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LUNAR STUDIES

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INTRODUCTION

Those aspects of studies of organic geochemistry of terrestrial samples, of meteorites, and those aspects of laboratory studies of the origin of life-related substances and systems which serve as preparation for lunar studies are described in this report.

ORIENTATION IN LUNAR RESEARCH PROGRAM

The Institute has been designated by NASA as a laboratory for the receipt of samples of the lunar surface when they become available. Both Dr. Mueller and Dr. Fox attended as invited participants in the NASA Lunar Exploration Program Review at Goddard Space Flight Center 31 January-3 February 1967. Dr. Harada attended a conference on preparation for lunar studies in Santa Cruz in July, and Dr. Mueller participated in one in Houston in September. In April, Dr. Fox was toured through the Lunar Receiving Laboratory by Mr. Don Flory.

Each of these researchers has been carrying out in his program studies related to lunar analysis, e. g., the analysis of amino acids and amino acid polymers in volcanic samples (Kilauea-Iki, St. Lucia, Surtsey, etc.), in various carbonaceous fractions, and in quartz samples obtained from southwest Africa.

Analysis of Volcanic Samples (Fox, Windsor)

Samples from many volcanic zones have now been extracted, hydrolyzed, and analyzed for amino acids. In order to diminish the possibility that these analyses were due to microbial contamination, the extracted samples (which were mostly nonporous) were crushed aseptically, again extracted, and the extract hydrolyzed and analyzed.

The amino acid pattern has been very similar for samples from Kapoho, Kilauea-Iki, Stromboli, St. Lucia, and Surtsey. Typically, such analyses have shown all of the amino acids of bacteria except methionine and cystine, and have displayed quantitative deficiencies in proline, phenylalanine, and tyrosine, except for the St. Lucia sample.

Accordingly, samples of lava were heated in the oven and treated with bacteria for varying periods of time at elevated temperatures. These were then extracted and analyzed. Some of the results, which are now being systematically scrutinized, suggest that the analyses of lava are very similar to those of lava treated with bacteria which have been heated extensively. Exemplary data are presented in Table I.

Of main interest in the table are the low or zero values of histidine, cystine, methionine, tyrosine, and phenylalanine which seem to apply to the polymers of amino acids in lava as well as to the samples on which were baked bacteria. The one result which is not consistent with those is the one for proline, which reveals the normal content for a bacterium. The total amount of amino acid in the bacteria-treated sample is 26 times that of the untreated sample. This result rules out the possibility that the bacteria were decomposed and the products volatilized to give merely the analysis of the lava.

The total result shown here raises seriously the possibility that analyses of lava truly represent analyses of bacteria which have fallen thereon and have been partially decomposed.

Analyses of many other volcanic samples have shown a high, ca. 50%, proportion of glycine in amino acid assays. Such assays were obtained in water from the soufriere at St. Lucia. The relative rates of decomposition of various amino acids in bovine serum albumin in such water have been studied as controls.

Increased Stability in Proteinoid Microspheres (Fox, McCauley)

Earlier experiments have shown an increase in morphological stability of microspheres at elevated pHs such as 8 by forming them from combinations of 2:2:1 acid proteinoid and neutral or basic proteinoids

Table I

Amino Acid Compositions of Hydrolyzates of

- a) Extracts of Lava from Kilaeua-Iki,
 b) Extracted Aseptically Crushed,
 Previously Extracted Sample,
 and of
 c) Lava Heated with Mixture of Bacillus subtilis, Rhodospirillum rubrum, and Sarcina lutea

Amino Acid	Original Lava (Mole %)	Aseptically Crushed Extracted Lava (Mole %)	Extracted Lava Following One Day of Baking with Bacterium (Mole %)
Lysine	0.64	0.34	1.51
Histidine	0.24	0.00	0.28
Ammonia	77	86	68
Arginine	0.72	0.13	1.13
Aspartic acid	2.72	1.50	3.58
Threonine	0.80	0.52	0.94
Serine	1.28	0.98	1.23
Glutamic acid	4.00	2.60	4.43
Proline	0.00	0.00	2.07
Glycine	4.64	3.38	9.05
Alanine	2.96	1.69	3.68
Cystine	0.00	0.00	0.00
Valine	1.52	1.04	2.07
Methionine	0.00	0.00	0.00
Isoleucine	0.88	0.59	0.66
Leucine	1.12	0.91	1.13
Tyrosine	0.00	0.00	trace
Phenylalanine	0.32	0.33	trace

(Fox and Yuyama, 1963). When lipid lecithin is incorporated, the resulting microspheres are yet more resistant to relatively higher pHs and to solution by washing with distilled water than are the others.

Figure 1 pictures a microsphere with a scar on its surface, believed to be the point at which some other microsphere or a bud was once attached. The uniqueness of this photomicrograph centers around the scar, for it clearly shows that the boundary of a microsphere does exist in a natural untreated microsphere.

To confirm this, the pH was increased with McIlwain's buffer, pH 6.8. The microspheres became hollow, leaving a boundary with the scar intact. Figure 2 shows the structure after hollowing.

Simulation of "Organized Elements", Microfossils, and Contemporary Terrestrial Organisms by Proteinoid Microspheres (Fox, McCauley, Ryan)

With the availability of neutral proteinoid, microspheres from such polymer have been compared with those from acid proteinoid. One difference that has appeared is rooted in the greater ease with which neutral proteinoid microspheres form vacuoles during their preparation (Fig. 3).

The same neutral microspheres also complex RNA readily. The marked difference has been observed in the fluorescence microscope of Dr. Charles Metz, when each type was made in the presence of Acridine Orange.

Prebiological Chemistry of Hydrogen Cyanide (Harada)

The importance of hydrogen cyanide in prebiological chemistry has been recognized increasingly. Hydrogen cyanide can be formed from various simple inorganic compounds by heat, electric discharge, ionizing radiation, and by short electromagnetic waves such as gamma ray, x-ray, and u. v. Since these energy sources would be available in the primordial world before and after the formation of the Earth, chemical reactions of hydrogen cyanide would be an important field of prebiological chemistry. Studies of pyrolysis of ammonium formate and formamide have been carried out in this laboratory, in which reactions hydrogen cyanide was produced as a pyrolytic product. Hydrolysis of the pyrolytic product shows several ninhydrin positive compounds. Among these, the formation of glycine, alanine, and aspartic acid is confirmed.

On the basis of these results, a possible pathway of amino acid formation by oligomerization of hydrogen cyanide has been proposed as shown in Fig. 4.

Chemical reactions of primitive gases which compose the proto-Earth could have taken place before the Earth's crust was formed through

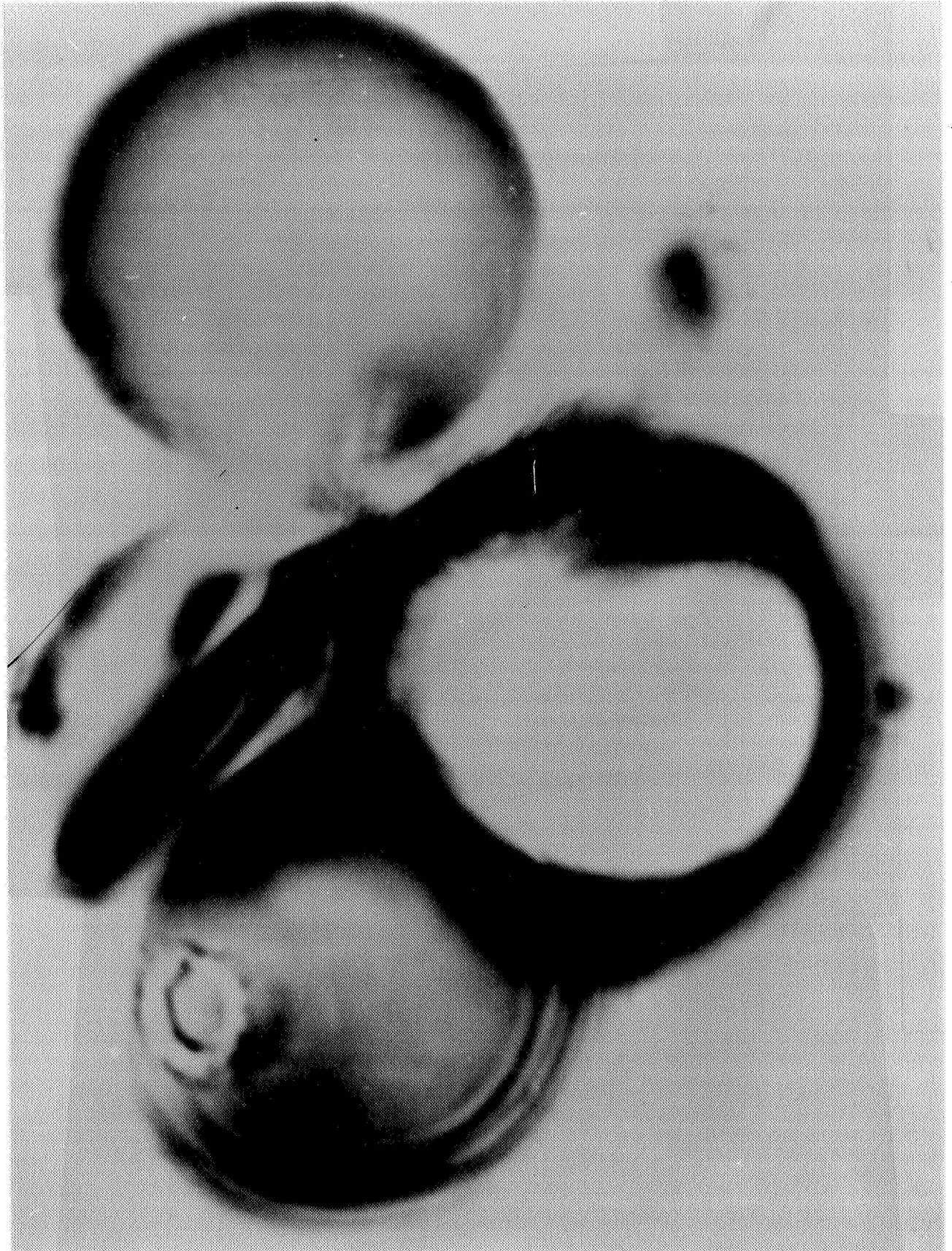


Fig. 1. Microsphere with a scar. The microsphere has a diameter of 14 microns, the scar has a diameter of 3.8 microns. These microspheres have not been treated in any way.

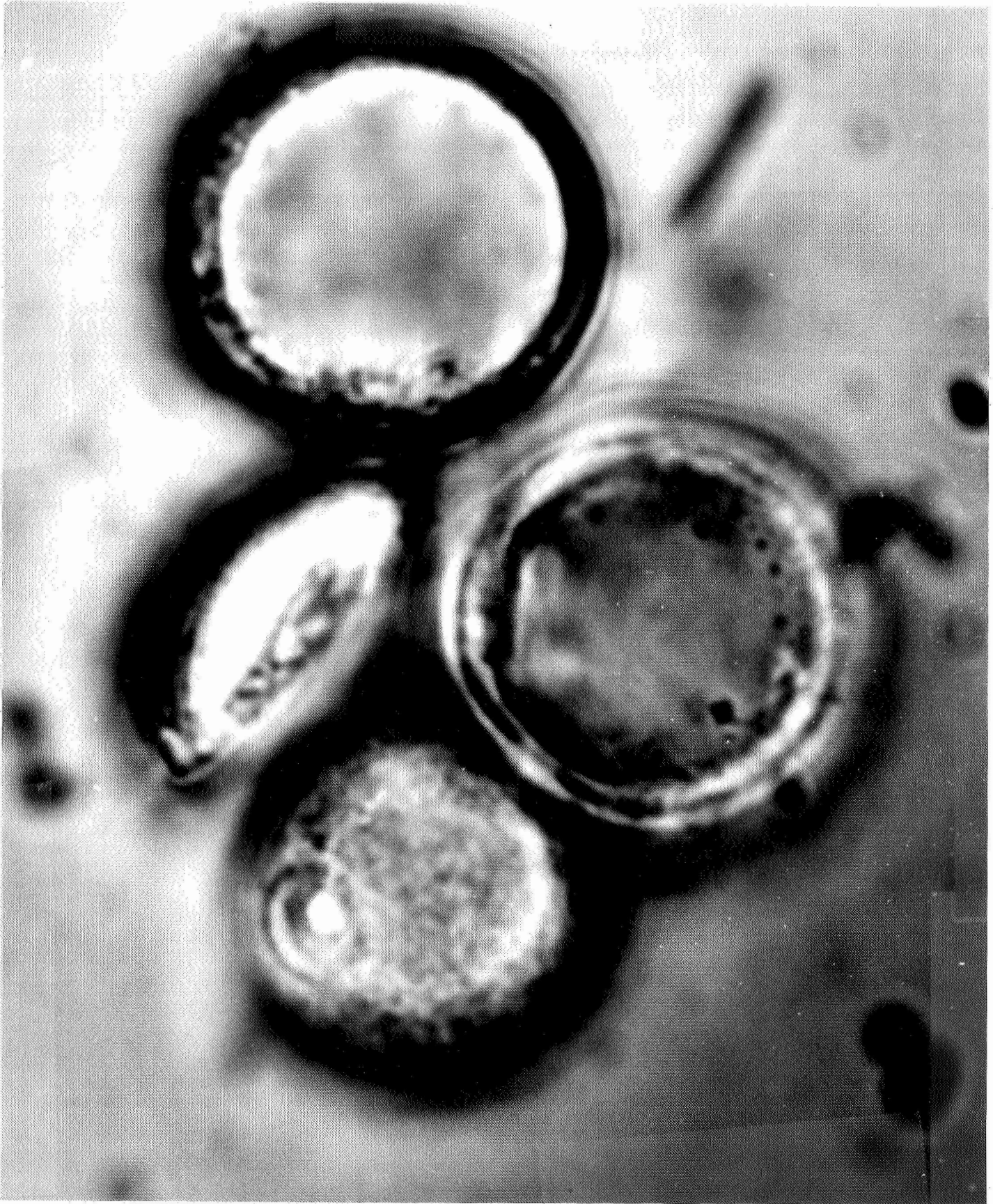


Fig. 2. Microsphere with a scar after hollowing brought about by raising the pH above 5.5 with McIlwain's buffer, pH 6.8.

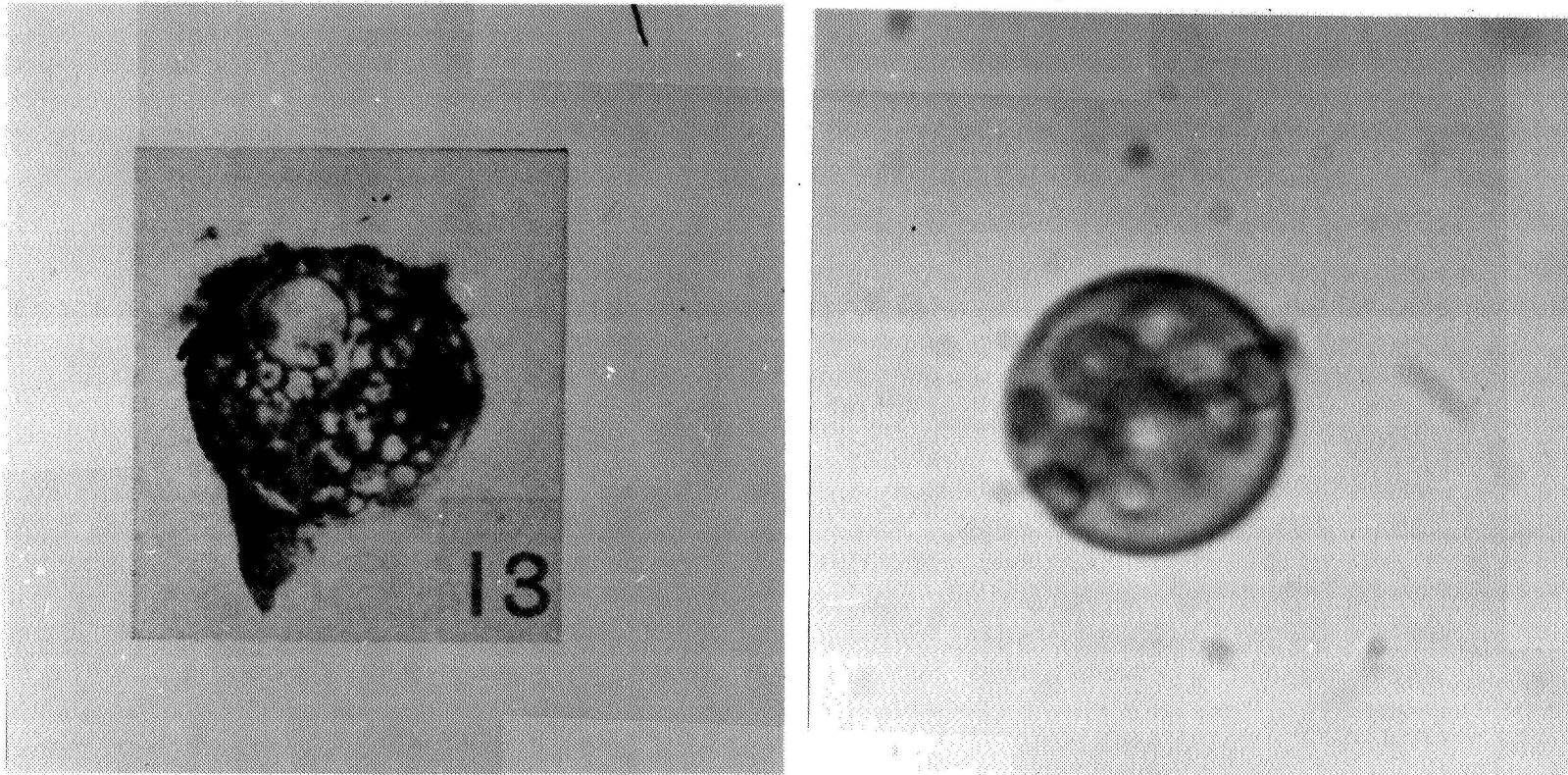


Fig. 3. Vacuolated microfossil on left, vacuolated neutral proteinoid microsphere on right.

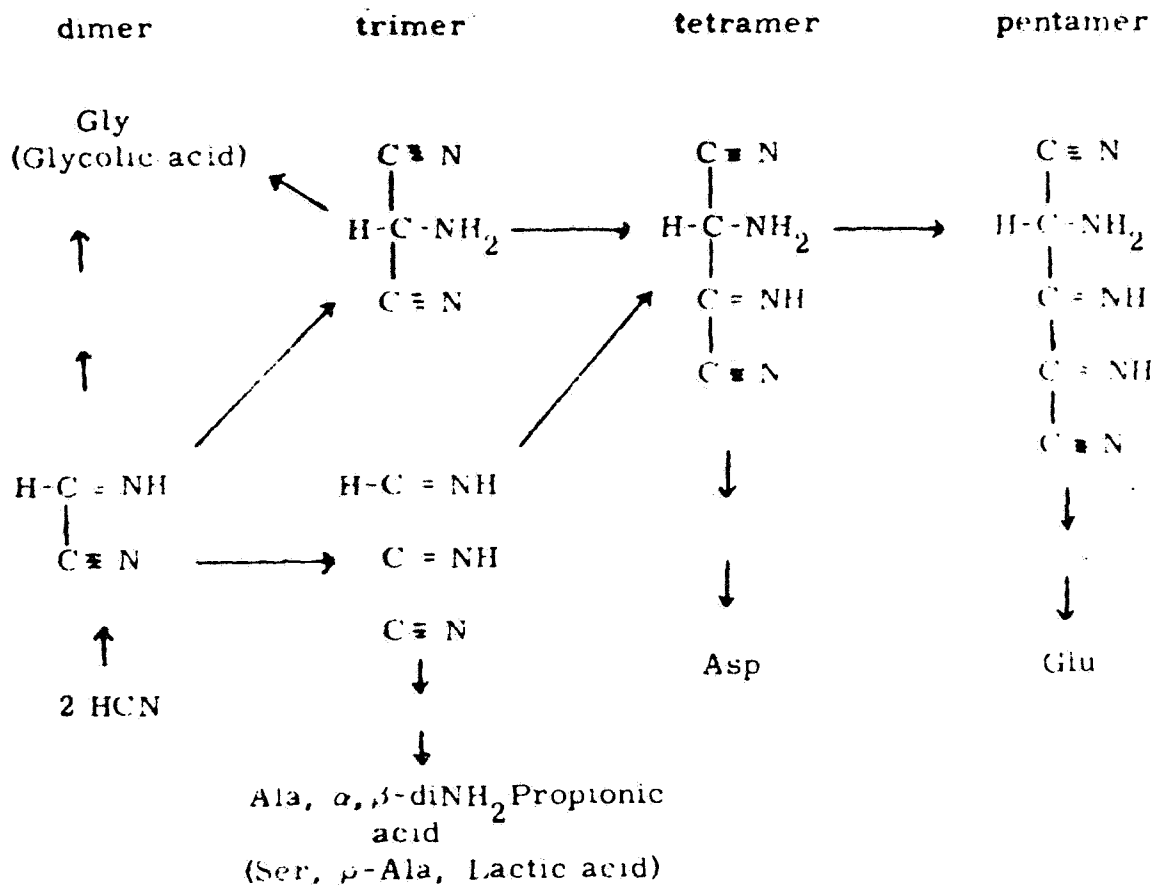


Figure 4

thermal energies due to the contraction process of the proto-Earth. Other energy sources might also have contributed.

Formation of several organic compounds from primitive gases could have taken place before the formation of the Earth's crust.

One of the most important precursors of the organic compounds would be hydrogen cyanide. The chemical study of hydrogen cyanide might be applied to the origin of organic compounds in the carbonaceous chondrites and on the Moon and Mars.

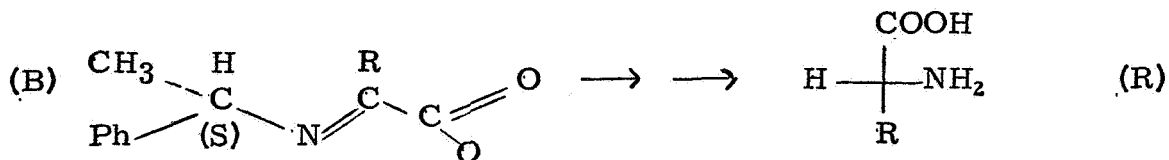
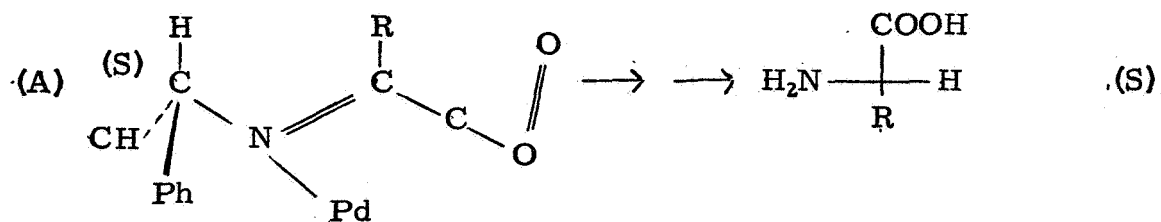
Optical Resolution of DL-Aspartic Acid Copper Complex by the Use of Biopolymers (Harada)

DL-Aspartic acid copper complex has already been resolved by preferential crystallization in this institute laboratory [K. Harada, S. W. Fox, *Nature*, 194, 768 (1962)]. The copper complex also could be resolved from its supersaturated solution by seeding with biopolymers such as cotton or wool. Crystallized aspartic acid copper complexes were found consistently to contain D-aspartic acid. The assumed mechanism of the resolution is a combination of asymmetric adsorption and a preferential crystallization of optically active compounds from the supersaturated solution.

This kind of resolution method throws light on new practical resolutions of organic compounds and also on concepts of development of optical activity in the prebiological world.

Syntheses of Optically Active α -Amino Acids from α -Keto Acids by Reductive Amination (Harada, Matsumoto)

In the previous study, the steric course of the synthesis of optically active α -amino acids from α -keto acids with (+) or (-) α -methylbenzylamine by reductive amination was proposed. To confirm the mechanism, solvent effects in the synthesis were studied. By the use of nonpolar solvent (low dielectric constant), the resulting amino acid showed higher optical purity, and low optical purity was observed by the use of polar solvents (high dielectric constant). To explain these results, it seems possible that two structures (A) and (B) are the most important conformers in the reaction. Structure (A) is an adsorbed state of the substrate and structure (B) is a conformation in solution condition. Structure (A) is an intermediate cyclic complex formed on the surface of palladium catalyst. The cyclic complex (A) is then adsorbed on the catalyst at the less bulky side. The hydrogenation resulted in S-amino acid when S-amine is employed. Structure (B) is a typical conformation of the substrate in solution and this was adsorbed on the less bulky side on the catalyst. Hydrogenation reactions yield R-amino acid when S-amino acid is employed. When nonpolar solvent is used,

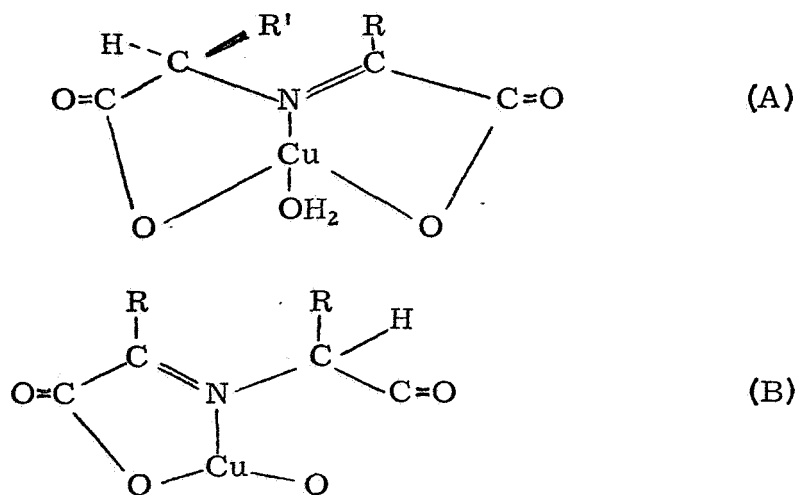


structure (A) might be predominant and structure (B) might be a small part (in conformation). When highly polar solvent is used, structure (B) might be increased and the reaction would result in less optical activity.

In the synthesis of glutamic acid from α -keto acid, when less polar solvent was used (alcohols), the resulting glutamic acid was S. When, however, polar solvent (H_2O) was employed in the reaction, R-glutamic acid was obtained. Thus the asymmetric synthesis in different solvents might be explained by the ratio of intermediate structures (A) and (B) in the solution.

Formation of Amino Acid from α -Keto Acid by Prototropy in a Metal Complex of Schiff Base Composed of α -Amino Acid and α -Keto Acid (Harada)

α -Keto acid could be converted to α -amino acid by heating with equimolar amounts of α -amino acid in the presence of Cu^{++} . The mechanism of the reaction could be explained by the formation of the Schiff base copper complex composed of α -keto acid and α -amino acid, as shown in structure (A). Prototropy between the structure (A) to (B) resulted in the conversion



of α -keto acid to α -amino acid. In the present study, factors determining the ptototropy, such as pH effect, size, and combination of the R, R' of (A) are now under investigation.

Asymmetric Synthesis of α -Amino Acid by the Strecker Synthesis
(Harada, Matsumoto)

Optically active alanine was synthesized by the Strecker method in which optically active α -methylbenzylamine functioned as the asymmetric center. In a similar reaction, optically active α -methylbenzylamine and acetaldehyde cyanohydrin resulted in optically active alanine.

To determine the steric course of the reaction, several investigations are now under way. In the asymmetric synthesis it was found that optically active α -phenylglycine could be used as an asymmetric source of the reaction.

This study and the two others described help to provide an evaluation of the validity of optical activity as a criterion of previous life on the Moon.

Organic Chemical Studies of the Carbonaceous Constituents of Meteorites
(Mueller)

The proportion of C and S volatilizable at 1000^o C in an N₂ atmosphere tends to decrease with the total of volatilizable constituents, as shown in Table II. These data seem to indicate the decrease of volatiles in the metamorphism of the carbonaceous complex, presumably towards the deeper and hotter zones of the closely comparable parent bodies. The latest results of this work indicate that the carbonaceous complex tends to turn more aromatic with the decrease of volatile matter. The analytical work is being continued on groups of meteorites not hitherto investigated, and the scope of it will be extended to determination of volatilization curves and the elemental and molecular composition of de-ashed carbonaceous complexes.

Differentiation of Bitumens (Mueller, Oh-hashi)

Microscopical and macroscopical evidence of a conclusive nature indicates that the bitumens associated with the highest temperature hydrothermal veins of the North Derbyshire Orefield, England, are differentiated into six immiscible liquids. The results of organic chemical analytical work demonstrate that the "foxite" phase contains well over 100 times more organic acid and 5 times more amino acid than is the mean percentage of the corresponding acids in the remaining differentiates. The separation of almost exclusively paraffinic molecules in the "ozocerite" may be of significance from the perspective of genesis of petroleum. The "elaterite" appears to contain the bulk of low molecular weight aliphatics. "Bernalite" is a polymerization product of mainly olefinic molecules. "Carbonite" contains the maximum of aromatic acid, and "cyclite" appears to consist mainly of condensed aromatic rings.

Table II
Analyses of Various Meteorites

	Volatiles	Vol. C% in C	Vol. S% in S
Carbonaceous chondrites, Type I	27.37%	94.9%*	63.2%
" " II	16.69%	87.2%	41.3%
" " III	5.12%	72.7%	32.1%
Enstatite chondrites	4.77%	78.3%*	39.8%
Ureilites	0.82%	12.6%*	1.2%*
Ordinary chondrites	0.23%	4.5%	1.5%

*Single data; for the others: mean values of 5 or more determinations

Preliminary microscopical studies of material collected in the course of field work, or obtained through exchanges with museums, indicate that several types of bitumen assemblages are invariably associated with hydrothermal veins. The localities are bitumen inclusions in fluorite from Kentucky; inclusions of bitumens in quartz from Herkimer County, New York; bitumens from a number of localities of Canada, the British Isles, Scandinavia, France, Italy, Russia, Chile, and New Zealand; and bitumens including condensed aromatics associated with mercury veins from California.

Quartz crystals from an igneous dike of Precambrian age from the Warmbad District of S. W. Africa contain numerous inclusions with a suit of five differentiated bitumens, water, and gas. Some of the inclusions also contain spherules of organic polymers. These closely resemble in morphology the microspheres of proteinoids, including the presence of a center, a boundary, and budding (see Fig. 5). In some of the inclusions, yellow spherules appear on cooling to +5° C, and they disappear again on heating to room temperature. Fibers with or without regular spiral patterns have been observed. The microscopically detectable properties of the most frequent, brown spherules are as follows: fluorescence: orange; birefringence: weak; density: higher than that of the water of the inclusions; softening: approximately 85° C. The above properties of the spherules rather closely resemble those of the "foxite" phase of Derbyshire, which proved to consist of polarized molecules, including amino acids.

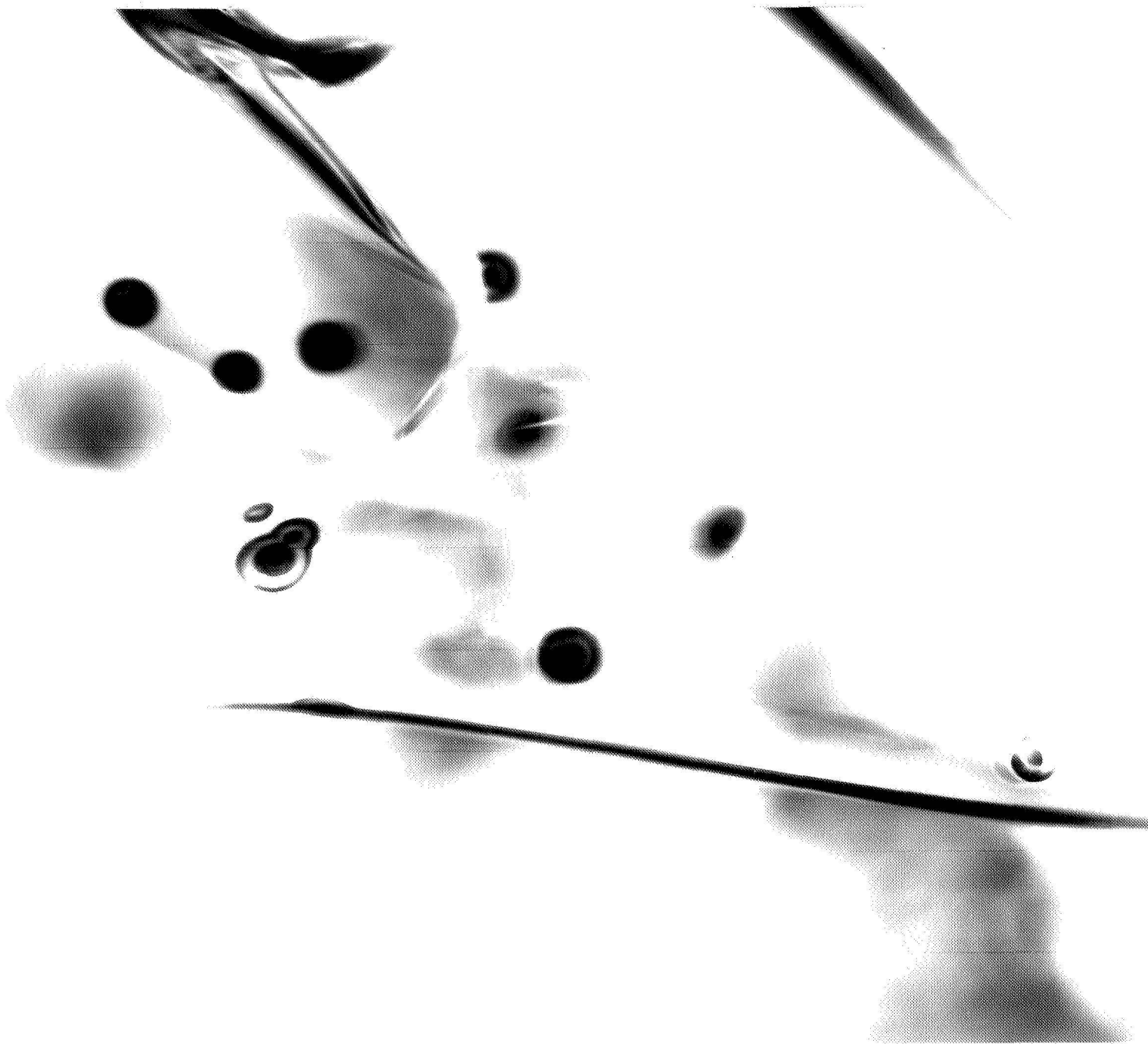


Fig. 5. Microspheres of organic polymers from a water and gas filled inclusion of quartz crystals of Precambrian age, from the Warmbad District of S. W. Africa. Combination of two photomicrographs focused at different levels of the same inclusion; note boundaries, centers, and budding. Ordinary light, 100X. The evidence that the polymer is organic is in the text.

In the course of recent statistical work, the diameters of the microspheres were found to grade towards one to three sharp maxima in some of the inclusions. In certain instances, up to 20,000 microspheres of close to identical diameters are arrayed in several types of regular, two-dimensional lattices. Yet in other inclusions, the budding of the microspheres occurs, as a good statistical rule, only above a certain critical diameter. Other inclusions contain small liquid microspheres in Brownian motion, which are compressed into polygonal patterns, without having been coalesced through the well over 1000 million years of geological history of the host crystals. Another type of rotational motility of some microspheres proved to increase with the intensity of illumination, but it is insensitive to changes of temperature. The melting points, solubility curves, etc. of the diverse organic phases within the inclusions are now being determined with the heatable microscope stage.

The presumably abiogenic gas condensates in carbonaceous meteorites and in the early Precambrian oceanic or coastal sediments proved to contain only a finely divided carbonaceous complex, and the experiments involving condensations of likely cosmic gas mixtures have also yielded products in which the amino acids are very considerably diluted with organic molecules of no substantial biological significance, such as paraffins, aromatics, etc. (see Ponnampertuma, C., *Abiological Synthesis of Some Nucleic Acid Constituents*, in The Origins of Prebiological Systems and of their Molecular Matrices, S. W. Fox, ed., Academic Press, New York, 1965, p. 221).

It seems that before the emergence of the proto-organism, additional processes involving the concentration of those molecules which constitute the bulk of the organism could have been crucial.

Moreover, differentiation is now shown to occur simply and spontaneously in the crust of the Earth, on the basis of the bitumens which have been differentiated within hydrothermal deposits. The degree of differentiation is actually relatable to the height of the temperature of mineralization experienced in Derbyshire. In the course of geological events, such processes of differentiation could have favored a concentration of amino acids to be condensed and polymer to be assembled, in the key prebiological processes. This analysis of the processes applies whether the microspheres found are proteinoid in composition or not.

Organic Geochemistry of Precambrian Carbonaceous Complexes of Juvenile Magmatic Origin (Mueller)

Several recent works deal with the carbonaceous substances in Precambrian sediments, but the literature contains no information regarding carbonaceous matter within the magmatic products. In the course of diverse geological field trips, material was collected from the following localities:

1. Black quartz of hydrothermal veins, which traverse the Fig Tree formation of the Transwaal-Swaziland borders, S. Africa.
2. Dark grey carbonatite, from dikes which have a radical distribution within the Premier Kimberlite Pipe, Transwaal, S. Africa.
3. Grey carbonatite from the Palaborwa Copper Mine, Transwall, S. Africa.
4. Fibrous carbon from the "carbon tracer" of the Western Deep Levels Gold Mine, Carletonville, Transwaal, S. Africa.
5. Thucholite from the pegmatite of Bodinen Mine, Sweden.
6. Graphite and graphitite from pegmatite veins of the Ratnapura area, Ceylon.
7. Shungite from intrusion-shale contact at Shunge, U. S. S. R.
8. Asphalt in granite from the Kiel District, Northern Germany.

The mode of distribution of the carbonaceous phase within the above specimens is being at present microscopically studied. Organic chemical work will follow.

The Organic Geochemistry of Molecules of Biological Significance in Members of the Coal Series (Mueller, Windsor)

The coals are particularly suitable for the determination of aging effects on amino acids, proteins, purine and pyrimidine bases, porphyrins, because of their high content of plant remains which accumulated under closely comparable geological conditions.

The results of the analytical work carried out so far indicate that amino acids are present in readily determinable quantities within members of the entire coal series, ranging from recent peat collected from the Everglades of Florida, to anthracite of the Pennsylvania mines.

Comparison of Distribution Patterns of Elements within Carbonaceous Meteorites and the Terrestrial Hydrothermal Deposits (Mueller)

The computation of data from the literature and from recent analytical work resulted in the conclusion that the redistribution ratios of minor and trace elements between mean carbonaceous chondrite/mean chondrite, on one hand, and mean hydrothermal deposit/mean Earth's crust, on the other hand, coincide to quite a remarkable degree. The only two anomalies, namely C and S, can be accounted for through the drastic change of volatilities of the compounds of these elements, when proceeding from the reducing conditions indicated through the chemistry of chondrites to the more oxidizing conditions which prevail within the Earth's crust. Further computation of analytical data is being carried out, in order to gain additional information regarding the mechanism of redistribution of volatiles in condensing celestial bodies.

Statistical Petrography of Chondrites (Mueller)

The close similarity of size distribution diagrams of terrestrial clouds and chondrites indicates a high degree of uniformity of spray condensation in general, which consists of the following two stages:

a) Primary condensation of spray, formation of fog particles, and microchondrules, respectively; b) collision-coalescence of spray particles of over 20 to 30 micron diameter, which leads to the rain drops in clouds and the spectacular series of "grape bunch-globular-partly coalesced-coalesced" chondrules in the meteorites. A statistical trend of coalescence of chondrules with decreasing volatiles of the chondrites has been observed.

Most of the terrestrial volcanic ashes contain only spray, which is derived from the mechanical subdivision of lava. However, it was found for the first time that welded tuffs, which formed through an exceptionally high temperature and extensive volcanic activity in the Quaternary at the San Pedro de Atacama district of Northern Chile, contain microspray with certain features of primary condensation from vapors, such as exsolution cores, incipient cohesion into "grape bunch chondrule"-type structures, etc.

The Mineralogy and Petrology of Ureilites (Mueller)

It was established in the course of the present work that the ureilites subdivide as follows: a) Type I contains the orthopyroxene of m. p. around 1300° C, coalesced into single crystals, but the olivine and nickel-iron of m. p. s in the range of 1400-1500° C preserved as aggregates of microchondrules of 10-50 micron diameter; b) Type II has all the above mineral constituents coalesced into single crystals within the 1000 micron diameter range. The above data and the recent observation of the presence of

inclusions of an ureilite Type I petrography in the Type II carbonaceous chondrite Cold Bokkeveldt, seem to support the hypothesis that the ureilites are impact shocked carbonaceous chondrites.

Distribution of Amino Acids in Fumarole Products (Mueller, Fox, Windsor)

The material collected from several types of fumarole vents and boiling springs from St. Lucia and Guadeloupe, of the Lesser Antilles, is being analyzed for amino acids. Plans are to extend the work in the future to the products of fumaroles, which are situated at relatively greater distances from the sea, from Chile, Central Africa, etc.

Theory of Cosmic Evolution (Mueller)

The main aim of the work in progress is to trace and specify the roots of biological evolution within the inanimate Universe in a relatively more rigorous manner. The new theoretical approach in question may lead toward a unified concept of the evolutionary process in general. Some of the hypotheses may be tested with critical experiments within the foreseeable future.

Comparative Studies of Carbonaceous Complexes (Mueller)

Studies of the carbonaceous constituents of meteorites have as a main objective establishing and elaborating well-defined interrelations between the percentage of constituents volatilizable at 1000° C (N₂ atmosphere) and the properties of the carbonaceous complex. The latest results of this work indicate that the carbonaceous complex tends to turn more aromatic with decrease of volatile matter.

Carbonaceous complexes separated into several immiscible liquids have been discovered for the first time by Professor Mueller in Derbyshire, England. The separation of amino acids and other polar molecules in one of the phases is of significance from the standpoint of the origin of life, which is constituted to a high degree of polar molecules. The separation of a paraffin-rich phase is important from the standpoint of genesis of petroleum. In the course of recent field trips, a number of other differentiated carbonaceous complexes have been discovered in Scandinavia, in California, and in S. W. Africa. The latter contain a considerable range of organic polymer microspheres, which show "budding", boundaries, and centers reminiscent of nuclei, as mentioned elsewhere in this report. Occasionally are found even more elaborate indications of self-assembly of matter, such as regularly spaced nets of up to 20,000 microspheres of equal diameters within ± 5%; fiber with regular spiral patterns, etc.

The detailed chemical investigation of hitherto unanalyzed Precambrian material, collected during recent field trips, is to be undertaken in the near

future. Such material includes thucholites from S. Africa and Scandinavia; carbonatites from Kenya and Transwaal; graphitite from Ceylon and shungite from Russia.

The metamorphism of the amino acid assemblages of coals with their age and their thermal history is being at present investigated.

Work is also continuing on the statistical petrography of chondrites and ureilites, and the crystallization and conversion mechanism of sulphur.

Recent work of more theoretical nature includes the reconstruction of the assemblage of mineral deposits of the Moon [Nature, 215, 1149-1151 (1967)], in which inter alia the basaltic character of the average lunar rock was predicted prior to the results of the analysis of Surveyor.