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INSTITUTE OF MOLECULAR EVOLUTION

UNIVERSITY OF MIAMI CORAL GABLES, FLORIDA

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30 September 1970

PRINCIPAL ADVANCES DURING THE YEAR

Dr. Fox

Components of Amino Acids from Interstellar Matter (Fox, Windsor)

Simple organic (carbon) compounds have been found in interstellar matter in the Galaxy. These have been identified by microwave spectroscopy, in studies at Green Bank, West Virginia and at Berkeley, California. The compounds are formaldehyde, ammonia, and water. They exist in enormous clouds in a number of locations in our Galaxy.

The possibility that these compounds might react to form amino acids (building blocks for protein) was shown in 1959 by subjecting such mixtures to ultraviolet radiation. Traces of amino acids had been detected. By heating formaldehyde and ammonia, in experiments at the Institute of Molecular Evolution, measurable (but small) yields of six to ten of the eighteen kinds of amino acid occurring in protein have been found, in 1969-70.

These experiments are the first in which substances known to exist in abundance in the Universe at the present time have been shown to function in a modelled first step in molecular evolution. The fact that formaldehyde and ammonia exist in abundance at the present time permitted doing experiments on a relatively highly factual basis. Early experiments such as those of Miller, and of Harada and Fox, employed hypothetical primitive atmospheres. The nature of those atmospheres was inferred on the basis of uncertain assumptions. In recent years, such postulated atmospheres have in fact been attacked as geologically indefensible. The facts from outer space indeed indicate that a highly reducing atmosphere was not needed to provide intermediates for the production of amino acids.

Since glycine, alanine, aspartic acid, glutamic acid, serine, and threonine are found in such experiments carried out at 185°, the entire sequence of cosmic reactants → amino acids → proteins (proteinoids) → primitive cells (proteinoid microspheres) is demonstrated to occur, from intermediates which are here now, under conditions which are widespread on the contemporary Earth. These conditions include temperatures lower than 200°. This theory of the evolution of molecules is thus on an unexpectedly rigorous basis, inasmuch as reactants and conditions for all steps (except precursors for some of the amino acids) exist now.

The particular amino acids obtained, and their proportions, bear a considerable similarity to those found in lunar fines from Apollo 11 and from hot flowing lava on Mauna Ulu. In these latter two cases, contamination by terrestrial organisms is a possible explanation; this seems unlikely, however. The lava was collected under conditions which minimized the possibility of contamination. The amino acid profiles themselves, and other data, are not typical of terrestrial contamination.

The production of amino acids from such inexpensive intermediates is being investigated as an economical method for the preparation of synthetic and artificial food.

Analysis of Lunar Dust (Fox, Hare, Harada, Windsor)

Samples of lunar dust from the Apollo 11 and Apollo 12 missions have been analyzed on Dr. Hare's ultrasensitive amino acid analyzer which is sensitive to .01 nanomole of amino acid. One sample was collected from beneath the surface at the Head Crater.

Samples were examined both for free amino acids and for precursors convertible to amino acids by hydrolysis (chemical reaction with water). Two amino acids, glycine and alanine, were found; these two amino acids were found also by Nagy, Hamilton, Urey, and their colleagues. The amino acids were reported as absent by two other groups of investigators. A public statement that another group failed to verify the finding when our methods were used is not correct. Our method consisted of extraction with hot water and use of the Hare analyzer. Both parts of this procedure are essential to obtaining the results. No other group, except that of Dr. Paul Hamilton, could have employed the full method since no other group had the apparatus.

When samples were examined for amino acid precursors, ours was the only one in three groups to find amino acids on hydrolysis. The Nagy-Hamilton-Urey, etc. group did no hydrolysis. Our finding was that of six amino acids. Glycine and alanine were again dominant. In some samples, aspartic acid, glutamic acid, serine, and threonine (total 50 ppb) were also found. In addition, a prominent peak in the basic amino acid region (but not lysine, arginine, histidine, or ammonia) was found.

All identifications are by rate of travel on a column and are subject to verification, in the usual sense of amino acid assays.

The two groups which did not find amino acids on hydrolysis performed their hydrolysis directly on lunar soil, whereas we hydrolyzed aqueous extracts (because our experiences with geochemical samples had taught us to do so). To verify the significance of this difference in procedure, we synthesized from formaldehyde and ammonia amino acid precursors in residues from lunar extraction. Such fortified lunar fines were divided into aliquots. Aliquots hydrolyzed directly yielded no amino acids. Hydryolyzates of water extracts of aliquots gave the same amino acids as are found from formaldehyde and ammonia alone.

These procedures and this instrument thus reveal at levels of <100 ppb a number of amino acids as such and as precursors in samples collected by Apollo 11 and Apollo 12. (Table I shows analysis following hydrolysis of aqueous extracts.)

Alternative explanations for the results are indigenous synthesis, organismic contamination by oxidized rocket fuel, and inhomogeneous samples. The profiles are not typical of terrestrial contamination. Oxidized 1,1-dimethylhydrazine does not give an amino acid pattern; the Apollo 12 trench sample was also collected at 200 meters from the vehicle. Some of the samples collected on the Moon were pooled and divided for investigators; inhomogeneities thus seem unlikely. The increasingly likely explanation is thus indigenous synthesis followed by incomplete decomposition by solar radiation. This indication is supported by the finding of Oro *et al*. that ^{13}C counts indicate that organic matter on the Moon is largely nonterrestrial.

As presented in Tokyo in September at the meeting of the International Association for Geochemistry and Cosmochemistry, *these results constitute the first evidence of the nonterrestrial occurrence of amino acids or of precursors hydrolyzable to amino acids*. Taken together with other findings, they indicate that simple compounds (such as formaldehyde and ammonia) are part of an interdigitated molecular evolution involving the Galaxy, the Solar System, and the Earth. This is illustrated in Figure 1.

Models of the Origins of Communication (Fox, Hsu, Brooke)

Proteinoid microspheres are models of primitive cells, as suggested by the conditions of their preparation, and by their properties. They have been found to possess intrinsically the tendency to form junctions between two or more. These junctions are new collar-like structures and are hollow. Endoparticles (small internal particles) transfer between microspheres. Since the endoparticles contain macromolecular information, the phenomenon is a model of the origin of communication.

The communication observed represents the beginning of neuronal processes. It has also many of the features of processes of inheritance. The model thus serves as a basis for understanding the simultaneous origin of intercellular and intergenerational communication.

Table I

Apparent Amino Acid Contents of Lunar Samples

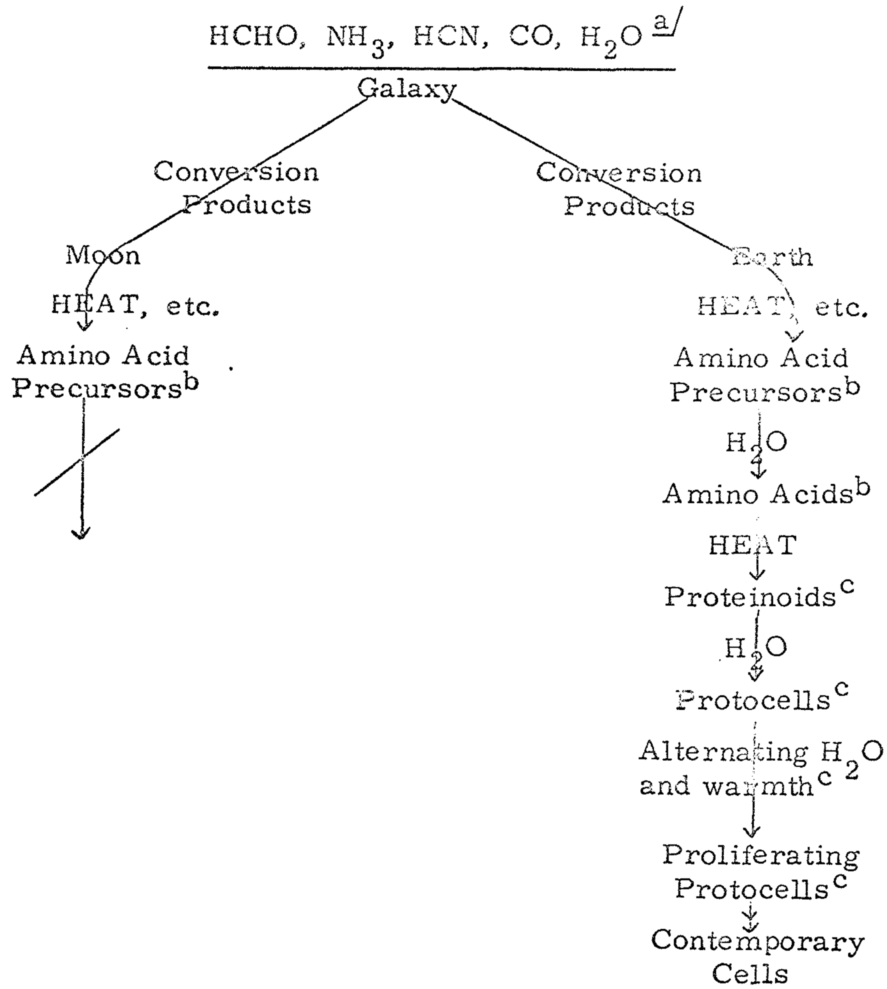
% Molar Composition[a]

Amino Acids in Hydrolyzate	Apollo 11 I No. 10086	Apollo 11 II No. 10086	Apollo 12 Trench No. 12033	Apollo 12 Surface No. 12001
Total ppb	53	37	19	69
Asp	5	5	<1	2
Thr	2	3	<1	–
Ser	9	10	<1	3
Glu	9	11	20[c]	6
Gly	50	52	37	70
Ala	25	19	12	3
Val	–	<1	<1	<1
Iso	–	<1	<1	<1
Leu	–	<1	3	<1
Tyr	–	–	–	2
Phe	–	–	–	2
BAA[b]	–	–	25	10

[a] Calculated without ammonia.

[b] A dominant peak in the basic amino acid (BAA) region, which does not correspond to a proteinogenous amino acid. RT between those of phenylalanine and histidine.

[c] Identity as glutamic acid uncertain.



[a] Microwave spectroscopy

[b] In laboratory under geological conditions from HCHO and NH_3 (Fox and Windsor, 1970)

[c] In laboratory under geological conditions (Fox, Harada, Krampitz, Mueller, 1970)

Fig. 1. Biochemical origins within cosmochemical unity. Organic compounds in the Galaxy can serve, as shown by experiment, directly to yield amino acids. They could also be converted to other kinds of matter which would in turn be convertible to amino acids. The evidence indicates that life could arise from organic compounds in the Galaxy in steps involving suitable alternations of heat and water. The data indicated that organic chemical evolution proceeded on the same pathway but was curtailed at an early stage, because of the absence of sufficient water.

Models of the origin of intracellular communication have also been recorded. These are of interest relative to processes in the cell which consist of messages from the nucleus to the ribosomes, etc.

The "intercellular" bridges are surprising in that they represent new structures without any internal synthesis of proteinlike material. That proteinoid of which the microspheres is composed must be very plastic. It also ages, and aging appears to be essential to the formation of firm bridges.

The formation of such structures has been observed for many years, but has been regarded as possibly optical artifacts. Staining and the constrained passage of smaller particles proves that the structures are not illusions in the microscope. Particles composed of polynucleotide and basic proteinoid also form bridges. These are models of interest to the contemporary transfer of information between cells, since contemporary cells rely heavily on polynucleotides (nucleic acids = DNA, RNA).

A Physical Basis for Nonrandom Communication (Fox)

The movement of endoparticles described above appears to be a kind of Brownian motion. Brownian motion is often thought of as being random, but we have questioned that it is truly random in this case, since the proteinoid material of which the endoparticles are composed is not truly random. The movement of the endoparticles is however constrained. They are released when they happen to reach a portal to another microsphere or a portal to the outside.

The position of the portals is nonrandom. Motion of the particles thus constrained by nonrandomly deployed passages results in motion considerably more nonrandom than Brownian motion.

Why Were Magnesium and ATP Selected in Evolution? (Fox, Ryan)

Experiments designed to elucidate the mechanism of protein synthesis and of the first protein biosynthesis have yielded a first explanation of the primacy of magnesium and of ATP as biochemical staples.

A number of divalent cations other than Mg have been tested for their activity in catalyzing the formation of peptide bonds in a test system incorporating ^{14}C-glycine. Other metals tested included Be, Mn, Zn, Mg, and Cd. Under the range of conditions tested, Mg was the only metal which did not precipitate with ATP. All of the other metals were, however, more active. In an evolving situation, the divalent cations would have had an opportunity to precipitate ATP before both agents

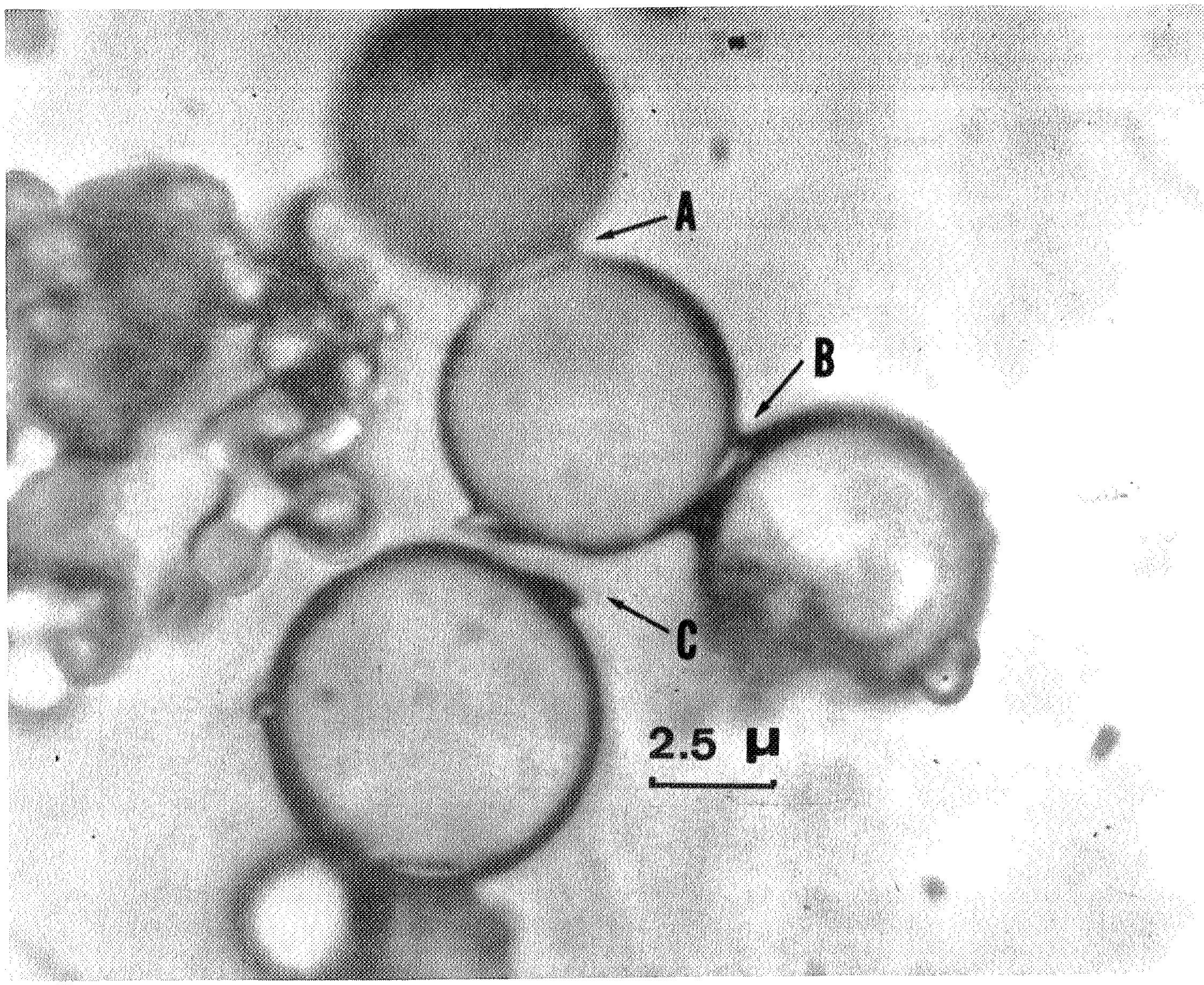


Fig. 2. <u>Junctions between proteinoid microspheres</u>.

Three proteinoid microspheres displaying junctions formed spontaneously. A is intact, B is cracked, C is separated.

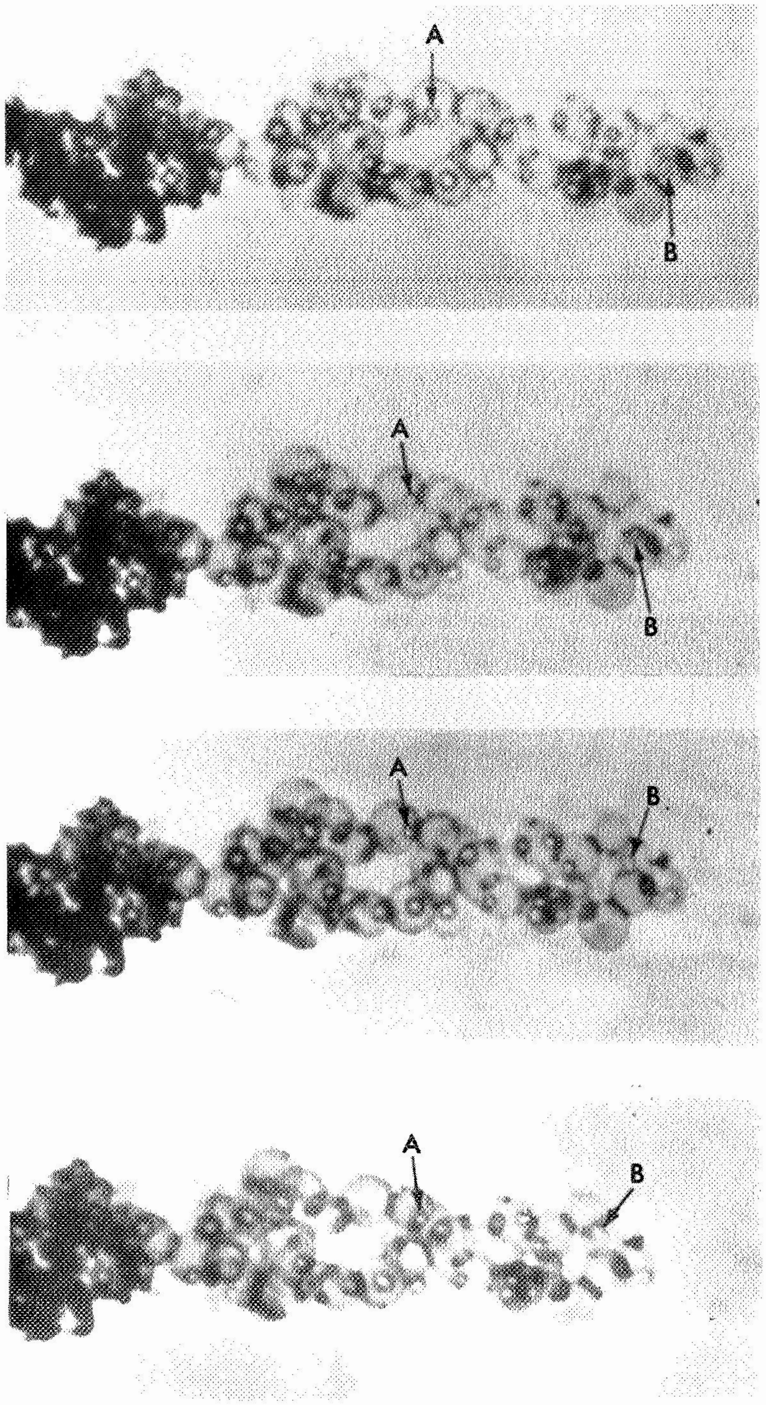


Fig. 3. Communication between proteinoid microspheres.

Transfer of endoparticles from one microsphere to another. Lapse between photomicrographs is 10 seconds.

had the opportunity to catalyze the formation of peptide bonds. Since magnesium does not so precipitate, its utility among those ions tested would have been unique.

ATP (adenosine triphosphate) was compared in the same model reaction with other nucleoside triphosphates (GTP, UTP, CTP) and found to be far more effective than the analogs. This observation explains why ATP is the nucleoside triphosphate chosen by evolution to play so many roles of energy transfer in organisms. The explanation of the superiority of ATP, at the molecular level, is more tenuous. That superiority is perhaps due to the presence in adenine of an amino group which might participate in and facilitate the mechanism of coupling of amino acids.

Evolutionary explanations are sometimes difficult to verify or reject. The above are offered as the first known explanations subject to further examination.

Proteins First or Nucleic Acids First? (Lacey, Fox)

This question is at the center of theoretical protobiogenesis and, in the minds of many, therefore at the center of theoretical biology. It has come into purview for renewed consideration, as indicated by recent discussions of this question in textbooks such as Dowben's General Physiology (1969), Moody's Principles of Evolution, 3rd ed. (1970), and Lehninger's Biochemistry (1970).

Without question, nucleic acids play a major role in contemporary organisms. The question of which came first is, however, a question for the primordial organism; it must of course be viewed in a more evolutionary manner than questions of the contemporary. In the beginning of the research program on protobiogenesis in this institute, a number of experiments were performed and papers published from the contextual premise of nucleic acids-first. Repeated analysis of the problem has led to substantial negation of the possibility of this premise. A condensation of the principal analyses follows.

Evolutionary

Biochemists traditionally use functions to search for structures. This is a reductionistic approach. Evolution must have proceeded in the opposite direction, employing structures to "search" for functions. Natural selection occurs at the level of function. Evolution must have been constructionistic, putting together components to yield functional systems, at which level selection occurs. The molecular evolutionist also proceeds from simplicity to complexity. As Jukes (Molecules and Evolution, 1966), one of us earlier (1959), and others have pointed out, nucleic acids can hardly be conceptualized as coding for proteins which did not already exist.

Biological

Beside the evolutionary explanation, another biological perspective of relevance is that the phenomena of the cell during its life cycle are predominantly phenomena of protein. Storage and readout processes from one life cycle to that of a descendant are primarily manifestations of nucleic acid. In this view, the first life cycle could perhaps have occurred with only protein; it could not have been expressed by nucleic acid.

Chemical

The proteins are chemically adapted to an evolving set of enzymes, and derivatively to metabolism. This follows since protein molecules possess the flexibility necessary for enzymic interaction with substrates; they permit sufficient variegation within a molecule and sufficient variation between molecules to allow for the evolution of an array of specificities.

Experimental

A relatively comprehensive physical model of the origin of living systems has been produced on the basis of (a) protein(oid)s-first, (b) a protein-governed cellular life cycle, (c) a proteins-first cellular inheritance cycle, followed by (d) refinement to a coded simultaneous biosynthesis of nucleic acids and protein. No such possibility has been demonstrated experimentally for nucleic acids first, nor do we visualize how to attempt to model a first step for this *a priori* possibility.

The experimental approach has also demonstrated a high degree of internal ordering of the reactant amino acid molecules. This result has experimentally obviated the need for a mechanism depending upon an outside agent, such as nucleic acids.

Models of the Origin of Nucleoproteinoid Microparticles Directing the Polymerization of Amino Acids (Fox, Nakashima, Lacey)

A number of attempts have been made to find ways in which polynucleotides would react selectively with amino acids using the molecular basis for the genetic code. Such experiments have been entirely or almost entirely fruitless. Interaction of polynucleotides with amino acids is evidently weak.

Studies published with Yuki in 1969 have shown that selective interactions do occur. These did not, however, reveal a direct relationship to the genetic code. Studies by Dr. Krampitz had also indicated that added polynucleotides could give peptides in the condensation of mixtures of amino acyl adenylates. These latter yields were extremely low, and the repeatability of the phenomena is also so low as not to be reportable in detail as yet. What is learned is that interactions can be found for polymer-polymer when they cannot be found for polymer-monomer.

Extending this understanding to use a system above the polymer level has given a set of results more in tune with the contemporary code. For this has been used a model of the evolutionary precursor of the ribosome, described earlier from this laboratory. This consists of nucleoproteinoid microparticles assembled from lysine-rich proteinoid and various homopolynucleotides. The peptide system is one involving the polymerization of amino acyl adenylates. The adenylates are universally intermediates of protein biosynthesis, but their evolutionary origins are incompletely modelled.

The particular adenylate favored in this model of organelle-controlled peptide bond synthesis is, under carefully identified conditions, directly consistent with the genetic code (a codonic relationship).

The typical results are shown in Figure 4. Such results have been obtained repeatedly, with several preparations of lysine-rich proteinoid. When the conditions are altered, other responses are found; this suggests that nature might have experimented with a variety of genetic codes. Some comparisons have shown that the particles employed are essential for the results obtained. The model is not closely equivalent to the contemporary ribosome in all of its characteristics; rather, it suggests a structure and related functions from which a contemporary type of ribosome might have evolved.

A Model of the Primordial Formation of Polynucleotides (Jungck, Fox)

The monophosphates of adenosine, cytosine, guanosine, and uridine have been condensed to oligomers in dimethylformamide solution, when imidazole is present as a catalyst in solution. These reactions do not proceed in aqueous solution; the yields are higher as water is more thoroughly excluded from the reaction. The particular significance of imidazole is that it appears in proteinoids and in contemporary enzymes in the sidechain of the amino acid histidine. Consequently, varied proteinoids can be studied as models of polymerases.

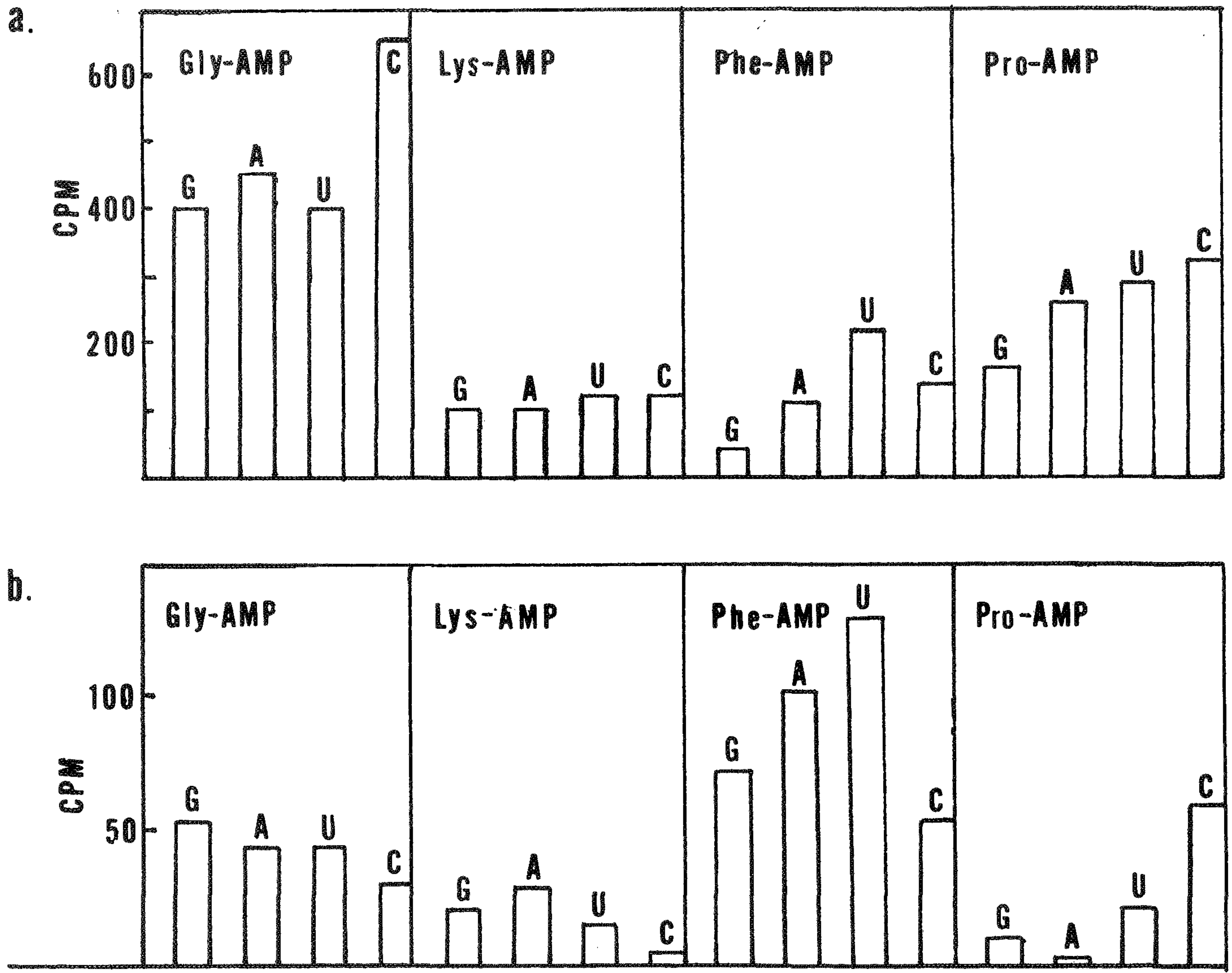


Fig. 4. Incorporation of radioactive amino acids into nucleoproteinoid microparticles preformed from lysine-rich proteinoid and different polynucleotides as shown. Amino acids are introduced as the adenylates. Initial polynucleotide concentrations: a) 0.75 mg/ml; b) 0.25 mg/ml. In a), which uses unsuitable conditions, Gly is anticodonic, Lys is nondescript, Phe is codonic, and Pro is codonic. In b), for which the conditions were discovered empirically, the relationships are all codonic.

Dr. Harada

Lunar Analyses

This is reported on earlier pages.

Chemical Reactions of Symmetric Molecules in the Presence of Asymmetric Molecules

Symmetrical substrates which contain C=C, C=N double bonds are hydrogenated in the presence of asymmetric molecules by using palladium catalyst. Optical purities of the resulting hydrogenated molecules were systematically examined. About 05 ∿ 10% of optical activities were found in these reaction systems.

Chemical Reactions in the Presence of Optically Active Quartz

In some reactions, weakly optically active products were obtained. However, this might be contamination of biological origin. Further studies are now under way.

Book on Recent Developments in Prebiological Chemistry

Planned for publication by Kyoritsu Shuppan Co., Ltd. in Summer 1971.

Dr. Mueller

1) Recognition of biomorphic organic particles and intensively phreochroic particles included in quartz crystals from Sillurian dolomite of Middleville, New York.

2) Recognition of microspheres of a reddish-brown resin from a Precambrian pegmatite vein at Brit, Ontario, Canada.

3) New observation of crystallographically ordered organic particles in oil-filled inclusions from fluorites of the Illinois-Kentucky Orefield. The position, the symmetry, and the crystallographic orientation of the crystalline organic particles are all determined by the host crystal. Repeating patterns of four-fold symmetry of up to 32 particles have been observed.

4) Several observations have been made on Apollo 11 and 12 fines, which are new, and have not been repeated by any other authors according to data in the most recently published literature. Most of the observations have been facilitated through the application of a new method of separation of globular spray particles from the fines--i.e. by repeated rolling of sieved and washed material on inclined sheets. Rare structures could be detected in the concentrates of 0.1% globules. The new observations are as follows:

A) Globules of spinel-porphyry glass in Apollo 11 fines, seem to indicate formation of the spinel through slow cooling from elevated temperatures, followed by quick quenching of the rest of the glass.

B) Rounded octahedra of magnetite in Apollo 11 fines rather closely resemble magnetite in type I carbonaceous chondrites.

C) Twin and grape bunch type partially sintered aggregates of glass globules resemble the "twin and grape bunch" chondrules in carbonaceous chondrites, which were recognized and described by G. Mueller [Astrophysics and Space Science 4, 3-43 (1961)].

D) Theoretically significant, rare rotationally derived forms of spray particles include crescents, rings, filaments, and dumbbells with a central (third) bulge which forms seem to indicate rotation of unusually homogeneous glasses. Tops and spindles seem to have been derived through viscous liquid droplets gyrating on the lunar surface.

E) Elongated rotational forms (prolate spheroids and dumbbells) with indications of centrifugal zoning, bubbles occupying the center, and glasses of relatively darker shade (presumably greater density), further iron sulphide and nickel iron concentrating towards the two extremities of the elongated particle. It was observed that the lobe of the particle, which contains more dense material, is the narrower one, as a general rule.

F) Elongated particles with crystalline terminations displaced in a clockwise, anti-clockwise direction from the long axis, indicating gyration in a resistant medium.

G) It was found statistically that spray particles with single fused or sintered stalks predominate. This seems to indicate that the spray particles may have rested or very gently impacted on the lunar surface in a hot stage.

H) It was statistically found that the mean elongation of spray particles is independent of diameter, and it increases with magnetism and density, indicating the possibility that the particles have been rotated through radiation and electromagnetic effects, and not through impact.

The foregoing observations seem to support the hypothesis that the bulk of the lunar sprays formed through irradiation of the equatorial lunar surface by unusually intensive solar flares. Devolatilization by these solar flares may also explain the fact that the lunar surface at the Apollo 11 and 12 sites is lower in organic substances than the mean for the terrestrial surface or for carbonaceous meteorites which are believed to originate from the close-to-surface zones of asteroids. According to the theory of genesis of lunar sprays through solar fusion, higher concentrations of volatiles, including organic substances, may be anticipated from those areas of the Moon which have not been close to the equator during any epoch of its history, and a rather sharp increase of organic substances may also be expected with increasing depth. It may be interesting to note that a process of solar volatilization of Na, K, S, and organic molecules from a mainly basaltic lunar surface was predicted by G. Mueller prior to the advent of Surveyor or Apollo data [Nature 215, 1149-51 (1967)].

5) A strewn field of macrospray particles of a new type of crystobalite-cordierite-sillimanite glass prophyry was observed during 1962, at San Pedro de Atacama, N. Chile. The results of recent studies indicated the presence of two additional new types of macrosprays: a garnet-mica glass porphyry, and a magnetite glass porphyry, the latter closely resembling a lunar glass porphyry mentioned above. Microspray particles have been observed also, and it was found that the microspray is of more basic chemical composition than the macrospray within the strewn field.

CURRENT PROJECTS

Dr. Fox

Demonstration of Darwinian selection in proteinoid microspheres.

Studies of compartmentalization in microspheres (this is especially significant relevant to the origins of metabolic pathways in primitive cells). Electron micrography is being arranged for.

Studies of taxis (electro-, thigmo-, chemo-, proto-, etc.) in proteinoid microspheres.

Studies of intercellular and intracellular communication in proteinoid microspheres. Such phenomena have been observed and recorded. The intercellular model is described elsewhere. The significance of the intracellular phenomena relates to nucleic ribosomes, messengers, etc.

Proliferation of proteinoid microspheres through binary fission and accretive growth.

Growth of proteinoid microspheres through internal synthesis of peptide bonds (amino acid adenylates).

Cyclical repetition of budding-and-growth proliferation cycle.

Development of energy-trapping mechanisms in proteinoid microspheres.

Evaluation of behavioral phenomena in proteinoid microspheres as manifestations of constraints imposed upon Brownian motion.

Communication of nucleoproteinoid vesicles between proteinoid microspheres.

Microspheres of homopolynucleotide and thermal polyamino acids as morphological models of organelles (ribosomes, nuclei, etc.).

Selective interactions of polynucleotides with thermal polyamino acids.

Models of nucleoproteinoid microparticles manifesting selective action on condensation of amino acyl adenylates.

Charging of homopolynucleotides by acylamino acid adenylic acid anhydrides and by amino acid imidazolides. This is a model for transfer RNA.

Synthesis of polynucleotides under hypohydrous conditions. Polymerization of a series of monoribonucleotides has been accomplished in solvent dimethylformamide with imidazole as catalyst. Since polyamino acids contain imidazole, this may lead to models of ligases.

Further explanation of why ATP has played a key role in evolution.

Further explanation of why Mg was chosen in evolution.

Formation in aqueous suspension of polynucleotides from aminoacyl adenylate intermediates.

Formation within microspheres of polynucleotides.

Analysis of the protein(oid)s-first theory.

Rigorous identification of suspected amino acids and other organic compounds in lunar fines.

Estimation of amino acids and other organic compounds in terrestrial volcanic lava. The possibility of such examination in sterile lava has increased due to on-the-site collection in September of flowing molten lava from Mauna Ulu on the island of Hawaii.

Synthesis of amino acids from intermediates recognized as constituents of interstellar matter.

Comparative study of methods of extraction, hydrolysis, and analysis of lunar fines for amino acids.

Dr. Harada

Studies on the chemistry of cyanohydrins and aminonitriles in connection with prebiological chemistry.

Further studies on the synthesis of α-amino acids by asymmetric transamination (pressure effect).

Studies on the racemization of L-amino acids during the course of the polycondensation process to form 2:2:1- or 1:1:1-L-proteinoid.

Studies on the racemization of α-amino acids in fossil shells and corals found in Florida.

Dr. Mueller

Chemistry of differentiated organic substances associated with hydrothermal veins of Derbyshire, England and elsewhere.

Morphology and chemistry of biomorphic organic particles within oil or water-filled inclusions of quartz crystals from the Precambrian of S.W. Africa, Canada, and elsewhere.

Amino acid abundances and distribution patterns of members of the coal series.

Petrology and statistical petrology of spray particles from lunar fines.

Petrology and statistical petrology of spray particles from terrestrial volcanic regions and meteorites.

Amino acid patterns of insects and other bio-remains, hermetically sealed in fossil resins from Mesozoic to Pleistocene age (with Dr. E. Hare, C. Windsor). This is a study of degradation of hermetically sealed proteins throughout geological timescales, under low temperature in the absence of any chemically active substances.

Amino acid patterns of coals and bitumens associated with the uranium deposits of Utah (with E. Hare, C. Windsor). A study of the effect of low intensity, long timescale radioactivity on amino acid patterns in coals and bitumens.

Crystallographically oriented organic particles from oil-filled inclusions in fluorites of the Illinois-Kentucky orefield. Chemical and crystallographic studies of the remarkable structures which seem to have been produced by a hitherto unknown process of self assembly.

THE "SYNTHETIC CELL" AND HEADLINES

Two of the favorite stories of the sensational press are A Cure for Cancer! and Synthetic Life in the Test Tube! The latter kind of publicity has been generated often enough, and in a sufficient variety, to warrant an analytical review of the substance of some of the reports.

Almost without exception, the reports of a synthetic cell, or of synthetic life, are not strictly speaking of synthesis. They are reports of experiments of reassembly of two or more components of disassembled living units. The report of a synthetic gene produced by Khorana is genuine, but it does not describe life in a test tube, since the unit of life consists of much more than genes. Genes function to hold the instructions for cells. The gene is a kind of molecular blueprint (necessary, but far from sufficient, for contemporary life).

Life or cells, however, are not molecules; they consist of or are, respectively, microsystems. Molecules can be synthesized; systems are assembled. The more accurate designation of artificial life is that of an assembly from appropriate molecules, which may in turn be synthetic. If the molecules are synthetic and the system can be and is assembled therefrom, the cell is truly a product of the science of the artificial. If the molecules are obtained from already living forms by disassembly, and the components can be and are brought into interaction to yield a functional unit, the process is one of reassembly.

At the level of nucleoprotein molecules, a principal early case of publicized "synthetic life" was that of Fraenkel-Conrat (who stoutly objected to the journalistic overinterpretations). In their studies, Fraenkel-Conrat and associates fractionated the tobacco mosaic virus into its component nucleic acid and protein, and then reassembled them. This was not a synthesis. As an assembly it was a reassembly. No reason is apparent to deny for the future the possibility of true synthesis of the nucleic acid and true synthesis of the protein. The synthetic macromolecules should then assemble with the same efficiency as do the components obtained from the tobacco plant.

Few experts would care to argue that a virus is alive; accordingly, what has been obtained in this case is a reassembled nucleoprotein particle (actually itself a microsystem), but not reassembled life. The virus lacks key properties of the accepted unit of life, the cell, e.g. metabolic use of energy. (It must enter a cell or it will not proliferate.)

An especially significant contribution of the Fraenkel-Conrat laboratory has been the finding that proteins and nucleic acids from different viruses could be hybridized.

The replication by Kornberg and co-workers of DNA (Phage ϕx 174 DNA) is also a reassembly in part. In order to obtain replication of the DNA molecule, Kornberg employs a polymerase (a polymerizing enzyme) from the same cell from which he obtained the DNA. The reassembly is thus not complete; it is carried to the point at which an enzyme catalyzes the replication of the DNA molecule.

An early assembly to units having some of the appearance of cells was that of Bungenberg de Jong, who introduced the coacervate droplet in the late 1920s. The polymers used to produce these units have, for example, often been gelatin and gum arabic. Although the gelatin is a partly degraded collagen, its source is an organism. Because of this, the coacervate droplets are models of cells. They cannot rightly be considered to be models of primordial cells, since the existence of prior cells was necessary for the existence of either gelatin or gum arabic.

The work of Oparin has been an extension of that of Bungenberg de Jong. While Oparin and his students have added to the literature some interesting experiments on inclusion of enzymes, etc., these experiments are lacking in relevance to the basic question, in quite the same way as those of Bungenberg de Jong. They do not explain the emergence of cells in the absence of cells to produce them.

A number of other investigators have reported on coacervate droplets, notably Liebl. Both Liebl and Oparin have used histones and enzymically synthesized polynucleotides to produce a kind of coacervate droplet, with properties of perhaps more general interest than those of the conventional droplet. The histone is, however, derived from organisms, so that reassembly is, again, the appropriate category. In a recent study, Miquel of the Ames Research Center and the workers at this institute have compared the morphology of units prepared from acidic proteinoid and histone with those made from acidic proteinoid and basic proteinoid. The morphologies appear to be almost identical. The latter unit is entirely artificial, or man-made, having been assembled from two polymers each of which is artificial.

The studies of Danielli are also reassembly studies; they have some unique aspects and promise. Danielli and associates transplant nuclei and cytoplasms from some _Amoebae_ to the membranes of others. In general, the more closely related the host amoebae are, the more successful is the reassembly. The reassembly is man-made or artificial. It is in no sense an act of, or sequence involving, man-made syntheses since the crucial, largely macromolecular preparations are the products of cellular synthesis.

Two kinds of experiment may be recognized as yielding true artificial cell-like systems. The earlier of these is that of the Mexican physiologist, A. L. Herrera (1868-1942). Herrera exposed to sunlight aqueous solutions of formaldehyde and ammonium thiocyanate. He obtained sulphobes, or "cells" with a number of properties of dynamic contemporary units. Herrera was, in his time and place, not equal to characterizing his sulphobes compositionally or in other ways. He could not know how to answer crucial questions, since at the time (the 1920s and 1930s) many of the crucial questions were not identified. In his selection of reactants, Herrera was also much ahead of his time. Ammonia and formaldehyde have been identified as components of galactic matter only since 1968. Moreover, Herrera included sulfur in his three reactants.

Like Herrera's sulphobes, the proteinoid microspheres are constructed from intermediates which in turn may be produced from the elements.

Are the proteinoid microspheres an example of artificial life in the test tube? The answer to this question necessarily requires a definition of life. Life is a term which has been described by Pirie and by others as meaningless. Pirie, however, has listed necessary criteria for life. The definitions vary with the expert, as has often been pointed out. According to some definitions, proteinoid microspheres are alive. Even before the ability to participate in their own proliferation was reported in 1967, I. Asimov had prepared a volume (Is Anybody There?) in which he stated that the proteinoid microspheres were beyond the state of being nonalive.

Perhaps the most rigorous statement that can be made about the proteinoid microsphere is that it is a model of a primordial living cell. How meaningful or valid is such a model depends upon how successfully it can be shown to be evolvable to a contemporary cell. The extent and nature of what remains to be done has been defined, a circumstance which itself indicates that the proteinoid microsphere is a considerable advance over previous models. The contemporary cell is itself varied, and difficult to define--a circumstance which will continue to confuse the answer to the primary question of exactly what has been accomplished.

A few comments can be made about evolvability in principle. The contemporary cell has discrete lipids, an internal synthesis of nucleic acid, and an internal synthesis of protein in a coded relationship, as well as being able collectively to utilize the energy of the Sun directly or indirectly. The reasons why the proteinoid microsphere did not require these mechanisms and how it might have acquired them have all been treated at length. Partial experimental support, or demonstration, has been published for the emergence of each of these.

In an article titled On Making and Recognizing Life [New Biol. 16, 41-53 (1954)], N. W. Pirie described the requirements for an eobiont and the necessary evidence. He then stated, "recognition and acceptance of this is likely to be slow." We can enter the possibility that the "feat" has already been accomplished; it can only be recognized after the act, and preferably by nonjournalistic individuals. Discussion of such possibility has begun to appear in textbooks and elsewhere, aside from newspapers.

RECENT TEXTBOOKS DESCRIBING RESEARCH OF THE INSTITUTE OF MOLECULAR EVOLUTION

Perhaps no index of recognition of the development of a new science is as significant as the appearance of the subject matter as a new chapter in a text for students in a traditional area. Recently this has occurred in textbooks in the fields of physiology, biology, and biochemistry, each written by outstanding authors. All are authors of high eminence as contributors to new knowledge. The texts are:

General Physiology, a molecular approach, by Robert M. Dowben, Harper and Row, 1969.

Introduction to Evolution, 3rd edition, by Paul Amos Moody, Harper and Row, 1970.

Biochemistry, by Albert L. Lehninger, Worth Publishers, Inc., 1970.

A large part of Dowben's first chapter, titled Introduction, deals with aspects of the "origins of life." Dowben states "Even if the earth had a cold origin, a transient high-temperature phase was possible; the required thermal energy could have come from the gravitational potential energy of accretion or from the decay of large quantities of short-lived radioisotopes. A requirement for the origination of life is the presence of organic compounds. Recent fascinating observations indicate that the earth may have acquired large amounts of organic material of cosmic origin early in its history. . . . While the possibility of contamination from extraneous sources is real, meteorites, particularly the carbonaceous chondrites, appear to contain abiogenic amino acids."

These comments are followed by a section prepared in collaboration with Rohlfing.

Moody's seventh chapter is titled "Life's Beginnings." He introduces it by stating "How did life on earth begin? The answer is that we do not know and probably never will. The origin of life occurred more than three billion years ago and was not the type of happening to leave a clear indication of its course of events in the fossil record. Why, then, do we discuss the question at all? The best we can do is to point out what might have happened. As we shall see, attempts to do that have added greatly to our knowledge of biochemical events of a type that may have occurred under primitive conditions both before and after recognizable living organisms appeared. Gaining knowledge of this kind is intellectually satisfying. But it also contributes to two areas of great interest at present: (1) the question of life on other planets and (2) the attempts to create life in the laboratory. The other planets differ from earth in many respects; may we expect that life in some form may be present? If so, what form may we expect life to have when atmospheric and other conditions are unlike those found on earth?"

The text and illustrations emphasize proteinoid microspheres.

Lehninger's final chapter is titled "The Origin of Life." He states, "Not too long ago, inquiry into the origin of life was considered to be a matter of pure armchair speculation with little hope of yielding conclusive information. But many scientific advances made in the last decade have given encouragement to the view that valid answers to some of these questions may be deduced and that at least some of the steps in the origin of biomolecules and of living cells may be simulated in the laboratory. In this final chapter we shall survey some of the experimentation and the shades of thought in this increasingly active field."

Lehninger then describes, among contributions from various laboratories, the thermal formation of polymers of amino acids, their nonrandom character, their catalytic activity (with a graph of pyruvic acid carboxylation), the cocondensation of aminoacyl adenylates, and the proteinoid microspheres. Among the illustrations included is the Budding and Replication of Microspheres.

Of particular interest may be the fact that these new textbooks represent three traditionally separate fields of science. The odds seem high that new textbooks on physics, geology, and cosmogony will take their lead from those in the life sciences in those areas of subject matter that now interface with life science.

Our view is that both the institute and the program of NASA can take pride in the degree to which research on "the origin of life" is becoming a permanent part of scientific knowledge through new textbooks of high quality.

In science itself, man can not only understand how he is formed of the biblical dust of the ground, he can now trace his origins to the dust of stars.

SPECIAL ACTIVITIES OF THE FACULTY

Invited Presentations

Dr. Fox

Lectures at Northwestern University and University of South Dakota, October, 1969.

"Experiments Explaining the First Cells on Earth," Scientific Colloquia of the Goddard Space Flight Center, October, 1969.

"A Constructionist View of the Protocell," in the 14th Annual Symposium of the Kalamazoo Section of the American Chemical Society, entitled Evolution--Molecules to Man, November, 1969.

"Synthesis of Proteins and Other Sources of Nitrogen," in the Symposium on The Total Synthesis of Food, Boston, December, 1969.

Presentation in Apollo 11 Lunar Science Conference, Houston, January, 1970.

Presentation in Chemo-Computer Engineering Workshop, NSF-ONR, Washington, April, 1970.

"The Primordial Sequence, Ribosomes, and the Genetic Code" in Symposium on Chemical Evolution and the Origin of Life, Pont-a-Mousson, April, 1970.

Lecture at University of Frankfurt, May, 1970.

Lecture to Democritus Atomic Energy Center in Athens, Greece, May, 1970.

"Does Alpha Relate to Omega?" in series on The Threat to Man, in Athens, Greece, sponsored by U.S. Information Agency, May, 1970.

American Chemical Society Lecture Tour through Oklahoma, Kansas, Arkansas, and Iowa, May, 1970.

"Selective Interactions of Polynucleotides with Thermally Synthesized Polyamino Acids" in Symposium on "Formation and Properties of Polyelectrolyte Complexes" in joint meeting of the Canadian Institute of Chemists and the American Chemical Society, Toronto, May, 1970.

"Amino Acids Obtained from Lunar Soil and from Laboratory Syntheses," in Session on Origin of Life, Paleobiogeochemistry in 1970 meeting of the International Association of Geochemistry and Cosmochemistry, Tokyo, September, 1970.

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Election to vice-presidency of the International Society for the Study of the Origin of Life.

Dr. Harada

"Origin and Development of Optical Activity of Bio-Organic Compounds on the Primordial Earth," at the Third International Conference on the Origin of Life at Pont-a-Mousson, France, April, 1970.

"Origin of Optical Activity" at the International Symposium on Hydrogeochemistry and Biogeochemistry, Tokyo, August, 1970.

Lectures on the "Chemical Basis of the Origin of Life" and on the "Asymmetric Synthesis of α-Amino Acids" at the following: Nuclear Center, Kyoto University, Takatori, Osaka, Japan; Institute of Scientific and Industrial Research, Osaka University, Osaka, Japan; Faculty of Science, Osaka University; Faculty of Pharmaceutical Science, Osaka University; Faculty of Science, Kanazawa University; Department of Chemistry, Tokyo Kyoiku University; Department of Chemistry, Hiroshima University; Medical School, Okayama University, August-September, 1970.

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The Individual and the Universe (by A.C.B. Lovell) was translated into Japanese by Dr. Harada and H. Ogo, and published July 1970 by Kyoritsu Shuppan Co. Ltd. The publisher has requested Dr. Harada to write a popular book on "Life in the Universe."

Dr. Mueller

Seminar at University of North Carolina, November, 1969.

"Lunar Research," two lectures, Concepcion, Chile, April, 1970.

Seminar on lunar research, Institute of Geology, University of Chile, May, 1970.

Four lectures at the University of Concepcion, April, 1970.

Geological Aspects of the Problem of the Origin of Life, Geological Society of Miami, October, 1970.

Contributed Papers

"Comparison of microstructures in carbonaceous chondrites with organic microspheres in terrestrial hydrothermal minerals," 32nd meeting of the Meteoritical Society, October, 1969, Houston.

"Hydrothermally differentiated carbonaceous complexes from the Illinois-Kentucky fluorspar district," 82nd annual meeting of the American Geological Society, November, 1969, Atlantic City.

"Differentiated organic phases from hydrothermal deposits of Derbyshire and elsewhere," S.E. regional meeting of the American Chemical Society, November, 1969, Richmond, Virginia.

A course of four lectures at the University of Concepcion, Concepcion, Chile, April, 1970.

SOME VISITORS

Dr. V. Liebl	Institute of Microbiology, Prague
Dr. John Griffith	Procter and Gamble, Cincinnati
Dr. B. Keil	Institute of Organic Chemistry, Prague
Dr. Walter Stoeckenius	University of California, San Francisco
Dr. Fritz Lipmann	Rockefeller University
Dr. Orville A. Smith	University of Washington
Dr. W. T. Liberson	V.A., Hines, Illinois
Dr. Werner Loewenstein	Columbia University
Dr. Reimar Krans	Federal Atomic Research, Jülich, Germany