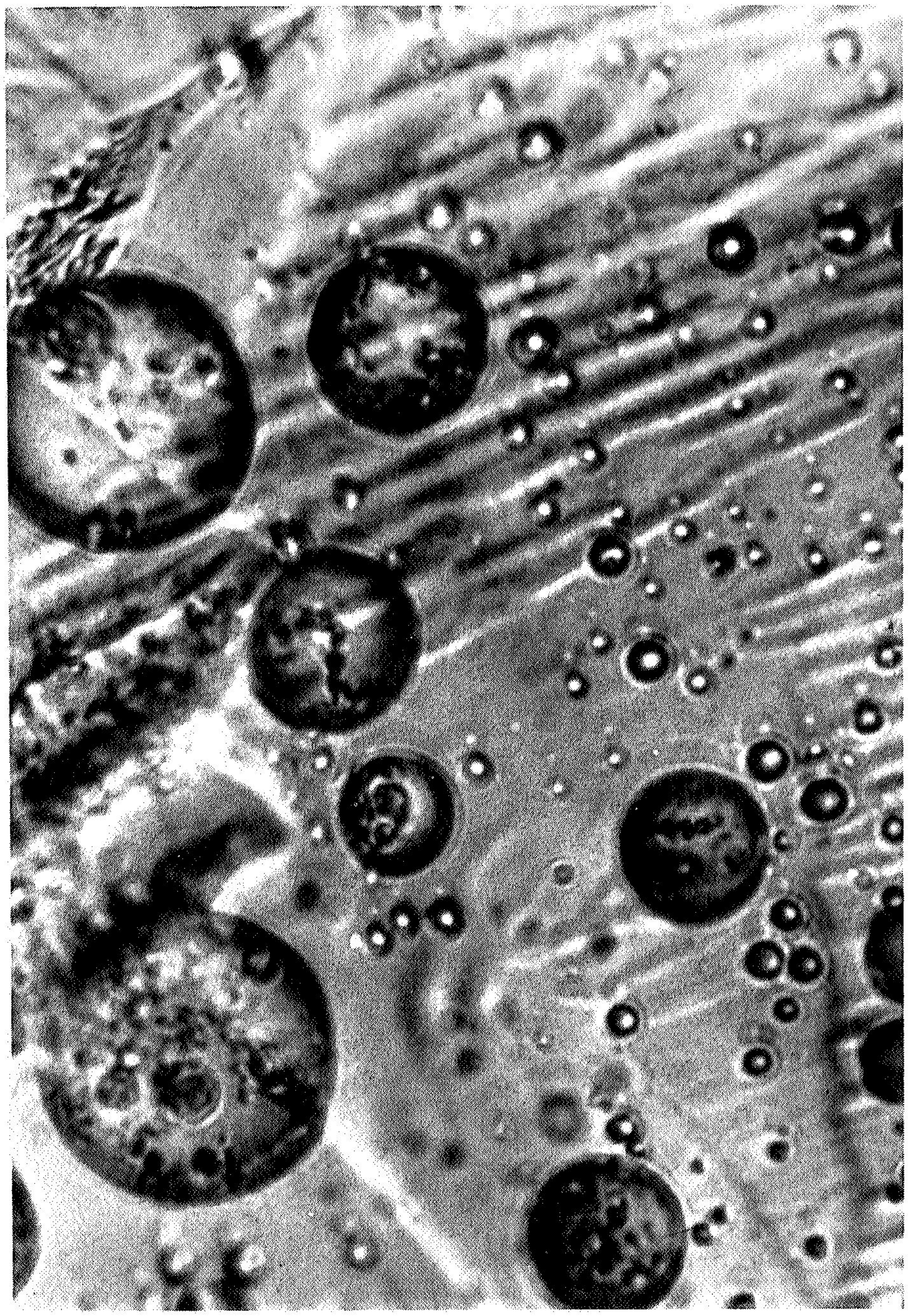


Fig. 5. Part of a large, water filled inclusion with approximately 40,000 microspheres, with randomly distributed diameters ranging from 2 to 60 microns. The results of statistical work indicate that budding is restricted to those microspheres which have diameters greater than 6 microns. Locality: Gaius Farm, Warmbad District, S.W. Africa. Phase contrast, 750X.

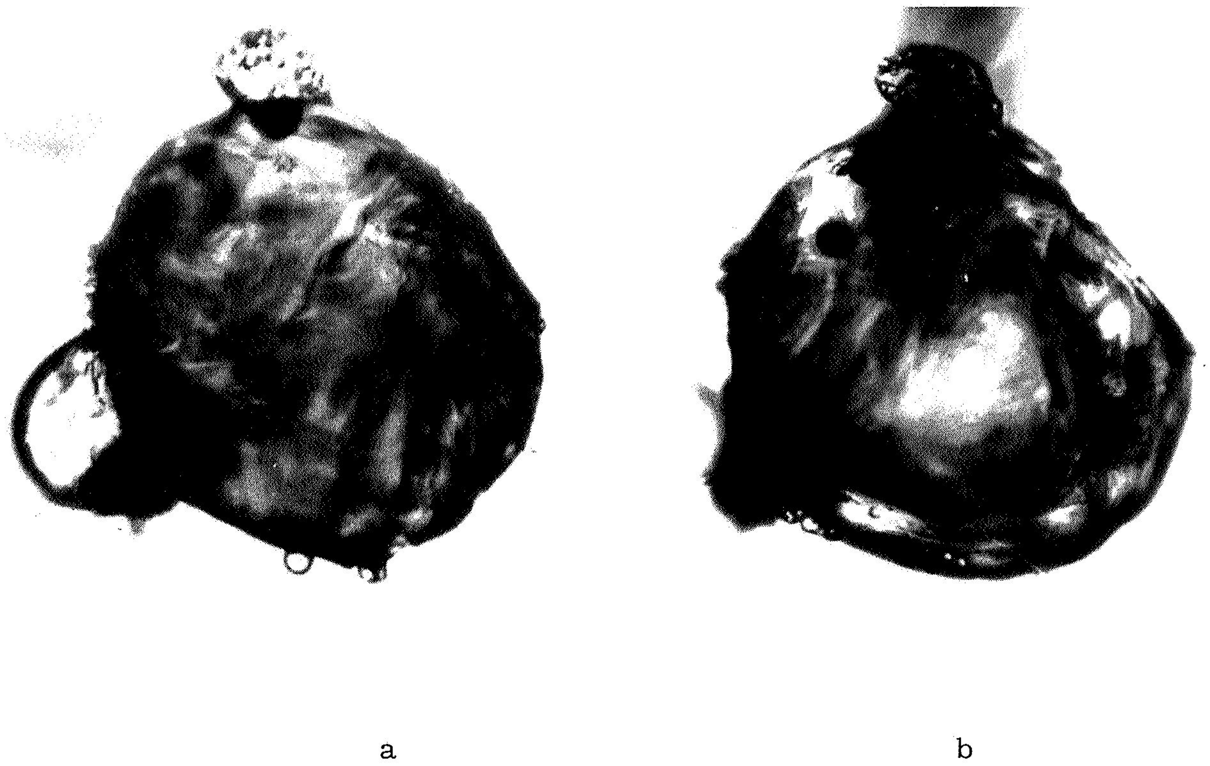


Fig. 6. Photomicrographs of the same complex microsphere in two different focal positions, which show the following structures: 1) presence of a darker brown phase with rather well defined boundaries within the center of the main (first generation) microsphere (b); 2) black hemispheres (a) and spheres (b); 3) two buds of the first generation (a, b); 4) several colorless buds of a second generation which appear both on the first generation buds and on the surface of the main microsphere. Althorn Farm, Warmbad District, S. W. Africa. 150X.

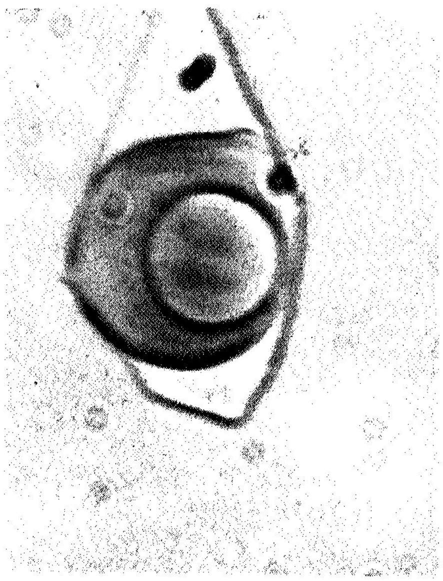


Fig. 7. Dark brown-red fluorescent microsphere with a yellow, green fluorescent nucleus; note also small single and paired brown microspheres. Bechuanaland, Africa. 1500X.

Fig. 8. Small inclusion with brown microsphere, which is partially covered by a dark brown sheath. A bud is seen protruding from the rupture of the sheath at the top, and material seems to bulge out from the rupture at the left. Bechuanaland, Africa. 1500X.

3. Sheathed microspheres. Some of the microspheres are covered by sheaths which are sufficiently thick for resolution with the optical microscope. Fig. 8 shows budding through rupture of the sheath covering the microsphere in two directions. There are indications along the lower half of Fig. 8 that the sheath may be a double "membrane".

4. Colonial microspheres. Groups of microspheres are rather common from the Gaius Farm locality; they are either irregular but equidimensional aggregates, or rows of microspheres resembling algae. The example illustrated in Fig. 9 is rather rare in containing both the irregular and the thread-like groups.

5. Coupling and indications for division. Preferential coupling is common among the small microspheres from the Althorn Farm locality (Fig. 10).

6. Diameter control. In certain inclusions the diameters of the microspheres may grade into 2 or 3 sharp maxima (Fig. 11), and it is interesting to note that the same quartz crystal may contain inclusions with well sorted and unsorted microspheres.

7. Gigantic lattices. The well graded microspheres in some of the inclusions may form gigantic two dimensional crystalline lattices in the cubic (Figs. 12, 13) or hexagonal (Fig. 12) packing. Some regular arrays of coupled microspheres have been also observed. The lattices of rather small microspheres show moiree patterns (Fig. 13), and similar moiree patterns also appear in inclusions, where the optical microscope cannot resolve the arrays of microspheres, which presumably cause the moiree patterns.

8. Indications for a process of division and coalescence. Within some inclusions from Gaius Farm, the microspheres are so soft, that they compact each other into a polygonal pattern (Fig. 14). It seems to be unlikely that such "polygonal" emulsions could have survived without coalescence since the formation of the quartz crystals in the Precambrian. The preservation of the microspheres through a process of constant division and coalescence seems to be the more likely possibility. The time lapse photomicrographs illustrated in Fig. 14 seem to indicate processes of division of the smaller structures of amoeboid habits, and coalescence of the same. Varying degrees of distortions of the close-to-globular, larger microspheres are demonstrated as well, but a well defined division of a larger microsphere into two individuals, or the coalescence of two smaller microspheres into a larger one, have not been so far demonstrated.

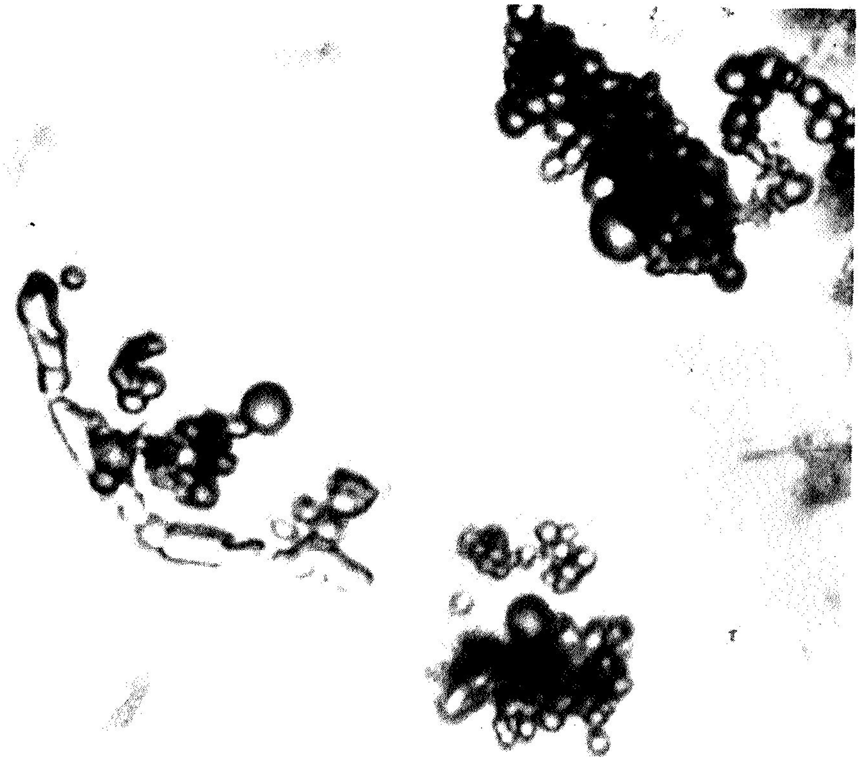


Fig. 9. Large, water filled inclusion with about 200 "colonies" of microspheres, and elongated microspheres, with a pronounced trend of chain formation. Gaius Farm, S. W. Africa. 1500X.

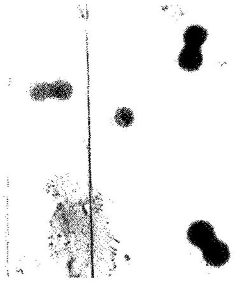


Fig. 10. Twinned microspheres.

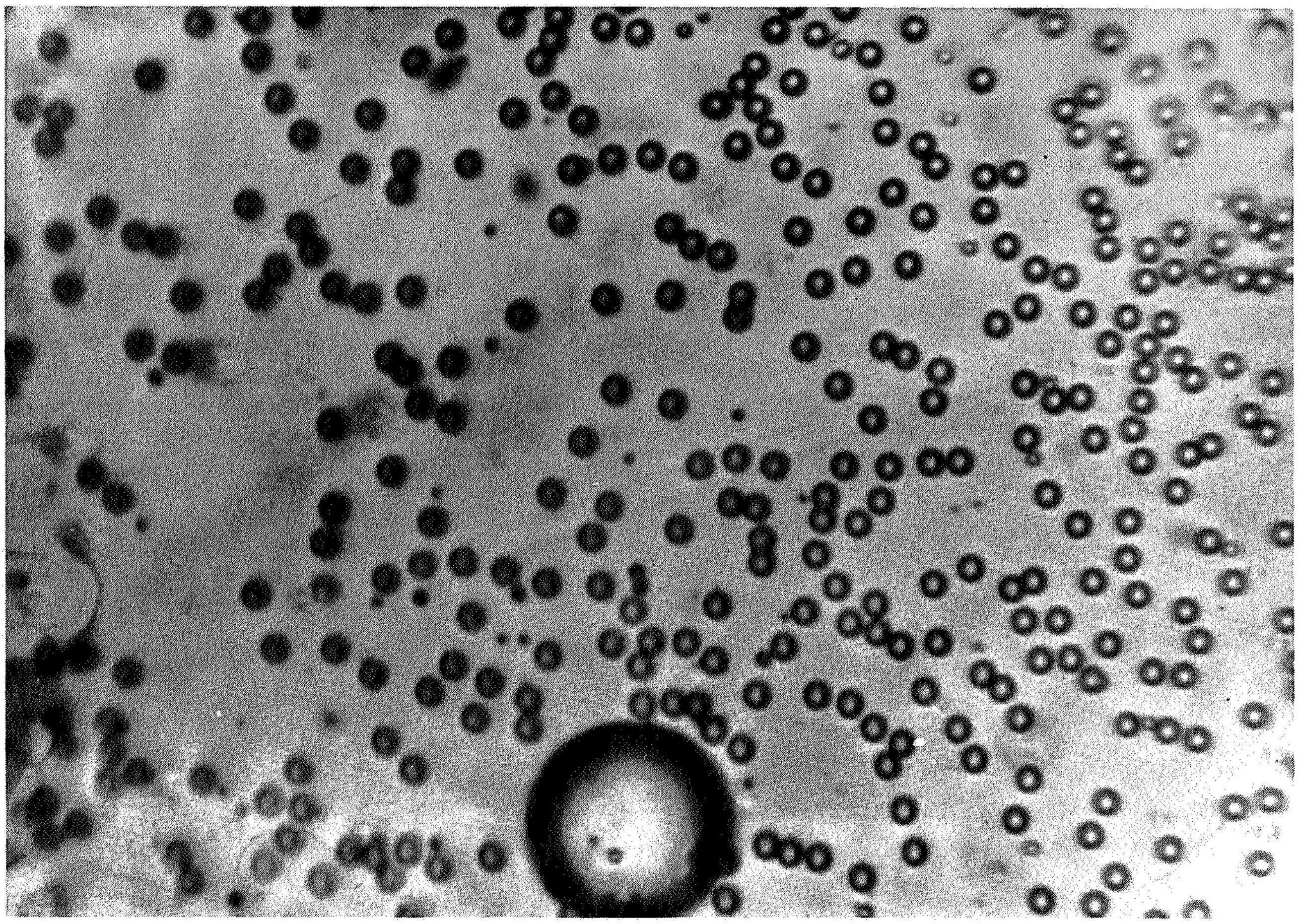


Fig. 11. Part of large, water-filled inclusion with about 16,000 microspheres. The microspheres subdivide into three well defined diameter ranges; a) some 30 microspheres with diameters between 12 and 180 microns, one of which appears at the bottom center of the photomicrograph; b) approximately 11,000 microspheres, which reveal a remarkable uniformity of diameters within the 4.2-5.0 micron range, with a sharp maximum at 4-6 microns. These microspheres are in slight Brownian motion, their distribution shows weak trends of alineation and coupling; c) a third group of small microspheres, the size distribution diagram of which reveals a rather flat maximum at 1.6 microns. Locality: Gaius Farm, S.W. Africa. 750X.

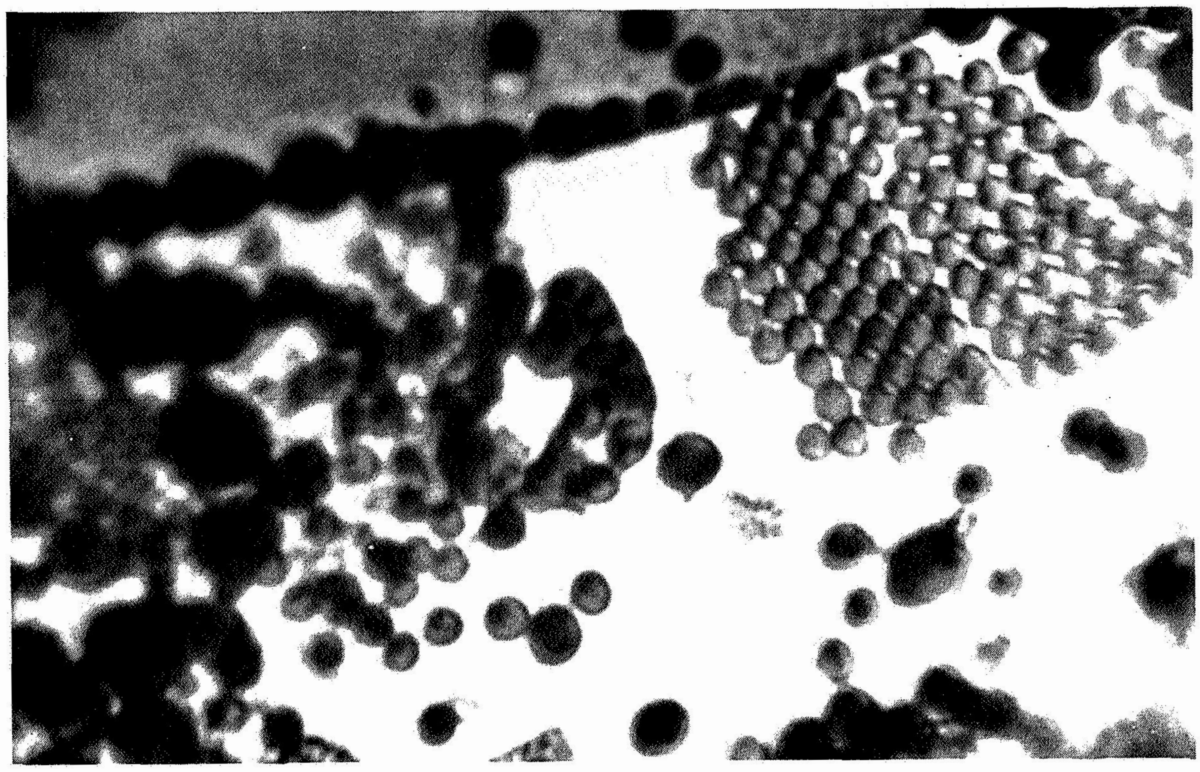


Fig. 12. Large inclusion with a brown, yellow fluorescent oily phase (top left) and water. The dark yellow to brown microspheres are present both in the oil and in the water. A chain of dark brown microspheres appears between the oil and the water. The water contains gigantic cubic and hexagonal lattices of microspheres (top right), which have a remarkable degree of uniformity of diameters. Gaius Farm, S. W. Africa. 750X.

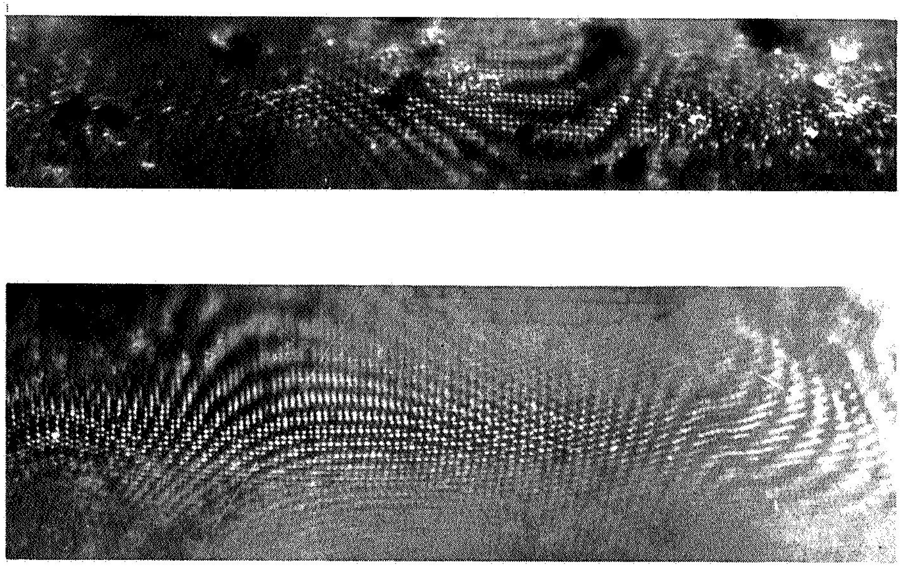
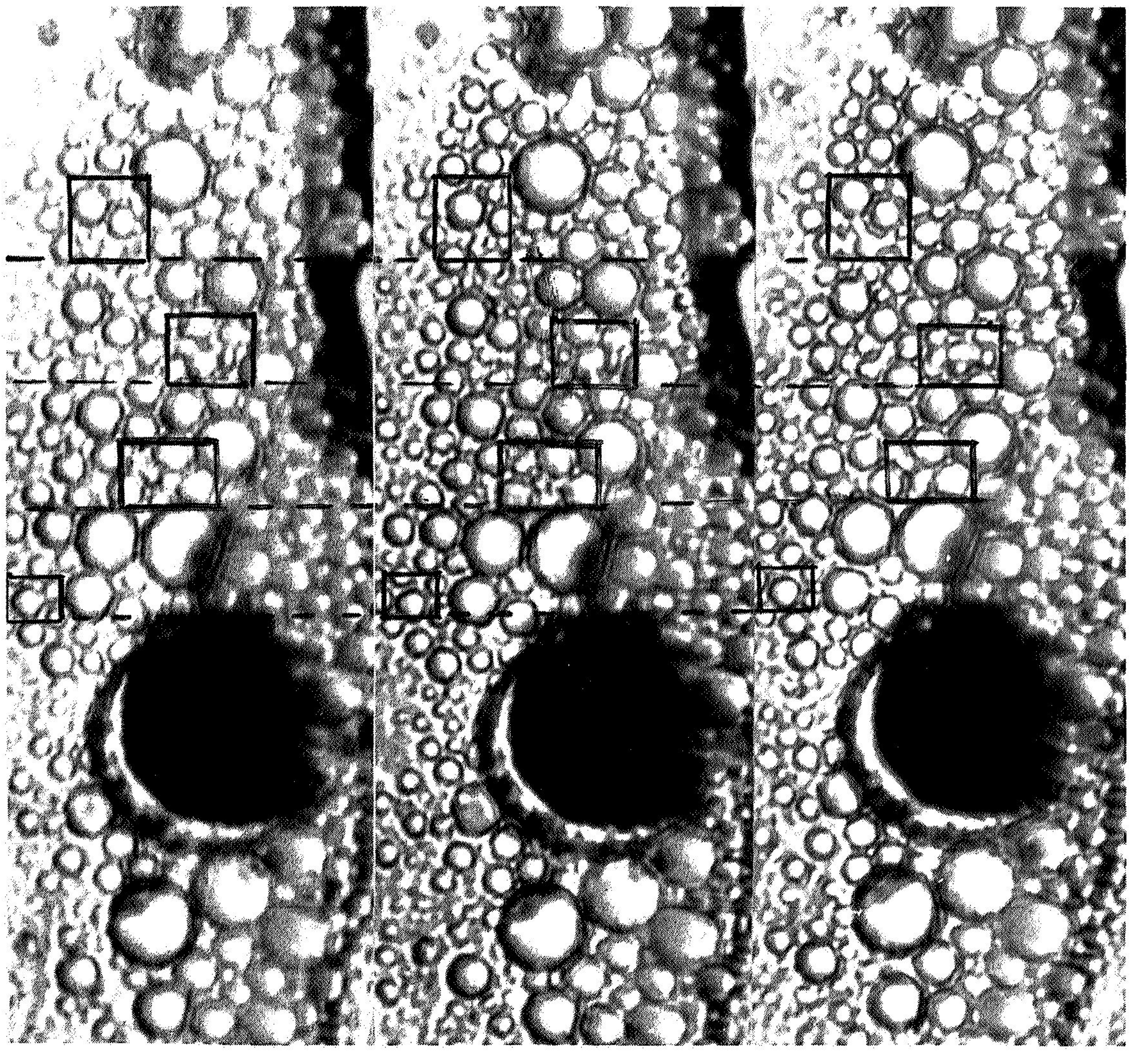


Fig. 13. Two photomicrographs of the same inclusion, at slightly differing focal positions. The inclusion contains approximately 180, 000 uniform microspheres arrayed in the order of the cubic lattice, and in certain portions as a regular paired pattern. The moiree effects are caused through the curvatures of the walls of the inclusion. Gaius Farm, S. W. Africa. 750X.



0 Sec. 5 Sec. 10 Sec.

Fig. 14. Three time-lapse photomicrographs of part of a large inclusion with pale green, blue fluorescent microspheres, and a larger black sphere, asymmetrically covered with the pale green phase. The microspheres are so soft that they crowd each other into a "polygonal" pattern. The set of time-lapse photomicrographs shows some indications for division (D) and coalescence (C). Gaius Farm, S. W. Africa. 1500X.

9. Spirals and curved ribbons. Structures which show curving at close-to-regular intervals are associated to quartz crystals which originate from a test pit situated approximately 100 miles to the east of Windhoek, S. W. Africa, just over the border of Bechuanaland (Fig. 15). The spiral structures in these crystals are associated with microspheres which have fluorescence and other optical properties well within the range of those from other localities of S. W. Africa.

10. Regular combinations between threads and microspheres. In a number of structures, organic material of essentially fibrous structure is associated with globular forms. Some of these structures are quite frequent within a given locality, indicating that the association is not fortuitous (Figs. 16, 17). Microspheres with a single spiral tail have been observed in recently acquired material from Althorn Farm; these crystals have not been sectioned so far for the preparation of photomicrographs.

The following four types of motilities have been observed:

1. Brownian movement. Some of the apparently soft microspheres may be squeezed into a "polygonal pattern" (Fig. 12) without apparent coalescence throughout the 1 to 3 eons-long geological history of the inclusion.

2. Rotation of some of the microspheres.

3. Mobility of granules or material of slightly differing refractive index within some microspheres.

4. It was found that intensive orbiting or vibratory motion of microspheres and other particles occurs in certain inclusion on strong illumination with any wavelength of visible light, but not on heating. This puzzling phenomenon may have an ultimate photoelectric explanation.

Finally, it was found that certain inorganic minerals, limonite and calcite in particular, may adapt remarkably biomorph morphology, if the inclusion contains an organic phase, in sharp contrast to the same minerals in neighboring inclusions, which contain no organics.

With the very kind cooperation of Prof. J. J. Wolken and his collaborators (Biophysics Research Lab, Carnegie Institute of Technology, Pittsburgh), the visible and near u. v. spectra of some of the microspheres were prepared through the flying spot method. The microspheres in opened inclusions proved to be insoluble in water. They swell in chloroform, and they dissolve with precipitation of some flaky matter in a mixture of ethyl alcohol and benzene. All these data indicate that the microspheres consist, essentially, of organic molecules. Organic chemical work on the bitumen assemblages of the inclusions is at present in progress.

Fig. 15. Rod-shaped inclusion of a brown substance with a typical pink fluorescence, which is covered with straight and curved spirals. These spiral structures are found in the locality of Bechuanaland, Africa. 150X.

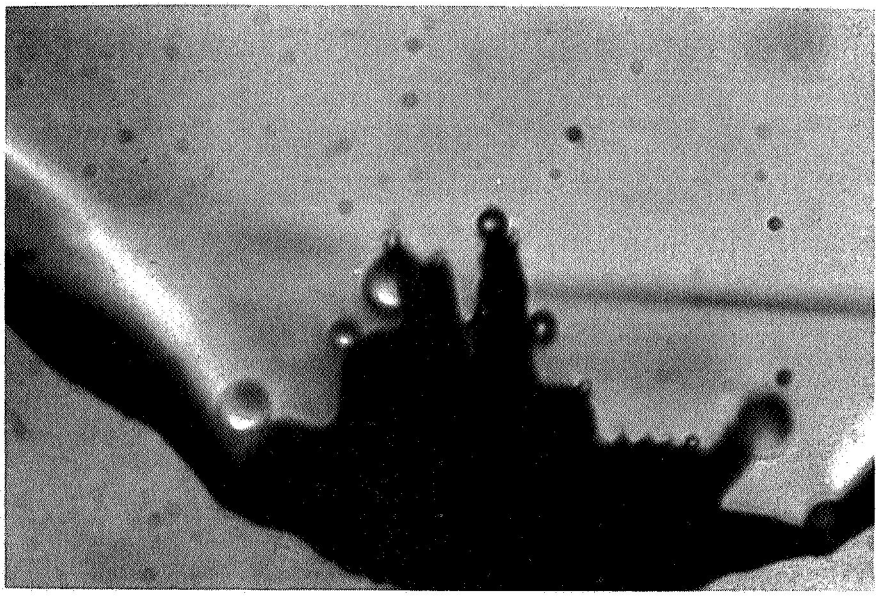


Fig. 16. A structure characteristic of the Althorn Farm locality; brown tubules terminating in colorless microspheres. 750X.

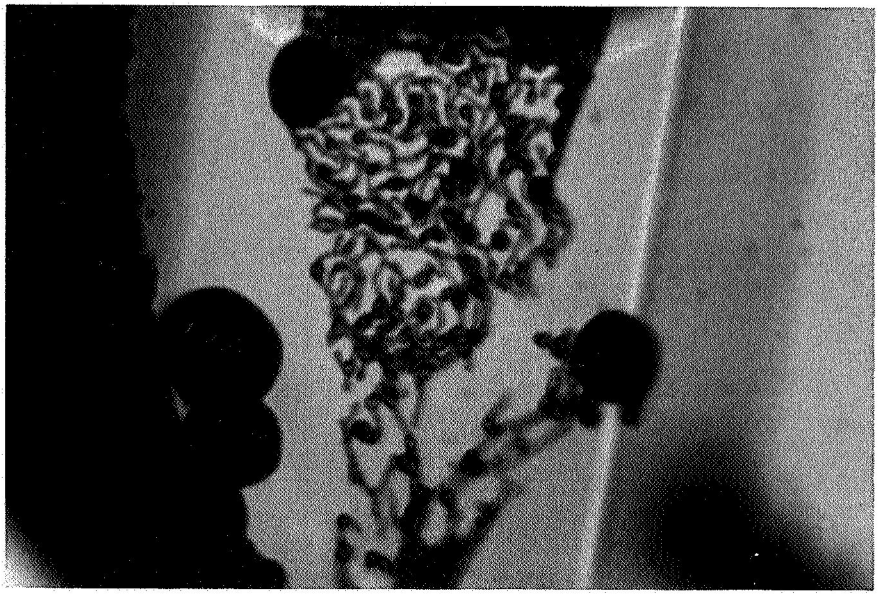


Fig. 17. A structure characteristic of specimens from Gaius Farm; approximately parallel, brown filaments with small microspheres. 1000X.

Assessment of Significance of Mueller's Microspheres (Fox)

These structures are morphologically interesting and probably have several kinds of geological relevance. They are also meaningful in widening the possibilities of misinterpretation of cell-like morphologies such as may be found preserved on the Moon or Mars. Their relationship to proteinoid microspheres or budded proteinoid microspheres is however not known as yet to be more than that of a formal morphological resemblance.

A uniform microspherical morphology may be regarded as a necessary but insufficient criterion of cells or precells. The proteinoid microspheres are known to be composed of protein-like material such as constitutes a major part of the structural material of true cells. The proteinoid microspheres have molecular structure and ultrastructure resembling those of true cells, they have selective properties in the boundaries, they can have double layers, they have been shown rigorously to proliferate through budding, and they have numerous other properties of contemporary cells (First Annual Report, IME, and Encyclopedia of Polymer Science and Technology, Vol. 9).

The geological microspheres are not known to have any of the properties of proteinoid microspheres other than morphology. In fact, some of them have been found to be soluble in organic solvents, a fact suggesting that they cannot be polyamino acid to a major degree.

Perhaps a principal significance of the geological microspheres is that they increase our appreciation of the likelihood that one may find in nature "populations" of uniform microspherical particles. Until evidence is obtained, geological microspheres should not be confused with proteinoid microspheres nor with fossilized lithosubstitution products thereof.

Differentiated Bitumens Associated with Hydrothermal Mercury Deposits from Central California (Mueller)

Bitumens have been collected from 7 mercury mines and from the Scrags Springs artesian area, which will be flooded by a new dam. The bitumen assemblage from the individual deposits proved to show differences in many interesting details. Preliminary microscopical and chemical investigations revealed the following common features:

1. The veins are of Mesozoic age, but the bitumen assemblage may be of deep-seated, juvenile origin, according to some geological evidences.

2. Sharp phase boundaries between a black asphaltite type mineral (of mainly aromatic composition according to data in the literature, and data obtained by the writer on Derbyshire bitumens) and mainly paraffinic green oils are evident both in the macroscopical and the microscopical scale. In some of the deposits a resinous, reddish-brown phase of mainly polar organic molecules (Foxite type phase) is also present. The unique chemical feature of this bitumen assemblage is the appearance of crystalline, intensively colored and fluorescing organic minerals, which consist, according to some data in the literature and preliminary work, of condensed aromatic molecules which readily sublime at atmospheric pressure such as derivatives of anthracene, naphthalene, etc.

3. "Froth veins" from Guadeloupe and Abbott mines have been briefly described in the literature. These consist of innumerable globules of opal or quartz. The center of each of these globules is occupied by a spheroid of viscous oil or asphaltite or foxite type substance of 10 to 500 microns diameter.

Chemical work on the material collected in the field is being continued.

Search for Microspheres and Differentiated Bitumens Associated with Hydrothermal Deposits or Intrusives (Mueller)

The continuation of microscopical and chemical work on the differentiated bitumens of Derbyshire, England, resulted in the discovery of some microspheres of Foxite.

A small quartz crystal acquired from Wards, Ltd. (locality, Herkimer County, New York) proved to contain a black, a brown, and a yellow fluorescent bitumenous phase, but no microspheres. Inclusions in fluorite from Kentucky have been found filled with a single phase of asphalt.

The presence of "two immiscible organic liquids" has been recorded in the mid-nineteenth century literature, on topaz from Scotland. This fact encouraged the search for bitumens in topazes from mainly Precambrian localities. Fluorescent and microscopical investigations have been carried out so far on topaz from Chile, S. W. Africa, Brazil, Mexico, and Ukraine, U. S. S. R. No bitumen was detected in any of the specimens.

Reconstruction of the Assemblage of Mineral Deposits of the Moon (Mueller)

The interpretation of rocket photography on the Moon, and computation with data on meteorites and terrestrial mineral deposits resulted in the prediction that the Moon may prove to be richer in deposits of Cr, $MgSO_4$, As, Sb, Se, Bi, Hg, graphite, diamonds, Fe, Co, Ni, and Pt Metals than the Earth. The lunar deposits of Li, Be, B, Ti, V, Nb, Mo, Rare Earth, Ta, U, CaF_2, Al, Mg, Zr, Sn, Au, and organic minerals may be inferior when compared to those of our planet, and the rest of the deposits would be approximately equal.

Geochemistry and Genesis of Chilean Nitrate (Mueller)

Many samples from the Chilean nitrate deposits were analyzed and experimental work carried out on the mechanism of concentration of salts within laboratory-scale models of the deposits. The results of the experimental work seem to support the "theory of genesis of nitrate deposits through capillary concentration". According to this theory, the nitrate, iodate, and other salts of relatively low concentration precipitate within the zones of capillary creep, which flank the western margins of the salt pans; due to the unique aridity of climate along the western extremities of the Childean desertic basin, the capillary concentrates are preserved.

NASA UNIVERSITY OF MIAMI SUPPORTING PROGRAM

(Fox)

The director of the Institute of Molecular Evolution is continuing as chairman of the Space Research Committee of the University of Miami, by request of the members of the committee and by appointment. This committee has deepened its study of the problems of the Survival of Man, and has made new plans for the coming year. Reports of the activities have been submitted elsewhere.

PUBLICATIONS OF THE INSTITUTE

Beginning with this issue of the annual report, lists of publications are not included. The listing is no longer necessary due to a NASA-sponsored project which performs the same function.

LECTURES AND OTHER ACTIVITIES OFF-CAMPUS

Dr. Fox

Seminars at University of Pennsylvania, Purdue University, University of Michigan, University of Tennessee, Duke University, and Eli Lilly and Company.

Lecture on "Can Man Start an Evolution?" at Le Comte du Nuoy Memorial Conference, University of Notre Dame, October, 1967.

Lecture on "Simulated Atmospheres and Models of the Synthesis of the First Organisms" in the Lloyd V. Berkner Memorial Symposium on Evolution of the Earth's Atmosphere, AAAS Annual Meeting, New York, December, 1967.

Lecture on "Self-Assembly of the Protocell from Self-Ordered Polymer" with and presented by Dr. Angus Wood, in Symposium on Self Assembly of Matter, as organized and chaired.

Lecture on Structure and Function of Proteinoids in Symposium on Protein Structure and Function at the University of Miami, January, 1968.

Banquet lecture to Food Industries Advisory Committee on Polyamino Acids and the Origin of Life, Naples, Florida, February, 1968.

Lecture on "Origin of Proteins, Cells, Coding Systems, and Polynucleotides" in Series on The Origins of Life at University of California at Irvine, March, 1968.

Florida Academy of Sciences Award Lecture on "A New View of the Synthesis of Life" at DeLand, Florida, March, 1968.

Lecture on "Experiments on the Origin of Protein" in Symposium on Proteins and Nucleic Acids, University of Houston, April, 1968.

Award lecture at Texas Christian University on "A New View of the Synthesis of Life" and closed TV lecture on "Self Assembly of a Model Protocell from Self-Ordered Polymer", April, 1968.

Lecture on "Self-Assembly of a Protocell from Self-Ordered Polymer" in Symposium on Evolution of Proteins in Annual Meeting of the Canadian Institute of Chemists, Vancouver, June, 1968.

Banquet lecture to annual meeting of the Phytochemical Society of North America, Tucson, June, 1968.

Lecture on "Methods of Producing Octadecatonic Polymers of Amino Acids" in the First Annual Symposium of the Peptide Chemists of the U. S. A. at Yale University, August, 1968.

Dr. Harada

Lecture on "The Sterically Controlled Syntheses of Dipeptides and their Stereochemical Courses" at the First Annual Symposium of the Peptide Chemists of the U.S.A. at Yale University, August, 1968.

(Of 100 peptide chemists at the invitational meeting at Yale, the two from this institute filled the "quota" from Florida.)

Dr. Mueller

Seminars at University of Cape Town, South West African Geological Survey in Windhoek, University of Houston, and University of Concepcion.

Paper on "Evidence of Redistribution of Volatiles along a Thermal Gradient within the Parent Bodies of Chondrites" at the 30th Annual Meeting of the Meteoritical Society, Ames Research Center, 25 October 1967.

A paper on "Trend of Coalescence of Microchondrules with the Decrease of Volatiles in Chondrites" at the 30th Annual Meeting of the Meteoritical Society, Ames Research Center, 26 October 1967.

Paper on "Process of Hydrothermal Differentiation of Carbonaceous Complexes" at the Meeting of the Group for the Analyses of Carbon Compounds in a Carbonaceous Chondrite, Ames Research Center, 26 October 1967.

Reconstruction of the Assemblage of Mineral Deposits of the Moon. Television program of B.B.C., London, 5 August 1968.

Paper on "Genetical Interrelations between Ureilites and Carbonaceous Chondrites" read before the International Symposium on Meteorite Research, Vienna, 7-13 August 1968.

HONORS

Dr. Fox

Florida Academy of Sciences Honors Medal and Citation as Outstanding Scientist of Florida, 22 March 1968. This citation recognizes the new subject matter area of molecular evolution as follows:

> "The Florida Academy of Sciences presents this certificate to Sidney W. Fox, recipient of the Honors Medal for 1968.
>
> This award is made in recognition of his fundamental researches in amino acids, proteins and molecular evolution and for his outstanding distinction as an internationally renowned biochemist, teacher, and science administrator".

Texas Christian University Distinguished Scientist of the Year, April, 1968.

Election to Fellowship in the American Institute of Chemists, September, 1968.