CURRENT STATUS OF CHEMICAL STUDIES ON THE ORIGIN OF LIFE

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1. Introduction

The prime goal of space biology is the search for extraterrestrial life. The discovery of life elsewhere in the universe would demonstrate that there is nothing unique about life on earth. Since the laws of physics and chemistry may be held as universal, the evolution of matter will be expected to follow a similar course in the entire universe. The search for life beyond the earth thus becomes only part of the scientifically broader question of the origin of life in the universe. The study of the beginnings of life on earth is the investigation of only one example of an event which must have occurred in the universe innumerable times. The earth is the model laboratory which should reveal the steps in this cosmic sequence of events. The retracing of the path by which life appeared on earth should give strong support to the theory of its existence elsewhere in the universe (PITTENDRIGH *et al.*, 1966).

The Oparin-Haldane hypothesis presupposes a long chemical evolution before the appearance of life (OPARIN, 1924; HALDANE, 1928). The accumulation of organic matter on the primitive earth and the generation of replicating molecules are two factors of prime importance in chemical evolution. This process may be considered to have taken place in three stages: the inorganic, the organic, and the biological. In the molecular approach to the problem of the origin of life, attempts have been made to elucidate how compounds of biological significance could have arisen on the earth before the first life appeared. The purpose of this paper is to review critically the basis for some of these studies, the experiments that have been performed, and the results obtained. A general solution to a problem of such cosmic proportions demands a multidisciplinary approach (BERNAL, 1951); however, since most meaningful experiments have been done in the field of chemistry, this aspect of the problem will be considered in detail here (OPARIN *et al.*, 1959; FOX, 1965a; ORO, 1963a; HOROWITZ and MILLER, 1962; PATTEE, 1965).

In the chemical approach to the study of this problem, one of the main objectives has been to unravel the path by which nucleic acids and proteins could have appeared before the origin of life. The tacit assumption is made that molecules which are important now were important at the time of the origin of life. Such a premise is not without foundation, for recent biochemical discoveries have underlined the remarkable unity of living matter. From the smallest microbe to the most highly organized mammal, there are two basic molecules, the nucleic acids and protein, whose interaction

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appears to result in that unique property of matter which is generally described by the word 'life'. This uniformity extends not only to molecular composition but also to metabolic pathways (ANFINSEN, 1959).

Contemporary biochemical pathways may also be regarded as a recapitulation of pre-biological syntheses. This analogy should perhaps not be extended too far considering that biological pathways have evolved through the ages by pressure of changing environments. According to HOROWITZ (1945), the first living entity was a heterotroph which reproduced itself at the expense of pre-fabricated molecules. A depletion of the supply limited further multiplication until a means was discovered to use other available substrates. The final step in the modern biological synthesis of a particular molecule must have evolved first.

2. The Time Scale

The most recent evidence suggests that the age of the earth is 4.75 billion years (TILTON and STEIGER, 1965). The age of the solar system is considered to be 5 billion years and that of our galaxy 7.4 billion years. This figure is consistent with Hubble's age of the universe of at least 12 billion years (DICKE, 1962). The most ancient evidence for organized elements in sediments has been given by BARGHOORN and SCHOPF (1966), who isolated bacteria-like particles from the Swaziland shale which has been dated at 3.2 billion years. Therefore, life must have made its first appearance during the first 1½ billion years of the earth's existence.

The evolutionary sequence presumably began with the origin of the universe and the formation of the elements from the primeval cloud of hydrogen gas (BURBRIDGE and BURBRIDGE, 1958). It is possible that conditions for organic synthesis were quite favorable during the proto-planetary stage of the solar nebula. The primordial molecules would have been mixed with inorganic particles which could have acted as catalysts (KUIPER, 1953). Chemical evolution, therefore, should take into consideration the very early periods of the universe (LEDERBERG and COWIE, 1958; FOWLER *et al.*, 1961). However, during the accretion of the dust particles in the solar nebula to form the planets, considerable heat must have been generated. Heat of compression and the heat generated from radioactive elements such as K-40 and U-238 would have given rise to temperatures in the neighborhood of thousands of degrees (LATIMER, 1950). Such temperatures might have been required to form the molten core of iron and nickel which exists within the earth. Under these circumstances, most of the organic matter would have been volatilized and would have escaped into space (MILLER and UREY, 1959). In considering chemical evolution on the earth it is therefore reasonable to start at the time that the accretion of the planet had already taken place and consider that atmosphere as the primitive atmosphere.

3. The Nature of the Primitive Atmosphere

A true understanding of the nature of the earth's primitive atmosphere is the logical starting-point for any discussion on the problem of the chemical origin of life. While it is difficult to answer by direct observation the questions that arise, evidence available from a number of sources indicates that a reducing atmosphere gradually gave way to the oxidizing atmosphere of today. Information gleaned from astronomy, astrophysics, chemistry, geology, meteoritic studies, and biochemistry can be used to elucidate this problem.

The present rarity of the noble gases in the earth's atmosphere in comparison to their distribution in the universe indicates that the primitive atmosphere of the earth was lost and that the atmosphere which is generally described as primitive was secondary in origin (BROWN, 1952). This atmosphere must have resulted from the out-gassing of the interior of the earth during planetary accretion. The secondary atmosphere must have been very similar to the first, and it is this atmosphere which will be considered in our discussion as the earth's primordial atmosphere (UREY, 1952a).

RUSSELL'S (1929) discovery that hydrogen is the single most abundant element in the universe indicates that the universe as a whole is reducing in nature. The main problem concerning the nature of the terrestrial atmosphere is, therefore, not how to explain why the early atmosphere was reducing but rather to explain how an atmosphere in which hydrogen was almost 1000 times more abundant than oxygen could have given rise to an atmosphere containing free oxygen (SUESS, 1965).

The distribution of elements in the sun indicates that most of the sun is made up of hydrogen (BROWN, 1949). Helium, carbon, nitrogen and oxygen are the next most abundant elements. Oxygen, nitrogen and carbon account for only 0.05% of the total mass of the sun, while hydrogen constitutes 87%. If the material of the sun represents the average composition of our solar system, the proto-planetary dust cloud must have been made up of an excess of hydrogen. Most of the smaller planets were unable to retain their early atmosphere, but the larger planets such as Jupiter and Saturn contain large amounts of methane, ammonia, and water (OPIK, 1962).

Analysis of meteorites which have been dated as being about 4½ billion years old are true examples of the primordial matter from which our planetary system was formed. In these meteorites, the metals are generally found in their reduced form (MASON, 1962).

The study of chemical equilibria sheds some light on the nature of the reactions which could have taken place in a primordial reducing atmosphere. The thermodynamic properties of carbon, carbon monoxide, carbon dioxide, methane, ammonia, and water are very well known (Table I). The composition of equilibrium mixtures

TABLE I

Chemical Equilibria

	$K_{25^\circ C}$
$C + 2H_2 \rightarrow CH_4$	8×10^8
$N_2 + 3H_2 \rightarrow 2NH_3$	7×10^5
$H_2 + \frac{1}{2}O_2 \rightarrow H_2O$	4×10^{41}
$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	7×10^{21}
$S + H_2 \rightarrow H_2S$	6×10^{15}

containing these materials has been satisfactorily determined (UREY, 1956, 1952b; MILLER and UREY, 1959). The equilibrium constants for the formation of methane from carbon and hydrogen, of water from oxygen and hydrogen, and ammonia from nitrogen and hydrogen are all of considerable magnitude (LATIMER, 1950). It is reasonable to suppose that in a large excess of hydrogen carbon, nitrogen, and oxygen existed in their reduced form. By considering the rate at which hydrogen escapes from the earth today it is possible to calculate the pressure of hydrogen on the surface of the earth about $4\frac{1}{2}$ billion years ago. By taking into account the loss of hydrogen required for the oxidation of iron, carbon, nitrogen and sulfur, etc., MILLER and UREY (1959) suggested the figure of 1.5×10^{-3} atmospheres as a reasonable concentration of hydrogen in the primordial atmosphere. According to the Urey equilibrium for the formation of calcium carbonate from calcium silicate, the CO_2 pressure would not be more than the equilibrium pressure of 10^{-8} atmospheres,

$$CaSiO_3 + CO_2 \rightarrow CaCO_3 + SiO_2 .$$

Assuming the figure of 1.5×10^{-3} atmospheres of hydrogen, the pressure of methane under these conditions would be 4×10^3 atmospheres which is very large. The conclusion then is that CO_2 could hardly exist in the presence of a trace of hydrogen. Similarly, the equilibrium data on carbon monoxide show that its concentration would also be negligible in the presence of even small amounts of hydrogen. The ammonia that would be formed from nitrogen and hydrogen would be rapidly dissolved in the oceans. The ammonia in the oceans would have been decomposed only when the hydrogen pressure fell below 10^{-5} atmosphere. From their discussion, Miller and Urey concluded that a reducing atmosphere consisting of small amounts of hydrogen and ammonia and a moderate pressure of methane and nitrogen would have constituted a reasonable atmosphere for the primitive earth.

LATIMER (1950) has suggested the possibility of nitrides and carbides having existed in the earth's mantle. If nitrides and carbides react with water, nitrogen and hydrocarbons could be formed. A certain amount of CO_2 could have been released by the reaction between iron carbide and iron oxide. The free energy for this equation is positive but the entropy is about 50 calories per degree. The reaction would take place at a temperature of 500 °C; but the carbon dioxide which would be formed would react with the silicates to give carbonates and thereby be removed from the atmosphere.

Where enough data are available to form some conclusions, the geological picture can also indicate the nature of the early atmosphere. According to RUTTEN (1962) "the difference between the primeval anoxygenic and the present oxygenic atmosphere is as clear cut as black and white". This categoric statement is based on several comparisons made between ancient and modern sediments.

In studying the ratio of ferrous iron to ferric iron in ancient and recent sediments, RANKAMA (1955) found that in the most ancient rocks, the ratio of ferrous to ferric was greater. In the quartz diorite and neighboring sediments, Rankama was able to identify iron sulfide and pitchblende, which could not have been deposited under oxidizing conditions. In the study of the middle-Precambrian rocks near Tampere, Rankama concluded that a reductive weathering process rather than an oxidizing one was in operation up to the last 2 billion years. RAMDOHR's (1958) studies of the ancient shales of South-Africa, Brazil, and Canada show the presence of sulfides and pitchblende mixed with quartz. In new sediments, however, only quartz is found. It may, therefore, be concluded that the ancient weathering process must have been a reductive one. LEPP and GOLDICH (1959) have studied the Precambrian and later times. Their investigations indicate that the Precambrian formations are siliceous, while in the later formations the iron and silica have separated. According to these authors, the difference between the Precambrian and later deposits can be attributed to the lack of oxygen. In support of this argument is the fact that recent iron deposits or red beds have iron in the oxidized form as ilmenite, $Fe_2O_3nH_2O$ (GUSSOW, 1959; GASTIL *et al.,* 1960). These geological observations apparently support the idea that for the first 2 billion years of the earth's existence the atmosphere was non-oxidizing.

RASOOL and MCGOVERN (1966) have calculated the exospheric temperature of the primitive atmosphere for the model suggested by Holland. Preliminary calculations indicate that for an exospheric temperature of 600 °K a methane atmosphere with small amounts of ammonia, nitrogen and hydrogen could be stable for as long as 1 billion years. HOLLAND (1961, 1962) has divided the history of the earth's atmosphere into three stages. The first covers the earliest period, prior to the formation of the core. During this time iron was probably present in the upper part of the mantle. Volcanic gases ejected during the first stage must have contained a large amount of hydrogen and the atmosphere was highly reduced. This stage could have lasted about $\frac{1}{2}$ billion years. The second stage began when iron was removed from the upper mantle. The out-gassing became less reducing. Carbon dioxide then took the place of methane. Free oxygen was present only in trace quantities. The presence of detrital uraninite in the mid-Precambrian suggests that the second stage lasted at least until 2 billion years ago. The third stage began when the rate of production of oxygen by photosynthesis exceeded the rate needed to oxidize reduced volcanic gases. Oxygen began to accumulate at an almost constant rate. On the basis of the oxygen requirements of Paleozoic insects, Holland suggests that a fairly high oxygen pressure obtained about 0.3 billion years ago. BERKNER and MARSHALL (1965) have developed convincing arguments to show that oxygen accumulated as a result of photosynthesis. These authors maintain that the sudden proliferation of life in the Cambrian era was due to the attainment of the Pasteur point, namely, a concentration of about 1% of the present oxygen pressure.

While most of the foregoing authors have adduced arguments to show that the early atmosphere of the earth must have been reducing, and that such conditions must have prevailed at least for about a billion years, there are others who disagree with this current view. RUBEY (1951, 1955) has suggested that the methane and ammonia atmosphere may not have lasted for more than 10^5 to 10^8 years. Since carbon dioxide is the primary gas evolved from volcanoes today, Rubey maintains that if the early earth's atmosphere was formed by an out-gassing from the earth's interior, carbon dioxide must have been the predominant feature of this atmosphere. Using similar arguments, ABELSON (1966) has postulated that the earth's primitive atmosphere consisted of carbon monoxide, carbon dioxide, nitrogen, and some hydrogen. In addition he maintains that ammonia must have been dissociated by ultraviolet light and could not have lasted for more than 20000 years. A further argument suggested by Abelson is that the earliest rocks should have contained an unusual amount of organic matter. It may be pointed out that very little analysis has been done of rocks more than 3 billion years old. Erosion and the heterotrophic growth of organisms already evolved would have removed a large amount of the organic matter formed by prebiotic synthesis. The fact that some of the oldest sedimentary rocks contain carbonates does not necessarily imply that carbon dioxide was the largest single component of the earth's atmosphere. The Urey equilibrium by which silicates absorb carbon dioxide and deposit carbonates could explain this situation. Perhaps the great weakness of both Abelson's and Rubey's suggestions is that the equilibrium data of the conversion of carbon dioxide to methane and the instability of carbon monoxide have been ignored.

Free oxygen is unique in our planetary system. The oxygen in the present atmosphere appears to have arisen from two sources: from the photodissociation of water in the upper atmosphere by short wavelength ultraviolet light and from plant photosynthesis (BERKNER and MARSHALL, 1965). Photosynthesis probably evolved when the ozone layer developed in the upper atmosphere and shielded the earth from the ultraviolet light emitted by the sun, thus preventing the further photochemical synthesis of organic compounds in the primitive environment. Heterotrophs which thrived on photochemically produced materials available around them were replaced by autotrophs which were able to photosynthesize their own food when the supply of abiogenic organic matter was depleted. This change may be diagrammatically represented by the hour glass. Only those organisms that could incorporate molecules such as the

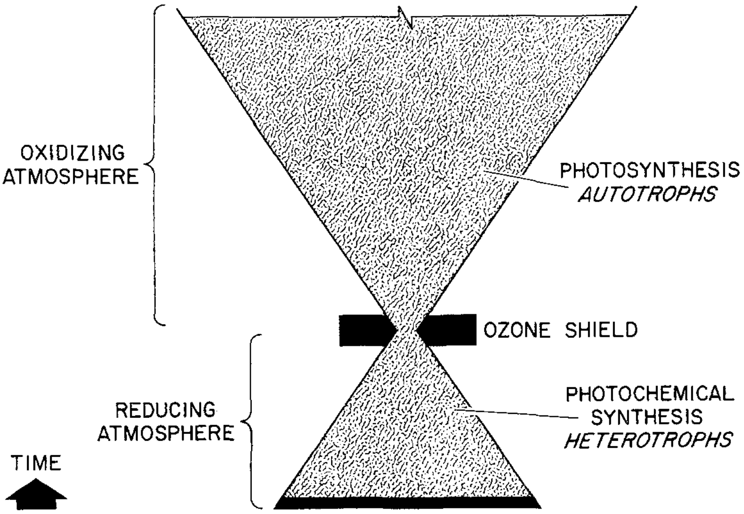


Fig. 1. Transition from reducing to oxidizing atmosphere.

porphyrins and make use of longer wavelength light were able to pass through the bottleneck. There was a wholesale massacre at this junction. The organisms that survived and developed further were able to evolve into the wide variety of life on earth today (Figure 1).

In biochemical reactions taking place in contemporary living organisms carbon has to be reduced before it can be incorporated into amino acids, purines, pyrimidines, etc. This is a universal fact of the biosphere (FRUTON and SIMMONS, 1961). It is a credit to the great insight of OPARIN (1924) that, even before the reducing nature of the universe was discovered astrophysically, he postulated the need of a primitive reducing atmosphere for organic synthesis. Most organisms have a mechanism to reduce nitrogen before it is first incorporated into living molecules. They are also without exception endowed with a device to eliminate nitrogen in the form of ammonia or urea (HUTCHINSON, 1954). This is perhaps a result of the evolution of biochemical pathways in the early reducing atmosphere.

Until the cell had achieved a highly organized and protected state, free atmospheric oxygen could not have been allowed, since its presence would have quickly oxidized the early precursors of life and absolutely prevented their evolution or even their existence (BERKNER and MARSHALL, 1965). The deleterious effect of oxygen on contemporary cell nuclei is also indicative that anoxygenic conditions may have prevailed during the early evolution of the cell (STERN, 1955). The existence of obligate anaerobes and the universality of anaerobic glycolytic pathways suggest that life arose in a reducing environment (HALDANE, 1928). As the environment changed from reducing to oxidizing in nature there may have been a selective advantage in the development of mechanisms for the removal of peroxides as a defense against their lethal effects. In addition a greater amount of energy was made available by aerobic metabolism (CALVIN, 1956).

4. Energies

The energies available for the synthesis of organic compounds under primitive earth conditions are ultraviolet light from the sun, electric discharges, ionizing radiation, and heat (MILLER and UREY, 1959) (Table II).

It is evident that sunlight is the principal source of energy. Solar radiation is emitted in all regions of the electromagnetic spectrum (Figure 2). A great deal of

TABLE II

Energy available for Synthesis of Organic Compounds

Source	Energy (cal cm^{-2}yr^{-1})
Ultraviolet light (2500 Å)	570
Electric discharges	4
Radioactivity	0.8
Volcanoes	0.13

this radiation, especially in the ultraviolet region, is shielded today by the ozone layer in the upper regions of the atmosphere. The question immediately arises as to the nature and intensity of the solar flux 4½ billion years ago and the extent to which this flux was available for organic synthesis.

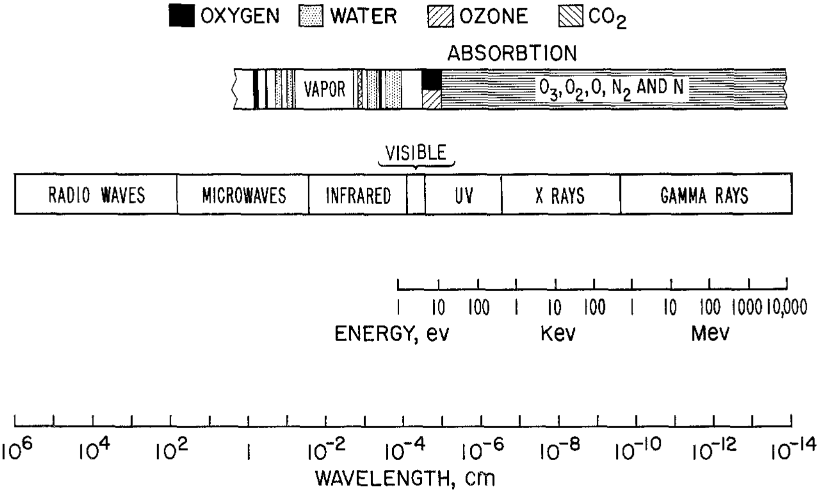


Fig. 2. Electromagnetic spectrum.

Since reaching its main sequence, the sun has been very stable (SAGAN, 1965). The temperature of the sun's surface 4½ billion years ago was almost as great as it is today. With this information the solar flux at different wavelengths has been calculated (Table III). These figures clearly demonstrate the dominant role that ultraviolet light must have played among primitive energy sources. The use of ultraviolet light should be considered as far more important than any of the other sources of energy that have been used in simulation experiments. However, on account of the intrinsic difficulties involved in using short-wavelength ultraviolet light this area has been somewhat neglected.

TABLE III

Solar Flux at Different Wavelengths

Å	Solar flux ($cm^{-2}sec^{-1}$)
2900	7×10^{14}
2800	4×10^{14}
2400	9×10^{13}
2000	2×10^{13}

The short-wavelength ultraviolet light must have had a profound effect on the primordial atmosphere. The absorption of methane extends to 1450 Å, water to 1650 Å, and ammonia to 2200 Å. The quantum yield for the photolysis of ammonia at 1470 Å is approximately 0.5. For water at 1650 Å it is 0.3; for methane, the quantum yield is equivalent to 0.35 to 0.5 (TERENIN, 1959). The dissociation of these molecules takes place in the shorter-wavelength region. However, the dissociation products may absorb at higher wavelengths. For example, in the case of methane, the CH_3 radical absorbs up to 2800 Å. The CH_2 and the CH radicals can also absorb in the same region (UREY, 1952b).

Next in importance as a source of energy are electric discharges such as lightning and corona discharges from pointed objects (SCHONLAND, 1953). These occur close to the earth's surface and therefore could have effectively transferred the reaction products to the primitive oceans. Since lightning can be easily simulated in the laboratory, many experiments have been performed using this form of energy.

The principal radioactive sources of ionizing radiation on the earth are potassium-40, uranium-238, uranium-235, and thorium-232 (SWALLOW, 1960). Potassium-40 seems to be quantitatively more important than the other three sources at present. Presumably, therefore, it must have been even more important in the past. The energy of potassium-40 is in the form of penetrating beta and gamma rays. In contrast almost 90% of the energy from uranium-238 and thorium-232 is emitted as alpha particles, which may not be penetrating enough to have a significant effect. Calculations show that the decay of potassium-40 in the earth's crust today gives rise to 3×10^{19} calories per year; 2.6×10^9 years ago this would have been 12×10^{19} calories per year. By comparison, this is only $\frac{1}{30}$th the amount of energy available from short-wave ultraviolet light below 2500 Å.

The distribution of energy is a matter of importance. Most of the radiation from radioactive sources is absorbed in solid matter. Since the earth's crust is about 30 km thick, this type of radiation could not have made a contribution to the synthesis of organic matter in the primitive atmosphere. However, local regions of high activity might have existed in the past. There may have been micro-environments where the catalytic action of the minerals present favored condensation reactions of the first molecules synthesized by ionizing radiation.

Heat from volcanoes was another form of energy which may have been effective (RITTMANN, 1962). The figure in Table I has been calculated on the assumption of 1 kM^3 of lava emission per year at 1000°C. It is reasonable to expect that volcanic activity was more prevalent on the juvenile earth than today. Besides volcanoes, heat could also have been available from hot springs around boiling mud pots.

Chemosynthesis by meteorite impact on planetary atmospheres has been suggested as a possible pathway for primordial synthesis of organic matter (HOCHSTIM, 1963). The reaction is probably the result of the intense heat generated momentarily in the wake of the shock wave following the impact. In a very preliminary experiment, these conditions were simulated by firing a ballistic missile into a mixture of methane, ammonia, and water vapor, some amino acids and ultraviolet light absorbing compounds were identified in the products (GILVARRY, 1963).

5. Amino Acids

The greatest amount of work in the field of pre-biological chemistry concerns the origin of amino acids. This may be due to the fact that amino acids are readily formed. A second reason may be that the emphasis on nucleic acid chemistry is only of comparatively recent origin. The protein molecule formerly occupied a pre-eminent position in biochemistry, and it is not surprising that efforts were made to look for its constituents. The development of chromatographic techniques facilitated the detection of minute traces of amino acids in complex mixtures.

Amino acids have been synthesized under simulated primitive earth conditions by the action of ultraviolet light, electric discharges, heat, and ionizing radiation. In some cases the starting materials were reactive intermediates. It is claimed that 14 of the 20 amino acids found in protein have been synthesized by abiogenic means. In one of the first experiments done simulating the synthesis of organic matter on the primitive earth, MILLER (1953) exposed a mixture of methane, ammonia, water, and hydrogen to an electric discharge from tesla coils for about a week. Several organic compounds were formed. Among these were the amino acids, glycine, alanine, β-alanine, aspartic acid, and glutamic acid. Of the initial carbon introduced as methane 2.1% had been converted into glycine.

ABELSON (1955–1956) studied the effect of electric discharges on 20 different mixtures of gases. Hydrogen, methane, carbon monoxide, carbon dioxide, ammonia, nitrogen, water, and oxygen. He found a number of amino acids including alanine, β-alanine, glycine, and sarcosine. The amino acids were formed only when the mixture was non-oxidizing. When the conditions were oxidizing, no amino acids were synthesized. These experiments indirectly confirm the hypothesis that non-oxidizing conditions were necessary to produce amino acids in significant quantities on the primitive earth. More recently, ABELSON (1966) has studied a variety of other mixtures containing carbon monoxide, nitrogen, and hydrogen. In every case, he was able to identify hydrogen cyanide as an intermediate. He suggests that hydrogen cyanide was the key intermediate in the synthesis of the amino acids, glycine, serine, alanine, and aspartic acid.

PAVLOVSKAYA and PASYNSKII (1959) exposed methane, ammonia, water and carbon monoxide to an electric discharge. The products they were able to identify were glycine, α-alanine, β-alanine, α-aminobutyric acid, glutamic acid, aspartic acid, and lysine. It appears that the depletion of hydrogen from the system enhances the synthesis of the amino acids. This is understandable since the methyl radicals will tend to react with hydrogen atoms and revert to methane, but in a system where the hydrogen is removed from the scene of reaction the forward reaction leading to more complex molecules will be favored. On the primitive earth, the hydrogen escaped into the atmosphere; whereas, in the laboratory the hydrogen is retained in the apparatus.

HEYNS *et al.* (1957) worked with various mixtures of gases. They, too, found that amino acids were formed when the conditions were non-oxidizing. When hydrogen sulfide was introduced they obtained ammonium thiocyanate, thiourea, and thioacetamide, but found no sulfur-containing amino acids. In the experiments in which methane was used, the amino acids formed contained exclusively two to four carbon atoms. However, when ORO (1963b) partially replaced methane with ethane, he was able to identify proline, valine, and leucine in addition to the glycine, alanine, aspartic acid, and asparagine that had been already synthesized.

GROSSENBACHER and KNIGHT (1965) sparked a 12-liter flask containing half an atmosphere of methane and ammonia over ammonium hydroxide for a period from 12 to 48 hours. At the end of this time they analyzed the products by an amino-acid analyzer and found aspartic acid, threonine, serine, glutamic acid, glycine, alanine, isoleucene, leucene, and lysine. In recent experiments done by PONNAMPERUMA and FLORES (1966), the exposure of a mixture of methane, ammonia and water to an electric discharge for 24 hours resulted in the conversion of over 95% of the methane into organic compounds; hydrogen cyanide accounted for 18% of the product. Several amino acids were identified: glycine, alanine, threonine, aspartic acid, valine, glutamic acid, leucine, isoleucine, and phenylalanine. These identifications were made after the hydrolysis of the end products. The retention time on an ion-exchange column was substantiated by gas chromatographic analysis using the N-trifluoroacetyl derivatives of the amino acids (GEHRKE *et al.*, 1965). PONNAMPERUMA and WOELLER (1967) exposed equimolar mixtures of methane and ammonia to an electric discharge in the form of an arc. Analysis of the volatile products by gas chromatography and mass spectrometry revealed the presence of several aliphatic nitriles, α-aminoacetonitrile, α-aminopropionitrile, and the C-methyl and N-methyl derivatives of the α-aminoacetonitrile were all found in the volatile fraction.

One of the first photochemical experiments to determine whether amino acids could be synthesized by the action of ultraviolet light was performed by ABELSON (1955–1956). A solution of ammonium formate in the presence of ammonium hydroxide, sodium cyanide and ferrous sulfate was irradiated at a wavelength of 2536 Å. Glycinonitrile was formed, which on hydrolysis gave rise to glycine. Ten % of the formate was converted into glycine. BAHADUR (1954) irradiated formaldehyde and certain inorganic salts such as potassium nitrate and ferric chloride with visible light. Aspartic acid, serine, and lysine were identified. This experiment may not be relevant since the use of nitrate is not consistent with the reducing conditions of the primitive earth. GROTH and VON WEYSSENHOFF (1960) exposed methane, ethane, ammonia, and water to radiation of 4070 Å and 2196 Å lines. Glycine, alanine, and aminobutyric acid were identified. At the first International Symposium on the Origin of Life, held in Moscow in 1957, TERENIN (1959) reported that when he irradiated methane, ammonia, and water in the Schumann region, he found alanine as well as other compounds.

REID (1959) described the use of the 1848 Å wavelength for the irradiation of a mixture of formaldehyde, ammonia, and carbon dioxide in the presence of some inorganic salts. Glycine and alanine were two amino acids that were formed. PAVLOVSKAYA and PASYNSKII (1959) claimed that when formaldehyde was irradiated in the presence of ammonium nitrate or ammonium chloride with a high-pressure mercury lamp, serine, glycine, glutamic acid, alanine, and valine were formed. More recently, DODONOVA and SIDOROVA (1961) used methane, ammonia, water, and carbon monoxide in the region between 1450 Å to 1800 Å. Several amino acids, including glycine, alanine, valine, and leucine were identified.

ABELSON (1966) had reported that hydrogen cyanide was formed when he exposed a mixture containing carbon monoxide and various proportions of nitrogen or hydrogen to an electric discharge. When the hydrogen cyanide in solution was exposed to a mercury lamp, several amino acids were identified; among these were glycine, serine, alanine, and aspartic acid.

There are numerous investigations of the effect of ionizing radiation on primitive mixtures of gases bearing on the problem of the origin of life (PONNAMPERUMA, 1966). One of the first experiments done with the use of X-rays in the search for amino acids was by DOSE and RAJEWSKY (1957). They irradiated a mixture of ammonia, water, methane, hydrogen, carbon dioxide, and nitrogen with X-rays and gamma rays and demonstrated by electrophoretic separation that some amino acids were produced. These were not identified individually but as a class.

HASSELSTROM *et al.* (1957) irradiated a solution of ammonium acetate with β-particles and identified glycine, aspartic acid, and diaminosuccinic acid in the products. PALM and CALVIN (1962) exposed a mixture of methane, ammonia, and water to 5 meV electrons from a linear accelerator, and found alanine and glycine among the end products. ORO (1963c), using the identical equipment, and in some cases replacing the ammonia by nitrogen, and the methane by ethane, reported the formation of several amino acids which were not individually identified. Recently, Dose and Ponnamperuma have used N-acetyl glycine as a starting material, and by exposing these to gamma rays were able to identify a whole range of amino acids (DOSE and PONNAMPERUMA, 1967).

In some of his early experiments, FOX *et al.* (1954) heated ammonium fumarate and ammonium malate and identified aspartic acid and alanine in the products. Subsequently, Fox and Harada used glucose, urea, α-hydroxy glutaric acid, and ammonia as starting materials. By the action of heat they obtained glycine, glutamic acid, aspartic acid, and alanine (FOX and HARADA, 1960). In recent experiments, HARADA and FOX (1964) studied the effect of heat on a mixture of methane and ammonia. Methane was bubbled through a solution of concentrated ammonium hydroxide and passed over quartz sand or alumina in a glass reaction tube which was heated from 900 to 1000 °C. The end products were absorbed in aqueous ammonia. When these were hydrolyzed and examined by the amino-acid analyzer, Fox claimed that 14 of the amino acids commonly found in protein could be synthesized: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucene, leucene, tyrosine, and phenylalanine. The yields in these experiments were extremely small with the exception of glycine and alanine.

A similar experiment was performed by ORO (1965). After passing a mixture of methane, ammonia and water through a hot tube at approximately 1300 °K, a number of amino acids were identified. Among these were aspartic acid, threonine, serine, glutamic acid, glycine, alanine, alloisoleucine, isoleucine, leucine, tyrosine, phenylalanine, and β-alanine. Among these acids, glycine and alanine were obtained in greatest yield.

LOWE *et al.* (1963) studied the action of heat on a mixture of hydrogen cyanide, ammonium hydroxide, and water, and identified aspartic acid, threonine, serine, glutamic acid, glycine, alanine, isoleucine, leucine, β-alanine, α-β-diamino-propionic acid, α-aminobutyric acid.

In some experiments performed by ORO *et al.* (1959), using an aqueous solution of formaldehyde and hydroxylamine hydrochloride at room temperature under slightly acidic conditions, large amounts of glycine and smaller amounts of alanine, β-alanine, serine, threonine, and aspartic acid were formed. When an aqueous mixture of formaldehyde and hydrazine were refluxed together, glycine, valine, and lysine appeared to be formed. In a further experiment at ambient temperatures glycine, alanine, and aspartic acid were spontaneously formed from hydrogen cyanide, ammonia, and water (ORO and KAMAT, 1961).

None of these experiments have demonstrated that the sulfur-containing amino acids could be formed. However, when CHOUGHULEY and LEMMON (1966), irradiated a mixture of methane, ammonia, water, and hydrogen sulfide, they were able to demonstrate the presence of cysteic acid, taurine, and cysteine in the end products.

The identification of so many amino acids in several experiments simulating primitive might lead one to believe that the problem of the origin of amino acids has been solved. However, one must bear in mind that in most of these identifications precise analysis still needs to be done. Unlike the analysis of a protein hydrolysate of biological origin in abiogenic experiments, a large number of very similar compounds are formed. The position on a paper chromatogram, the retention time, or elution time on ion-exchange columns, may not be sufficient evidence for an identification. In some cases amino acids which do not occur naturally may be synthesized. These may have the same *Rf* values as natural ones. Further analysis is required to establish the true nature and structure of these compounds. Except in one case, where the identification has been corroborated by ion-exchange chromatography, gas chromatography and mass spectrometry (PONNAMPERUMA and FLORES, 1966; PONNAMPERUMA and WOELLER, 1967), in most other instances the identification has been based on either paper chromatography or ion-exchange chromatography alone.

Little is known about mechanisms of these syntheses. Apart from MILLER's (1957b) experiments, in which the possibility of a Strecker synthesis was suggested, no attempt has been made to find out how amino acids have arisen. Miller suggested two alternative proposals. First, the aldehydes and hydrogen cyanide were synthesized in the gas phase by the spark. These aldehydes and the hydrogen cyanide react in the aqueous phase of the system to give amino and hydroxynitriles, which were, in turn, hydrolyzed to amino and hydroxy acids. The second suggestion made was that the amino and hydroxy acids were synthesized in the gas phase from the ions and radicals that are produced in the electric discharge. Miller's work has shown that most of the amino acids were synthesized according to the first hypothesis, since the rate of production of aldehydes and hydrogen cyanide by the spark, and the rate of hydrolysis of the amino-nitriles were sufficient to account for the total yield of amino acids. Miller's experiments on the mechanism of electric discharge synthesis of amino acids indicate that a special set of conditions is not required to obtain amino acids. A process or combination of processes that yielded both aldehyde and hydrogen cyanide could have contributed to the amount of α-amino acids in the oceans of the primitive earth (MILLER and UREY, 1959) (Figure 3).

$$\mathrm{RCHO + NH_3 + HCN \rightleftharpoons RCH(NH_2)CN + H_2O}$$

$$\mathrm{RCH(NH_2)CN + 2H_2O \rightarrow RCH(NH_2)COOH + NH_3}$$

$$\mathrm{RCHO + HCN \rightleftharpoons RCH(OH)CN}$$

$$\mathrm{RCH(OH)CN + 2H_2O \longrightarrow RCH(OH)COOH + NH_3}$$

Fig. 3. Strecker synthesis of amino acids.

The experiments with methane and ammonia in the absence of water to give rise to nitriles has shown that an alternative mechanism may also be valid (PONNAMPERUMA and WOELLER, 1967). Thus, if α-aminonitriles are preferentially synthesized by the reaction between methane and ammonia, and these nitriles react with water, hydrolysis can take place to give rise to amino acids. In this case the amino acids are being formed in the absence of aldehydes, SANCHEZ *et al.* (1966b) have also suggested a possible synthetic pathway for aspartic acid through cyanoacetylene.

6. Purines and Pyrimidines

The first synthesis of purines under simulated primitive-earth conditions was carried out by ORO (1960), who demonstrated that adenine could be synthesized from a concentrated solution of ammonium cyanide. The overall reaction can be depicted as 5 molecules of hydrogen cyanide in the presence of ammonia giving rise to adenine. This synthesis has been confirmed by LOWE *et al.* (1963). ORO's (1961b) detailed study has shown that 4-aminoimidazole 5-carboximide and formamidine were the probable intermediates in this reaction (Figure 4). Subsequently, Oro was able to synthesize guanine and xanthine by heating aqueous solutions of amino-imidazole carboximide between 100 and 140°. Yields of 1.5% were obtained for both purines. It is possible that this is a way in which the purines were synthesized on the primitive earth, but the concentrations used by Oro were drastically high for prebiotic conditions. If genuine prebiotic conditions can be satisfied, such as the use of lower concentrations, these reactions will be of great value in understanding the origin of purines on the prebiotic earth.

Adenine was also synthesized by PONNAMPERUMA *et al.* (1963) by the electron irradiation of methane, ammonia, and water. Since hydrogen cyanide has been detected in these reactions, the possible pathway may be the same, namely, the base catalyzed reaction of hydrogen cyanide. In the electron irradiation of methane, ammonia, and water, the yield was about 0.01% of the starting methane. This is considerable and is realistic under primitive-earth conditions. The interesting observation was made that the production of adenine was enhanced by the absence of hydrogen. This is not surprising, since methane carbon must be oxidized in order to appear finally in the purines. In any event, the high concentration of organic matter on the prebiotic earth probably arose when most of the hydrogen had escaped from this atmosphere. Although many efforts have been made, purines and pyrimidines have not been definitely identified in the end products of electric discharge experiments.

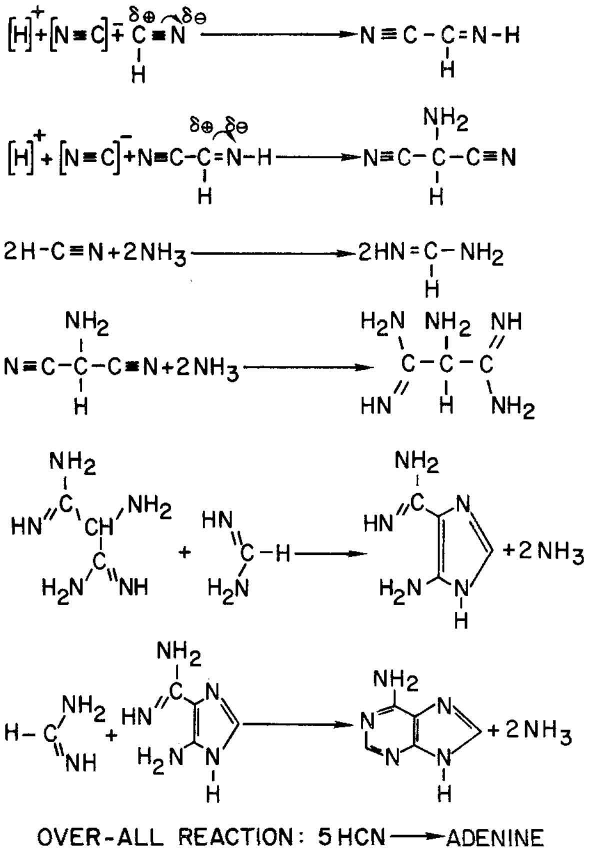


Fig. 4. Mechanism for formation of adenine from HCN.

Since a large amount of hydrogen cyanide is formed, it is difficult to understand why the purines could be absent in such experiments.

In further experiments, it was shown that when a dilute solution of hydrogen cyanide was exposed to ultraviolet light, both adenine and guanine were formed. The yields of adenine and guanine were small, but more adenine was formed than guanine. Urea was found in large yield (PONNAMPERUMA, 1965).

Recent investigations by SANCHEZ *et al.* (1966a) have shown that aminomalononitrile is an important intermediate in the synthesis of adenine. The monoaminomalononitrile is transformed into 4-aminoimidazole-5-carboximide, either by direct reaction with formamidine or, what is most interesting for our studies, the photochemical rearrangement of the hydrogen cyanide tetramer. By hydrolysis, it gives the 4-aminoimidazole-5-carboximide, which can condense under milder conditions with aqueous cyanide, formamidine, or cyanogen to produce a variety of purines, adenine, hypoxanthine, diaminopurine, and guanine (Figure 5).

Very little work has been done on the synthesis of pyrimidines. FOX and HARADA (1961) had shown that by heating malic acid and urea, uracil could be formed. While urea is readily formed in primitive earth experiments, there is no evidence for the presence of malic acid. More recently, SANCHEZ *et al.* (1966b) have shown that cynoacetylene is a major nitrogen-containing product of the action of an electric

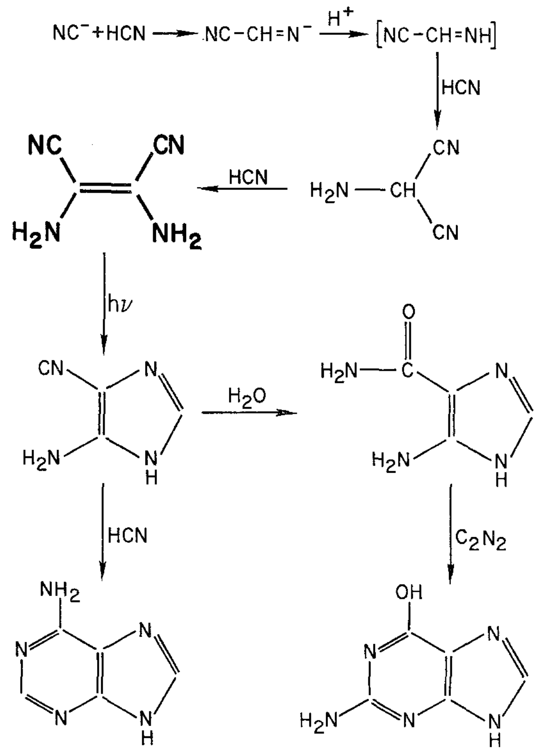


Fig. 5. Adenine and guanine from HCN tetramer. (After ORGEL, 1966.)

discharge on a mixture of methane and nitrogen. A number of pyrimidine syntheses are possible starting with cyanoacetylene. By fusing cyanoacetylene with urea, cytosine has been obtained. Similarly, uracil has been prepared from propiolamide.

When an aqueous solution containing 1.0 M potassium cyanate and 1.0 M cyanoacetylene was heated to 100°C for one day, a 5°/₀ yield of cytosine was obtained. Very significant is the fact that when the same mixture was allowed to stand at room temperature for seven days, cytosine was obtained in 1 °/₀ yield.

ORO (1963a) had theorized that the formation of pyrimidines could take place from C-3 molecular species found in comets. One of these C-3 derivatives is malonamidesemialdimine or its isomer β-aminoacrylimide, which by condensation urea could be expected to yield uracil. He further considered acrylonitrile, β-aminopropionitrile, and β-aminopropionimide as possible intermediates. When each of these compounds was allowed to react with urea in aqueous ammonia systems, the formation of small amounts of uracil was consistently observed. In theory, the three pyrimidines found in nucleic acids could conceivably have been formed in aqueous systems under possible primitive-earth conditions by the mechanisms described above. The carbon suboxide, C_3O_2, which is found in cosmic bodies may also be a suitable pathway for pyrimidine synthesis (ORO, 1961a).

7. Monosaccharides

Despite the advances which have been made in the primordial chemistry of amino acids, the origin of an equally important set of biological building blocks, the monosaccharides, has remained in a state of uncertainty.

As early as 1861, BUTERLOW (1861) showed that aqueous formaldehyde will undergo condensation to a mixture of sugars in the presence of alkali. Shortly thereafter, LOEW (1886, 1887, 1889) succeeded in isolating a mixture of optically inactive hexoses from this alkaline-induced condensation mixture. In addition to hexoses, the lower molecular-weight monosaccharides are also formed in this alkaline induced condensation of formaldehyde. Glycolaldehyde has been isolated and is apparently the product of the condensation of two molecules of formaldehyde (EULER and EULER, 1906) (Figure 6).

Other condensation products which have been demonstrated include glyceraldehyde, dihydroxyacetone, erythrose, and various pentoses (ORTHNER and GERISCH, 1933).

Loew's experiments indicated that this alkaline-induced condensation of formaldehyde proceeds best in the presence of alkaline earth hydroxides and in the presence of weakly basic hydroxides of lead and tin. The condensation is also characterized by what has been termed a 'lag-phase'. This delay in the time of reaction can be eliminated by the addition of glycolaldehyde, dihydroxyacetone, or higher sugars (LANGENBECK, 1942; MAYER and JASCHKE, 1960a, 1960b). It has also been observed that condensation of formaldehyde to a sugar mixture does not take place in the presence of tetramethylammonium hydroxide, a strong base, unless the salt of an alkaline earth metal is introduced (AKERLOF and MITCHELL, 1963).

$$2\,CH_2O \longrightarrow CH_2OH \cdot CHO$$

$$CH_2OH \cdot CHO + CH_2O \longrightarrow CH_2OH \cdot CO \cdot CH_2OH$$

$$CH_2OH \cdot CHO + CH_2OH \cdot CO \cdot CH_2OH \longrightarrow CH_2OH \cdot CHOH \cdot CHOH \cdot CO \cdot CH_2OH$$

$$\begin{aligned} 2\,CH_2OH \cdot CHO \longrightarrow\ & CH_2OH \cdot CHOH \cdot CHOH \cdot CHO \\ \text{OR}\ & CH_2OH \cdot CO \cdot CHOH \cdot CH_2OH \end{aligned}$$

$$2\,C_4 — \left[C_8\right]? \longrightarrow C_3 + C_5$$

Fig. 6. Synthesis of sugars from formaldehyde.

The condensation of two molecules of formaldehyde to form glycolaldehyde thus appears to be a slow process. However, once this primary product is formed, higher sugars are rapidly produced via reversible aldol condensation and enolization reactions. Since aldol condensations are known to be base catalyzed and not dependent on the presence of any particular cation, the catalytic activity of divalent metal cations is probably involved in the formation of glycolaldehyde. The mechanism of formation of glycolaldehyde from formaldehyde has not yet been elucidated nor carefully scrutinized, although it has been discussed briefly by WANZLICK (1962) and BRESLOW (1959).

The proposal by MILLER (1957a) that the amino acids in the discharge experiments could have arisen via a Strecker synthesis focussed attention on formaldehyde as a possible precursor of sugars in prebiological chemistry. Formaldehyde has been reported in the products obtained when methane, ammonia, and water mixtures were subjected to an electric discharge (MILLER and UREY, 1959), β-irradiation (PALM and CALVIN, 1962), or γ-irradiation (PONNAMPERUMA and FLORES, 1966). Although sugars have not yet been detected in experiments using methane, ammonia, and water as starting materials, the ultraviolet irradiation of formaldehyde can lead to ribose and deoxyribose as well as other sugars (PONNAMPERUMA, 1965). ORO and COX (1962a) have investigated the formation of 2-deoxyribose from glyceraldehyde and acetaldehyde and from formaldehyde and acetaldehyde. They reported that 2-deoxyribose and its isomer, 2-deoxyxylose, were formed in yields of about 5% from glyceraldehyde and acetaldehyde in aqueous systems. The reaction is catalyzed by divalent metal oxides or ammonia. 2-deoxyribose was also identified as a product when the aqueous starting mixture was composed of formaldehyde, acetaldehyde, and calcium oxide.

Several difficulties are encountered in proposing the use of formaldehyde as the precursor of monosaccharides. HOROWITZ and MILLER (1962) have pointed out that the high concentrations of formaldehyde used in some of the experiments were unrealistic in primitive-earth experiments. Objections have also been made to the use of very basic solutions. ABELSON (1966) maintains that the concentration of free ammonia in the seas and atmosphere was never very large and that a strongly alkaline ocean never existed. Formaldehyde polymerizes very readily to give paraformaldehyde and may therefore be removed from circulation.

In an effort to circumvent some of these objections, GABEL and PONNAMPERUMA (1967) have recently used a simulated hydrothermal spring as a reaction medium. Aqueous solutions of formaldehyde of various concentrations were refluxed over kaolinite. The products were separated into trioses, tetroses, pentoses and hexoses. At a formaldehyde concentration of 0.5 M only trioses, tetroses and pentoses were formed. When the concentration of formaldehyde was reduced to 10^{-2} M, hexoses were also formed. Ribose was identified in the end products. The simulation of the hydrothermal spring, although possibly occurring only in microenvironments on the primitive earth eliminates the need for postulating a basic primitive ocean, and would have provided an opportunity for the breakdown of the paraformaldehyde. The photochemical synthesis of sugars, however, needs further investigation and may provide another reasonable pathway for sugar synthesis.

8. Nucleosides and Nucleotides

The satisfactory synthesis of nucleic acid bases and sugars under primitive-earth conditions have led some investigators to explore the abiogenic formation of nucleosides and nucleotides.

When ribose, adenine, and phosphate in dilute aqueous solution 10^{-3} molar was exposed to ultraviolet light, adenosine was formed (PONNAMPERUMA *et al.*, 1963). The phosphate appears to have acted as a catalyst in this reaction. The mechanism has not been investigated. However, it may appear that a sugar-1-phosphate is formed and the phosphate is then replaced by the purine. The synthesis of deoxyadenosine has also been in experiments where adenine and deoxyribose were exposed to ultraviolet light in the presence of hydrogen cyanide. If it is mediated by hydrogen cyanide it may be a plausible scheme for the appearance of nucleosides under primitive-earth conditions (PONNAMPERUMA and KIRK, 1964). When adenine and the deoxyribose in the presence of cyanide was allowed to stand at room temperature deoxyadenosine was synthesized in 1% yield. In the presence of phosphate the yield was increased to 5%. When ultraviolet light was used in the presence of cyanide, a 7% yield was observed. This reaction, however, did not take place when the other bases were used.

It has been suggested that the earth's primordial reducing atmosphere was at least slightly transparent between 2400 Å and 2900 Å, and that the photo activation of ultraviolet light-absorbing purines and pyrimidines was a possible step in the formation of nucleosides and nucleotides (SAGAN, 1961). To test this hypothesis, four experiments were performed in which adenine, adenosine, adenosine monophosphate, and adenosine diphosphate, respectively, served as the starting material (PONNAMPERUMA *et al.*, 1963). The conversion of adenine to adenosine, adenosine to adenosine monophosphate, the monophosphate to diphosphate, and the diphosphate to triphosphate, was established. Adenosine was not produced in a detectable amount in the absence of a phosphorous compound. While adenosine was produced in the presence of both phosphoric acid and ethyl metaphosphate, the nucleoside phosphate were detected only with the use of ethyl metaphosphate (SCHRAMM *et al.*, 1962). Although it is unlikely that ethyl metaphosphate could have been a common source of phosphorus on the primitive earth, the result clearly established that the process could occur abiologically. Besides, it is possible that another more abundant phosphorous compound, could have substituted for ethyl metaphosphate.

MILLER and PARRIS (1964) have worked with apatite since it is by far the most abundant form of phosphorus on the earth. Using hydroxyapatite and cyanate salts they have produced pyrophosphate. Apatite is insoluble, as is calcium pyrophosphate, so that this synthesis must therefore take place on the surface of the apatite. The yield of pyrophosphate was maximum at a *ph* of $6\frac{1}{2}$, in that 27% of the cyanate salt added to the system was converted. NEUMAN and NEUMAN (1964) have also outlined the possible role of apatite in the condensation reactions leading to nucleosides and nucleotides.

In the synthesis of nucleotides, the heterogeneous reactions which may have taken place on a dried-up ocean bed have also been examined. In simulating these conditions, an intimate mixture of the nucleosides was heated with an organic phosphate. Several phosphates were used in this reaction. Among them were disodium monohydrogen, trisodium, sodium ammonium monohydrogen, ammonium dihydrogen, diammonium monohydrogen, monocalcium, and tricalcium monophosphates and phosphoric acid (PONNAMPERUMA and MACK, 1965b). When the intimate mixture of the nucleoside and the phosphate was heated, phosphorylation took place. The mononucleotides were identified in the end products. The best yields in this reaction were obtained at about 160°. However, a small yield was obtained at temperatures as low as 50°. These experiments simulate an environment with a relative absence of water. However, water is not incompatible with this reaction and does not hinder it unless present in large excess. The conditions under which this reaction proceeds may therefore be described as hypo-hydrous. Amongst the monophosphates that were formed there were equal amounts of the 2′, 3′, 5′, and cyclic monophosphates. It is possible that the presence of certain amino acids might direct the synthesis preferentially to yield a particular isomer.

WAENHELDT and FOX (1967) phosphorylated the nucleosides, adenosine, cytidine, guanosine, uridine, deoxycytidine, and thymidine with polyphosphoric acid at temperatures ranging from 0 to 22 °C in yields ranging from 25% to 45%. Deoxyadenosine and deoxyguanosine are not phosphorylated under these conditions. The yields in these experiments are impressive, but the use of concentrated polyphosphoric acid precludes these experiments from being considered as authentic models of prebiological reactions.

9. Hydrocarbons and Fatty Acids

Although hydrocarbons have been produced in large quantities by the action of various forms of energy on methane, veiy few detailed studies are available in this field. In experiments performed to compare the effects of different kinds of discharges on methane, very interesting results have been obtained (PONNAMPERUMA and WOELLER, 1964). In the high-intensity arc, the product was a clear yellow fluid, which gave chromatograms of well-spaced peaks. On the other hand the semi-corona yielded a colorless distillate which was poorly resolved by gas chromatography. In the arc discharge, benzene was the most abundant product, and next in order of magnitude was toluene. In contrast, benzene and toluene were virtually absent from the products of the semi-corona discharge. Some of the prominent peaks have been identified by mass spectrometry, as 2,2-dimethyl butane, 2-methypentane, 3-methypentane and 2,4-dimethylhexane and 3,4-dimethylhexane.

When the compounds beyond C_9 were examined, it was found that in the semi-corona discharge, the aliphatic fraction consisted of an irresolvable mixture with a maximum around C_{17}. Mass spectrometric and NMR data indicate that these are cyclohexyl derivatives. The use of a molecular sieve indicated that there were no normals or isoprenoid type molecules formed in this system. It is interesting to note that some hydrocarbon deposits have been found which are similar to the spark discharge hydrocarbons (PONNAMPERUMA and PERING, 1966, 1967). A mechanism for obtaining straight-chain hydrocarbons and fatty acids has been suggested by WILSON (1962). This method involves crowding the reaction chains of fatty acids onto a surface so that only the ends are available for reaction, thus preventing branching.

When a mixture of methane and water was exposed to a semi-corona discharge and the products analyzed by gas liquid chromatography and mass spectrometry, several monocarboxylic acids from C_2 to C_{12} were detected. Those above C_6 were characterized as branched carbon chains (ALLEN and PONNAMPERUMA, 1967). One area of great interest in the study of chemical evolution is the formation of the normal fatty acids containing 12 or more carbon atoms which are constituent fatty acids of the complex lipids. Although the reaction conditions employed in the semi-corona discharge of methane and water would have favored the production of branched chains owing to the stability of secondary carbon radicals, it is surprising that the formation of only as few as 11 of the possible C_1 to C_{12} isomers were formed. And by contrast to this, highly isomeric mixtures of hydrocarbon are produced when methane alone is subjected to a semi-corona discharge. The present evidence shows that certain C_8 to C_{12} acids which are not excessively branched could have accumulated by the action of a semi-corona discharge passing through methane over an aqueous surface.

10. Porphyrins

It is generally assumed that organisms could not have survived the transition from the early reducing atmosphere to the oxidizing atmosphere of today without porphyrins

(GAFFRON, 1955; PONNAMPERUMA, 1966). Presumably pyrroles were abiologically synthesized early in the course of chemical evolutions. CHICHIBABIN (1915) showed that when acetylene and ammonia are passed through a heated tube, pyrroles are formed. Earlier, MEYER (1913) had demonstrated that acetylene and hydrogen cyanide gives rise to pyrroles. SZUTKA *et al.* (1959) have made several attempts to synthesize porphyrin-like substances under presumed primitive-earth conditions. In some experiments, benzaldehyde was heated with pyrroles to produce to porphyrin-like material (ROTHEMUND, 1936). In other experiments, he has been able to obtain the tetraphenylporphyrin by the radiation of a mixture of benzaldehyde and pyrroles by gamma rays (SZUTKA, 1959). Similar results have been found by using electric discharges (SZUTKA, 1965). More recently the use of δ-amino laevulinic acid has been condensed to form pyrrolic compounds (SZUTKA, 1966).

11. Polypeptides

In an effort to understand how protein-like molecules first appeared on earth, many experiments have been performed, in which two or more amino acids were condensed to produce dipeptides or polypeptides. Since amino acids were among the first important compounds synthesized in experiments designed to study the problem of the origin of life, it is to be expected that greater strides have been made in the study of their polymerization than in the formation of any other biologically important polymer. It must be borne in mind that organic chemists have long been interested in the synthesis of polypeptides, and a large body of literature is available on peptide synthesis (ZERVAS, 1966). However, most of these methods require elaborate chemical procedures involving protective groups and non-aqueous conditions. The condensation of amino acids in chemical evolution, on the other hand, should have been achieved in simple and straightforward reactions.

Fox was a pioneer in initiating studies on the thermal polymerization of amino acids (FOX and HARADA, 1958). His efforts are significant since it has been generally believed that even mild heating of an amino acid results in its degradation. The heating of amino acids ordinarily results in products such as deketopiperazines and other pyrolytic matter. In the presence of large proportions of aspartic acid or glutamic acid, however, copolymeric peptides are formed. Anhydropolymers can be obtained consisting of all of the 18 amino acids usually present in proteins. The polymerization of these amino acids is generally done at a temperature of 180–200 °C. In the presence of polyphosphoric acid it can be effected at temperatures below 100 °C. These random polymers have been described as proteinoids. The molecular weights increased from 3600 in a proteinoid made at 160 °C to 8600 in one made at 190 °C. The maximum molecular weight which has been obtained was 80000.

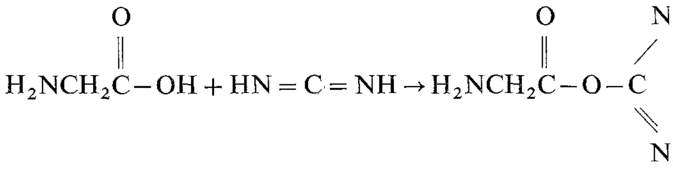
The presence of peptide bonds has been inferred from several observations. The polymers are ninhydrin-negative but biuret-positive. They are hydrolyzed to amino acids by concentrated hydrochloric acid. They are attacked by various proteolytic enzymes (FOX, 1960). The chromatographic patterns of the mixed proteolytic fragments are specific for the proteases used. Additional evidence for peptide bonds is also available from infrared absorption maxima at 3300, 3080, 1650 and 1150 cm^{-1} (Fox and Harada, 1960).

It has been argued that the heat available from volcanoes may not have been sufficient to play a significant role in the primordial synthesis of polypeptides (Horowitz and Miller, 1963). However, thermal polymerization of amino acids through vulcanism could have been an important source of peptides and polypeptides in micro-environments where the suitable temperatures could have been maintained.

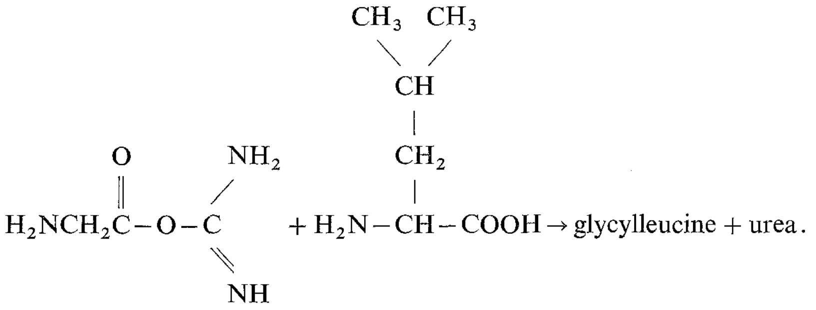
Using heat as a source of energy, Oro and Guidry (1961) reported the interesting observation that glycinamide or glycine could be thermally polymerized in concentrated ammonia. Although these experiments demonstrated that anhydrous conditions are not a necessity for thermal polymerization of amino acids, it is doubtful whether large concentrations of ammonia existed on the primitive earth. These polymerization reactions may be significant for chemical evolution on the larger planets where great concentrations of ammonia and water are known to exist.

Several other investigators have reported the synthesis of peptides directly from amino acids. Glycylleucine and leucylglycine were formed from a dilute aqueous solution of glycine and leucine when subjected to ultraviolet irradiation in the presence of cyanamide (Ponnamperuma and Peterson, 1965). Exposure of aliphatic acids to far ultraviolet irradiation is known to promote homolytic scission of the C-H bonds on α-methylene carbon atoms followed by dimerization of the resulting radicals to substituted succinic acids (Pfordte and Leuschner, 1959). Irradiation of ammonia below 2000 Å produces NH and NH_2 radicals (Schnepp and Dressler, 1960). Since both di- and tripeptides were formed, the role played by the ultraviolet radiation may have been to increase the concentration of carbodiimide, a tautomer of cyanamide which can act as a condensing agent for amino acids. Cyanamide and dicyanamide are known to be formed upon ultraviolet irradiation of dilute cyanide solutions and by the β-irradiation of a mixture of methane, ammonia, and water (Schimpl *et al.*, 1965):

$$H_2N-CN \rightarrow H\dot{N}-C\equiv N(\leftrightarrow)\,HN=C=\overset{H}{\dot{N}} \rightarrow HN=C=NH \qquad (1)$$

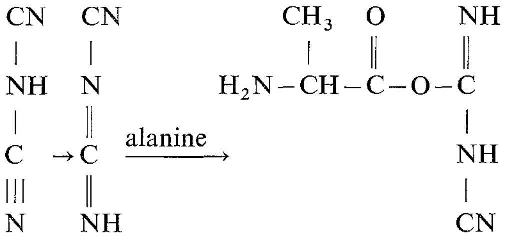


$$H_2NCH_2\overset{\overset{O}{\|}}{C}-OH + HN=C=NH \rightarrow H_2NCH_2\overset{\overset{O}{\|}}{C}-O-C\begin{array}{l}\diagup NH_2\\ \diagdown\!\!\diagdown NH\end{array} \qquad (2)$$



$$H_2NCH_2\overset{\overset{\displaystyle O}{\|}}{C}-O-C\begin{smallmatrix}\diagup NH_2\\ \diagdown\!\!\diagdown NH\end{smallmatrix} + H_2N-\underset{}{\overset{\overset{\overset{\displaystyle CH_3\;\;CH_3}{\diagdown\;\diagup}}{\overset{\displaystyle CH}{\overset{|}{CH_2}}}}{\overset{|}{CH}}}-COOH \rightarrow \text{glycylleucine} + \text{urea}. \quad (3)$$

In a similar experiment STEINMAN *et al.* (1965a) reported the formation of the di- and tripeptides of alanine when a dilute solution of the amino acid was treated with dicyanamide. The reaction, although favored by acidic conditions, also proceeded to a measurable extent at a neutral pH. STEINMAN *et al.* (1965b) proposed a mechanism similar to the condensation step above:



$$NC-NH-C\equiv N \rightarrow NC-N=C=NH \xrightarrow{\text{alanine}} H_2N-\overset{\overset{\displaystyle CH_3}{|}}{CH}-\overset{\overset{\displaystyle O}{\|}}{C}-O-\overset{\overset{\displaystyle NH}{\|}}{C}-NH-CN$$

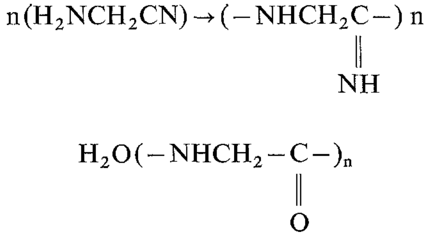
There are two other reports of the direct polymerization of amino acids. OTOZAI *et al.* (1954) claim to have synthesized polyglycine by subjecting a 10% glycine solution to an electric discharge. However, the polymeric product was not well characterized. BARKER *et al.* (1959) found polymeric material after subjecting a 0.1% solution of glycine to γ-irradiation. These authors do not claim to have made a polypeptide, since their evidence points only to the production of a radical zwitterion formed by the scission of a C-H bond on the α-methylene carbon atom.

DOSE and PONNAMPERUMA (1967) used N-acetyl glycine as starting material in some experiments and by the use of both γ-rays and β-rays demonstrated that a number of peptides could be formed. There are some reports to indicate that small peptides could be formed in experiments starting with methane, ammonia, and water (PONNAMPERUMA and FLORES, 1966). In one of these experiments, 9 amino acids were formed. Preliminary evidence indicates that at least 5 were linked up together in the mixture of organic compounds obtained as the product. LOWE *et al.* (1963) have also reported the presence of peptides in the complex mixture formed by heating ammonium cyanide.

The formation of peptides from a mixture of methane, ammonia, and water was first reported by ELLENBOGEN (1958). He subjected his mixture to ultraviolet irradiation in the presence of suspended particles of inorganic minerals. According to Ellenbogen a large amount of organic material including polypeptides could have been synthesized during the cold accretion process which is believed to have occurred during the formation of the primordial earth. The existence of some high molecular-weight organic material is postulated even before the crust of the planet was actually formed.

Since the thermodynamic barrier to spontaneous amino acid polymerization is not easily overcome (Blum, 1962) a sequence of events leading to polypeptide formation has been postulated by Kliss and Matthews (1962), in which hydrogen cyanide dimerizes to aminocyanomethylene, $H_2N-\dot{C}=C=\dot{N}$, a 1,3-biradical. Additional polymerization would then lead to polymeric peptide precursors, which would be converted to polypeptides by water. To test this hypothesis Matthews and Moser (1966) subjected methane and ammonia to an electric discharge. The residue, after being heated with dilute acid in order to hydrolyze the imino groups, was characterized as a polypeptide. More vigorous hydrolysis resulted in the formation of glycine as well as seven other amino acids.

There is an alternative to the Kliss and Matthews (1962) explanation for the formation of peptides from methane, ammonia, and water. Ponnamperuma and Woeller (1967) have shown that the volatile liquid resulting from an anhydrous mixture of methane and ammonia being subjected to an electric discharge contains aminoacetonitrile, α-aminopropionitrile, and methylaminoacetonitrile. Akabori (1955) proposed a hypothesis concerning the formation of what he called the 'fore-protein' from aminoacetonitrile, which would give rise to a polyglycine. This type of condensation



$$n(H_2NCH_2CN)\rightarrow(-NHCH_2\underset{\underset{NH}{\|}}{C}-)\,n$$

$$H_2O(-NHCH_2-\underset{\underset{O}{\|}}{C}-)_n$$

reaction was first reported by Krewson and Couch (1943) and was later extended by Short *et al.* (1949). Hanafusa and Akabori (1959) successfully used this reaction to produce di- and triglycine. Akabori *et al.* (1956) maintains that the 'fore-protein' consisted primarily of glycine. He demonstrated that the α-methylene groups of polyglycine can condense with aldehydes via a base catalyzed reaction to produce a variety of side chains. Recently, Reuter (1966) reported the synthesis of di- and triglycine from aminoacetonitrile, which was absorbed on clay and then irradiated with ultraviolet light in the presence of dicyanamide.

12. Polynucleotides

If one subscribes to the genetic model of the first living organism, the synthesis of a polynucleotide under simulated primitive-earth conditions would be a considerable leap forward in our understanding of the origin of life (MULLER, 1961). The in vitro synthesis of a nucleic acid molecule by KORNBERG (1958) has heightened the prospect that the first polynucleotide could have occurred abiogenically. BEADLE (1960) has suggested that this first replicating molecule could have been simpler than contemporary polynucleotides consisting of an adenine-thymine polymer which by mutation subsequently developed into the nucleic acid we know today.

Several attempts have been made to synthesize nucleic acids by non-enzymic means. High-energy phosphates may be considered a necessary prerequisite for this step. LIPMANN (1965) had suggested the possible role of carbamyl phosphate. SCHRAMM *et al.* (1962) have shown that polynucleotides can be synthesized at moderate temperatures, from their monomers. The $3' - 5'$ diester linkages which are common to RNA and DNA appear to be present.

CONTRERAS *et al.* (1962) studied the γ-irradiation of ribomononucleotides in aqueous solution and found a decrease in absorbancy at 260 μ. Alkaline and enzymatic hydrolysis of the product produced a hyperchromic effect. The irradiated product had a lower *Rf* value than the mononucleotides. On this basis they suggested the possible radiation-indiced polymerization of ribomononucleotides.

SCHWARTZ and FOX (1967) have shown that cytidylic acid could be polymerized to di- and trinucleotides using 85% polyphosphoric acid. It is hard to make a case for a high concentration of polyphosphoric acid on the primitive earth. However, the situation could have prevailed in micro-environments. In the thermal phosphorylation of nucleosides by inorganic phosphates, it has been shown that some oligonucleotides are formed. Electrophoresis, paper chromatography, ion exchange chromatography and ultraviolet absorption studies show that the di-, tri-, and tetranucleotides of uridine can be synthesized in this manner (PONNAMPERUMA and MACK, 1965a). The temperature required for this reaction is about 150 °C and the yields are small. If the yields can be increased and the reactions take place at low temperatures, the finding may be very significant for chemical evolution.

13. Precellular Organization

The two most extensively discussed concepts relating to precellular organization are OPARIN's (1964, 1965) coacervates and FOX's (1965b) microspheres. The association of the two names with these precellular models is justified since both men have championed their models exceptionally well and have been primarily responsible for experimentation carried out on their respective models.

Coacervation is a process in which a large part of the colloid of a sol separates as a second phase. The original phase system is depleted in colloidal material whereas the second phase is greatly enriched. If the new phase separates as amorphous droplets, the droplets are called coacervates. These droplets may or may not coalesce to a coacervate layer depending upon the amount of the second phase which is suspended in the original sol. Coacervation can be induced by various changes in the sol including hydrogen ion concentration and temperature (BUNGENBERG DE JONG, 1949).

Oparin has used natural and unnaturally occurring high molecular-weight polymers and various mixtures of polymers to form coacervates. He has incorporated enzymes into the coacervate systems and has demonstrated an enhancement of activity in the coacervate drops above that which the enzyme would have in solution. Oparin does not in any way imply that the coacervates he and his associates have studied were the actual precursors of the protocell; but he does maintain that the process of coacervation, involving simpler biologically important materials, could have lead to the formation of this protocell.

FOX and YUYAMA (1963) and FOX *et al.* (1962) have shown that when thermally produced polypeptides are rehydrated in warm water, cooling or salting out will produce a myriad of microspherules which morphologically closely resemble micrococci. These microspherules are called proteinoid microspheres.

It is Fox's contention that biologically important polymers have a thermal origin and that the formation of proteinoid microsphere represents a plausible pathway by which the first protocells were produced. The catalytic activity of thermally prepared polypeptides and microspheres has also been demonstrated (FOX, 1965b; ROHLFING, 1967).

Although there is some dispute over whether thermal polymerization of amino acids was the prime source of polypeptides, this should not detract from the usefulness of the proteinoid microspheres as a model for a protocell. If microsphere formation is a general property of polypeptides, then microspheres could be the most general model of a protocell. It has been argued that proteinoids are more stable than coacervates and represent, therefore, a more valid model of a protocell. Oparin however, maintains that the instability of the coacervates is an advantage since it makes them more responsive to their environment.

A radical departure from the afore-mentioned morphological concepts has recently been proposed by GABEL (1965). Based upon the idea that an excitable membrane preceded the formation of a protocell, this hypothesis attempts to trace the evolutionary development of a proto-life excitable membrane. From a critical examination of the properties of an excitable membrane (ABOOD and GABEL, 1965), it can be envisioned that a three-dimensional polyphosphate coordination complex with alkaline earth cations (Ca and Mg) as central atoms could serve as a primordial model for a proto-life membrane. The polyphosphates would be provided by leaching of primordial igneous phosphate deposits which would have separated from the cooling magma as polyphosphate salts. The positions in the complex to be occupied by alkali metal cations (Na and K) would depend on the ion exchange characteristics of polyphosphates. The formation of this inorganic proto-life membrane would be in accord with the laws of thermodynamics and would possess a potential gradient similar to the potential gradient of present-day biological membranes. It is further postulated that biologically important monomers and polymers, being polydentate ligands themselves would participate in the formation of this macromolecular coordination complex and eventually assume structural and metabolic functions as the source of polyphosphates became depleted.

It is almost generally accepted that self-replication was the prerequisite function of any precellular living entity (MULLER, 1966). Although this hypothesis does not preclude the evolutionary occurrence of a self-replicating protein, current knowledge of biological self-replication would imply that a polynucleotide should have been the first free-living proto-life form. An interesting approach to the formation of this 'primordial gene' has been given by CAIRNS-SMITH (1965), who has proposed that the template for a self-replicating system could have been clay crystallites which possess a repeating pattern of lattice imperfections. These lattice imperfections replicate themselves as a necessary part of the crystallization process and would, therefore, be self-selecting. These 'genographs' could hold large amounts of information and orient or control the absorption of large macromolecules on their surfaces. Cairns-Smith maintains that the theoretical information density of such crystallites is comparable to deoxyribonucleic acid. Eventually this information would be transferred to the evolving macromolecules themselves.

14. Discussion

Aside from seeking to obtain synthesis of biologically important molecules under conditions which can rationally be called primordial, the general areas of importance are the stability and accumulation of these materials and their eventual organization into self-replicating macromolecules. Although the stability and accumulation of organic materials can be studied in the laboratory under simulated environments, corroborative evidence can be obtained through the systematic study of organic fossils. An analysis of the organic materials of ancient sediments should prove to be extremely useful in elucidating the stability of organic compounds under actual geological conditions and may help to pinpoint the time at which life first appeared. The study of organic matter on the moon and in meteorites is of significance in this context.

Optical activity has been viewed by many investigators as the most general characteristic which biologically derived chemicals possess (WALD, 1957). Although racemization is thermodynamically favored, the geological sediments and samples from extraterrestrial sources should be closely examined for optical activity. If optical activity is not found, the data will be of little value; but if samples containing optically active organic materials are discovered, they would be the most meaningful indication for the existence of life when the sediment was laid down (STRYER, 1966).

Of paramount importance, because it bridges the gap between chemical evolution and biological evolution, is the problem of precellular organization. In essence the problem is to envision how the now biologically important chemicals spontaneously organized themselves into a three-dimensional matrix, which would eventually acquire the characteristics of a readily agreed-upon life-form. This brings us to the very heart of the matter, that is, the definition of life itself. Furthermore, chemical evolution may not have necessarily lead to biological evolution. There can be no doubt that chemical processes occurred and are now occurring on every celestial body and within the interstellar gases. However, it is an open question whether chemical evolution always leads to biological evolution. Even if future investigations succeed in the actual synthesis of life, these experiments will still remain at best nothing more than hypothetical models of what may have happened.

Yet, the problem of precellular organization still retains its importance and will continue to be one of the most fascinating areas for future investigations. The purpose of investigation into this area should be to develop many rational models for precellular organization, devise experiments to test them, and then proceed to evaluate the feasibility of each model. Hopefully, this course of events will eventually clarify the question of how life could have originated. It is probable that several models may be feasible. This again should be no reason to discourage efforts to gain further insight into this problem; for the information which will be uncovered may very well serve as a useful frame of reference for the study of extraterrestrial life if and when it is ever encountered.

It is difficult to believe that life is a chance occurrence which is characteristic only of this planet. Even if present Martian conditions indicate that there is only a slender chance for the existence of any form of life, a careful if not painstaking effort should be made to determine whether Mars ever supported any life-form. Each in its turn, the planets must be explored to determine the extent and nature of chemical evolutionary processes upon it. If the earth can be considered as a laboratory for the study of evolutionary processes, then indeed the solar system is a laboratory on a more magnificent scale. If evidence for extraterrestrial life is not found within our solar system, the challenge will remain for future generations to extend these explorations and seek an answer to the problem of the nature of life in order that man may understand himself.

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