

# THE FORMATION OF ORGANIC COMPOUNDS ON THE PRIMITIVE EARTH

By Stanley L. Miller

*Department of Biochemistry, College of Physicians and Surgeons, Columbia University,  
New York, N. Y.*

## INTRODUCTION

One of the most fundamental problems of biology is posed by the question "How did life arise on the earth?" The theory of evolution offers an explanation for the development of complex multicellular living organisms from unicellular organisms, but this theory does not explain the development of the first organism. To assume that life arose from inorganic matter presents overwhelming difficulties. One must assume, not only that a self-duplicating organism could be made from inorganic matter, but that the organism could contain the complex apparatus needed to synthesize all of its components and energy requirements from carbon dioxide, water, and light.

In his celebrated book *The Origin of Life* (1938), Oparin proposed that spontaneous generation of life would be facilitated if the ocean contained a large amount of complex organic compounds similar to those present in living organisms. These compounds would serve both as structural components and as the energy supply for the first organisms. Oparin also suggested that the earth had a reducing atmosphere of methane, ammonia, water, and hydrogen in its early stages, and that organic compounds might be formed under these conditions.

Urey (1952a, 1952b), in considering the problem of the formation of the solar system, also proposed that our planet originally had an atmosphere of methane, ammonia, water, and hydrogen. Unlike Oparin's qualitative arguments, Urey's reasoning was based on thermodynamics. Methane and ammonia are the thermodynamically stable species of carbon and nitrogen in the presence of excess hydrogen.

Experimental support for these theories has come from studies of the action of electrical discharges on these gases (Miller, 1953, 1955, and 1957). These results will be summarized, and some of their implications will be discussed.

As a basis for discussion, the following model of the earth in its early stage is proposed. The atmosphere was reducing, and the oceans covered an appreciable fraction of the surface of the earth. The temperature is assumed to have been less than 100° C. The sources of energy for the production of the initial organic compounds were ultraviolet light, electrical discharge, and high temperatures (under local conditions such as those produced by volcanoes). Although the level of radioactivity was higher than at present, the energy available was still quite small, and there is no evidence that the intensity of cosmic rays was ever sufficient to compare with the energy from the sun.

The energy from ultraviolet light probably would be greater than that from electrical discharges. Because of the difficulties of working with ultraviolet light in the region in which these reduced gases would absorb (<2000 Å.), I decided to work with electrical discharges.

## SPARK DISCHARGE: RUN 1

An approximation of the proposed model is shown in FIGURE 1. The apparatus is made of Pyrex, with tungsten electrodes. The water in the small flask is boiled to promote circulation and to bring water to the region of the spark. The products of the discharge are condensed and flow through the U-tube, which prevents circulation in the wrong direction. The nonvolatile

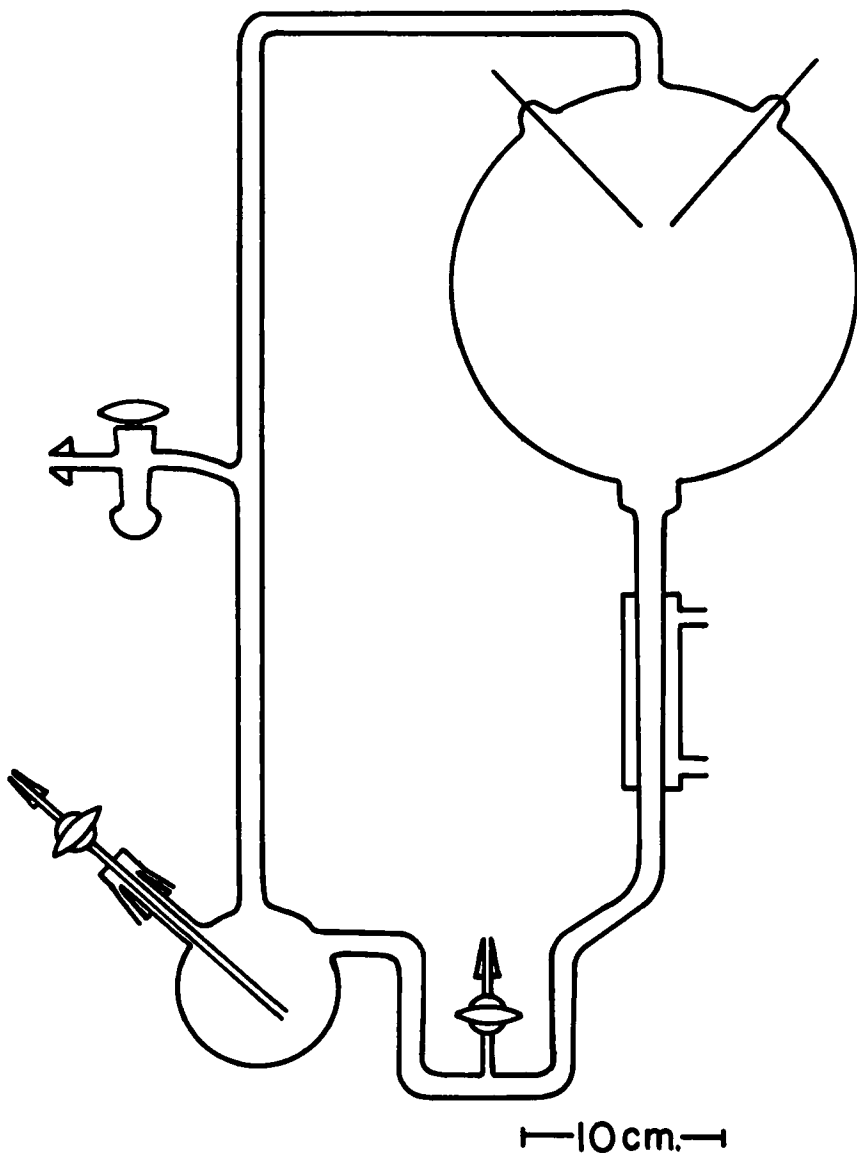


FIGURE 1. Spark-discharge apparatus.

compounds accumulate in the small flask. The spark discharge is produced by a high-frequency Tesla coil that has a peak of 60,000 volts.

The pressures of hydrogen, methane, and ammonia were 10, 20, and 20 cm. of Hg, respectively. The water was boiled, and the spark was operated continuously during a run. Colloidal silica that originated from the action of ammonia on glass, together with a yellow polymer, formed in the boiling flask during the run. The yellow polymers have a strong ultraviolet absorption, but have no peaks above 230  $\mu$ . The nondialyzable compounds were hydrolyzed and chromatographed. Only a very small amount of amino acid was present in this fraction.

#### *Analysis of Products*

Analysis of the gases remaining at the end of a run showed that carbon monoxide, carbon dioxide, and nitrogen were present in addition to the initial gases.

The organic compounds were separated into acidic, basic, and ampholytic fractions by various ion-exchange resins. The different acids were separated by chromatography on silica (Bulen *et al.*, 1952) and the amino acids by chromatography on Dowex 50 (Stein and Moore, 1949). The compounds were identified by  $R_f$  values on ion-exchange resins, silica, and paper. Some of the compounds were characterized further by preparation of derivatives and the comparison of their melting points and mixed melting points with authentic samples of the derivative.

The optical rotation of a sample of alanine was  $0.000 \pm 0.003^\circ$ . If this sample had been either pure enantiomorph, the rotation would have been  $0.12^\circ$ . The yields of compounds from the various runs are shown in TABLE 1.

#### *Absence of Purines and Pyrimidines*

The mixture of compounds from a run similar to Run 1 was evaporated to dryness, and the whole sample was chromatographed on a column of Dowex 50 ( $H^+$ ) (Wall, 1953). The eluent showed no 260  $m\mu$  absorption maximum where the naturally occurring purines and pyrimidines would be eluted, and paper chromatography of the evaporated fractions showed no spots under ultraviolet radiation. It is concluded that the presence of any purine was less than  $0.2 \times 10^{-5}$  mols and of any pyrimidine was less than  $0.1 \times 10^{-5}$  mols.

#### *The Effect of Adding Ferrous Ammonium Sulfate*

Since iron is one of the more abundant elements on the earth, and would be present as both the native metal and as ferrous compounds, an experiment was performed to compare the organic compounds synthesized by a spark discharge in the system with and without added ferrous ammonium sulfate. About 16 per cent of the iron had been oxidized to ferric by the end of the run. The organic compounds were the same as in Run 1, and the quantitative values were only slightly different.

TABLE 1  
YIELDS OF COMPOUNDS (MOLS  $\times 10^6$ )

	Spark Run 1	Silent Run 3	N <sub>2</sub> run Run 6
Glycine.....	63 (2.1)*	80 (0.46)*	14.2 (0.48)*
Alanine.....	34	9	1.0
Sarcosine.....	5	86	1.5
$\beta$ -Alanine.....	15	4	7.0
$\alpha$ -Aminobutyric acid.....	5	1	—
<i>N</i> -Methylalanine.....	1	12.5	—
Aspartic acid.....	0.4	0.2	0.3
Glutamic acid.....	0.6	0.5	0.5
Iminodiacetic acid.....	5.5	0.3	3.9
Iminoacetic-propionic acid.....	1.5	—	—
Formic acid.....	233	149	135
Acetic acid.....	15.2	135	41
Propionic acid.....	12.6	19	22
Glycolic acid.....	56	28	32
Lactic acid.....	31	4.3	1.5
$\alpha$ -Hydroxybutyric acid.....	5	1	—
Succinic acid.....	3.8	—	2
Urea.....	2	—	2
Methylurea.....	1.5	—	0.5
Sum of yields of compounds listed.....	15%	3%	8%

\* Percentage yield of glycine, based on carbon placed in the apparatus as methane.

#### SILENT DISCHARGE: RUN 3

An experiment was performed using a silent electrical discharge (ozonizer) instead of a spark. As seen in TABLE 1, the yields are about one fourth those obtained with the use of the spark, but the products are similar.

#### SPARKING A MIXTURE OF METHANE, NITROGEN, WATER, AND HYDROGEN: RUN 6

The equilibrium constant for the reaction  $N_2 + 3H_2 = 2NH_3$  is  $7 \times 10^6$  atm.<sup>-2</sup>, at 25° C., which predicts that the nitrogen would remain as ammonia instead of N<sub>2</sub> until the partial pressure of hydrogen fell below 10<sup>-2</sup> atm. by escape into outer space. However, the disruptive effect of ultraviolet light and electrical discharges might result in a steady-state concentration of ammonia less than the equilibrium value. To see which organic compound would be formed under these conditions, a mixture of methane, nitrogen, hydrogen, and water was subjected to the spark. The same products are formed as in Run 1, but the yields are somewhat less.

#### THE MECHANISM OF SYNTHESIS

There is the question of whether the compounds observed in this system were synthesized by microorganisms. To check this point, blank runs were made with the same gases but with no spark. The production of amino acids was less than 10  $\mu$ g. There is the possibility that the microorganisms might synthesize amino acids from some of the products of the discharge. To check

this point the apparatus was filled with water and with the reduced gases, and then sealed, autoclaved for 18 hours at 130° C., and sparked for one week. The yield of organic compounds was the same as that of the runs performed without autoclaving. In addition, the temperature of the apparatus was maintained at 80 to 100° C. during the run; the alanine was racemic; and the organic compounds do not represent the distribution one would expect if they had been produced by living organisms. For these reasons it is stated with confidence that the organic compounds in the system were synthesized without the aid of microorganisms.

The next problem in attempting to understand the chemistry of the system is to determine which compounds are formed in the electrical discharge, and which reactions occur in the solution phase of the system. The following alternative hypotheses will be made for the synthesis of the products.

(1) Hydrogen cyanide, aldehydes, acrylonitrile, aliphatic nitriles, amines, and part of the polymers are synthesized in the electrical discharge, and the amino, hydroxy, and aliphatic acids are formed by hydrolysis of the respective nitriles in the solution.

(2) All of the products identified were synthesized in the gas phase from radicals and ions formed in the electrical discharge.

In order to determine a few of the direct products of the electrical discharge, samples were withdrawn from the U-tube during the course of a run. Hydrogen cyanide was detected qualitatively by the Prussian blue test and estimated by titration with  $\text{AgNO}_3$ . Formaldehyde was detected qualitatively by chromotropic acid and acetaldehyde by *p*-hydroxydiphenyl (Neidig and Hess, 1952). The total aldehydes (and ketones) were estimated with 2,4-dinitrophenylhydrazine (Lappin and Clark, 1951).

FIGURE 2 shows the concentrations of ammonia, hydrogen cyanide, and aldehydes in the U-tube and amino acids in the 500 ml. flask during the sparking of a mixture of methane, ammonia, water, and hydrogen. It is seen that the concentration of ammonia decreased steadily during the run, mostly because of the decomposition of the ammonia to hydrogen and nitrogen in the discharge. The hydrogen cyanide concentration rose to  $4 \times 10^{-2}$  M and, after 120 hours, apparently little more was synthesized in the spark. Thereafter, the hydrogen cyanide present was hydrolyzed to formic acid or decomposed in the spark. The aldehyde concentration rose to about  $10^{-3}$  M and declined after 120 hours. The concentration of amino acids rose during the run and leveled off after about 140 hours.

Several repetitions of this experiment gave concentrations of these compounds of the same order of magnitude, but the values were not reproducible in detail. Probably the most important variable that could not be controlled was the operation of the spark.

#### *Hydrolysis of Aminonitriles and Hydroxynitriles: Run 4*

Hydrogen cyanide, aldehydes, and ammonia are known to react to give aminonitriles and hydroxynitriles. It is uncertain whether the conditions of this experiment will hydrolyze these nitriles to the corresponding acid. A solution of 63 mmols  $\text{NH}_3$  (corresponding to 25 cm. Hg pressure), 20 mmols

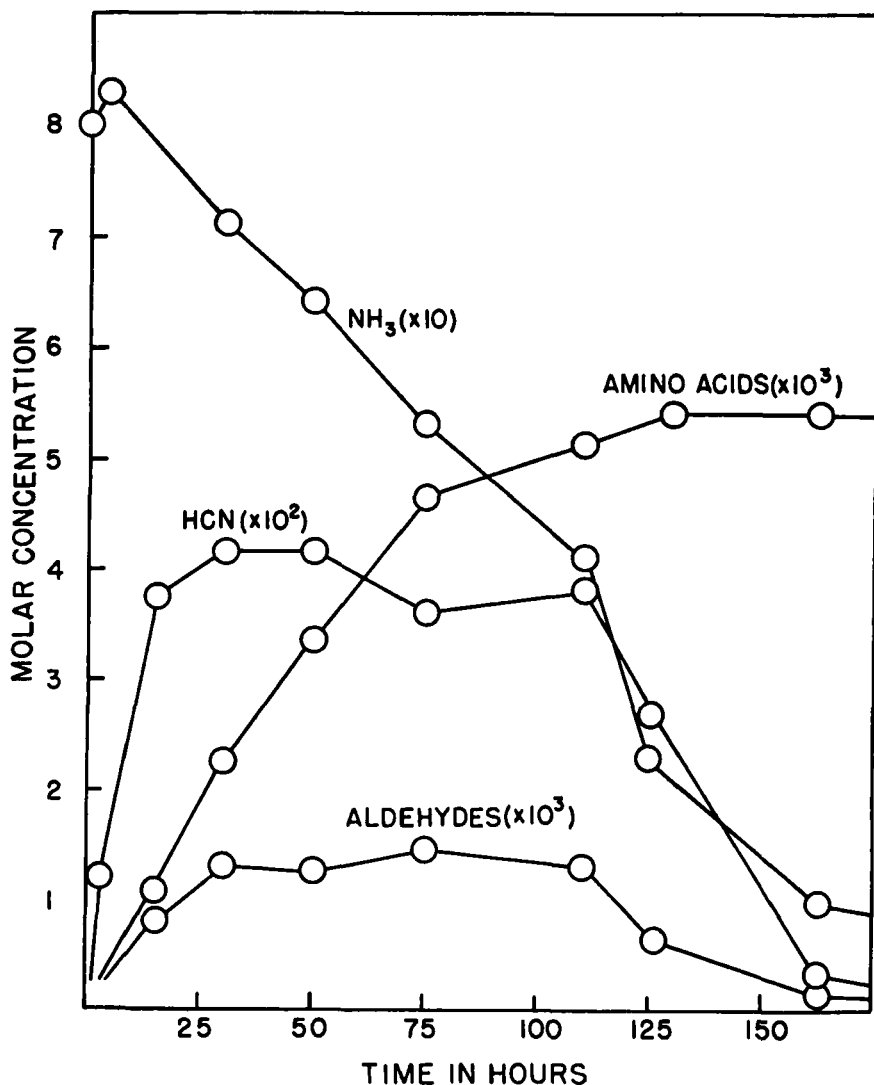


FIGURE 2. The concentrations of ammonia, hydrogen cyanide, and aldehydes in the U-tube, and of amino acids in the 500-ml. flask while sparking a mixture of methane, ammonia, water, and hydrogen in the apparatus shown in FIGURE 1.

hydrogen cyanide, 6.1 mmols formaldehyde, 3.64 mmols acetaldehyde, and 1.16 mmols propionaldehyde (in 325 ml. H<sub>2</sub>O) was boiled in the apparatus for one week. The glycine, glycolic acid, iminodiacetic acid and iminoacetic-propionic acid accounted for 52 per cent of the formaldehyde; alanine, lactic acid, and iminoacetic-propionic acid accounted for 58 per cent of the acetaldehyde;  $\alpha$ -amino-*n*-butyric acid and  $\alpha$ -hydroxybutyric acid accounted for 36 per cent of the propionaldehyde. This experiment shows that aminonitriles and

hydroxynitriles can be hydrolyzed in this system and, further, that the acids are formed in good yield from the aldehyde.

By titrating samples withdrawn from the U-tube for hydrogen cyanide during the course of the run, the rate constant for hydrolysis of hydrogen cyanide to formic acid is estimated to be  $0.1 \text{ hr.}^{-1}$ . Similarly, by determining the amino acid concentration in the 500 ml. flask at various times, the rate constant for hydrolysis of the aminonitriles is estimated to be  $0.2 \text{ hr.}^{-1}$ .

From these rate data and from the concentrations in FIGURE 2 the yield of formic acid from hydrolysis of hydrogen cyanide is calculated to be 3.6 mmols, and the yield of amino acids from hydrolysis of the aminonitriles is calculated to be 1.4 mmols. These values agree within the experimental error with the observed yields of 2.4 mmols of formic acid and 1.2 mmols of amino acids. Thus, in the case of the spark discharge, the rates of hydrolysis under the conditions of the experiment are sufficient to account for the total yields of formic acid and amino acids observed.

### *The Ratios of Products*

Further evidence that the amino and hydroxy acids are synthesized through the corresponding nitrile can be obtained by consideration of the ratios of products predicted by this mechanism.

It can be shown (Miller, 1955) that if the reaction of the aldehyde, hydrogen cyanide, and ammonia to form the aminonitriles and hydroxynitriles is a rapid and reversible equilibrium, and that the hydrolysis is a first-order irreversible reaction, then the ratio of hydroxy acid to amino acid at the end of the run will be

$$R_1 = h_i H_i / k_i K_i (\text{NH}_3) \quad (1)$$

where  $H_i$  and  $K_i$  are the equilibrium constants for the formation of the hydroxy- and aminonitrile from aldehyde<sub>*i*</sub>, and  $h_i$  and  $k_i$  are the respective rates of hydrolysis. Similarly, we have

$$\begin{aligned} R_2 &= N\text{-methylamino acid/amino acid} \\ &= m_i M_i (\text{CH}_3\text{NH}_2) / k_i K_i (\text{NH}_3) \end{aligned} \quad (2)$$

where  $M_i$  and  $m_i$  are the equilibrium constant and rate constant of hydrolysis of the methylaminonitrile.

$\beta$ -Alanine cannot arise from a Strecker synthesis as with the  $\alpha$ -amino acids. A reasonable mechanism would be from a Michael addition of ammonia to acrylonitrile, acrylamide, or acrylic acid. One would expect that hydrogen cyanide and methylamine would also add to give, after hydrolysis, succinic acid and *N*-methyl- $\beta$ -alanine. This last compound was not detected during the analysis, since it does not react with ninhydrin. The rate of formation of the nitrile of  $\beta$ -alanine and succinonitrile would be  $k_{\text{NH}_3}(\text{NH}_3)(\text{CH}_2=\text{CHCN})$  and  $k_{\text{HCN}}(\text{CN}^-)(\text{CH}_2=\text{CHCN})$ , respectively, where the  $k$ 's are the rate constants for addition. Assuming that the addition is irreversible, and that the

TABLE 2  
 RATIOS OF PRODUCTS

	Spark Run 1	Silent Run 3	N <sub>2</sub> run Run 6	Aldehydes HCN, NH <sub>3</sub> Run 4
Glycolic Glycine .....	0.89	0.35	2.3	0.73
Lactic Alanine .....	0.91	0.48	1.5	0.33
Hydroxybutyric Aminobutyric .....	1.0	1.0	—	0.55
Sarcosine Glycine .....	0.08	1.07	0.11	—
Methylalanine Alanine .....	0.03	1.4	—	—
Succinic $\beta$ -Alanine .....	0.25	—	0.29	—

nitriles are hydrolyzed by the end of the run, then

$$(\text{succinic acid})/(\beta\text{-alanine}) = k_{\text{HCN}}(\text{CN}^-)/k_{\text{NH}_3}(\text{NH}_3) \quad (3)$$

This treatment is easily generalized to include additions to acrylonitrile and acrylic acid. The ratios of products are given in TABLE 2.

If the ratio  $hH/kK$  does not depend on the aldehyde, then EQUATION 1 predicts that the ratio of the hydroxy acid to the amino acid should be the same for the different aldehydes in a given run. The agreement is good for the spark discharge and silent discharge except for the hydroxybutyric/aminobutyric in the silent discharge. In Run 4 the hydrolysis of the aminonitriles and hydroxynitriles was necessarily the mechanism for synthesis of the respective acids. There is less agreement of the ratios than with the electric discharges, but the agreement is within the errors of the experiment.

Similarly, the ratios of methylamino acid/amino acid are nearly the same for Runs 1 and 3. The succinic acid/ $\beta$ -alanine ratio is the same in Runs 1 and 6.

The ratios of various products are in qualitative agreement in all cases and in quantitative agreement (within the experimental error) in most of the cases. The similarity of products in Runs 1 and 4 is striking (except for the expected absence of  $\beta$ -alanine and succinic acid, since no acrylonitrile was added), which suggests that the products were formed by the same mechanism.

Since the production of aldehydes and hydrogen cyanide is sufficient to account for the observed yield of amino acid, there can be little doubt that most of the amino and hydroxy acids were formed from the nitriles in Run 1. However, these experiments do not exclude the possibility that a small per-



centage of the amino acids were formed directly in the spark, entirely by radical reactions.\*

The synthesis of the products expected from acrylonitrile and the agreement of the ratios of these products in the different runs provides strong indirect evidence for the synthesis of  $\beta$ -alanine and succinic acid by  $\beta$ -addition and, in turn, that acrylonitrile or its derivatives were synthesized in the electric discharges.

If cyanate were formed in the electrical discharge, then both urea and methyleurea could be expected by reaction with ammonia and methylamine (Wöhler synthesis). The direct synthesis of the simple ureas in the electrical discharge is also quite reasonable.

#### DISCUSSION

Assuming that the earth initially had a reducing atmosphere, do the experimental results obtained in this very simple system show that amino acids and other organic compounds would be present in the oceans? The experiments on the mechanism of the electric discharge synthesis of amino acids indicate that a special set of conditions or type of electrical discharge is not required to obtain amino acids. Any process or combination of processes that yielded both aldehydes and hydrogen cyanide would have contributed to the amount of  $\alpha$ -amino acids in the hydrosphere of the primitive earth. Therefore, electrical discharges are not critical for the synthesis of amino acids, and the similar results could be expected from ultraviolet light.

The ultraviolet light emitted by the sun as black-body radiation amounts to 85 cal.  $\text{cm}^{-2}\text{yr}^{-1}$  for wave lengths less than 2000 Å. and 1.6 cal.  $\text{cm}^{-2}\text{yr}^{-1}$  for wave lengths less than 1500 Å. (Urey, 1952b). Superimposed on the black-body radiation is a strong Lyman  $\alpha$  line at 1216 Å. of 1.9 cal.  $\text{cm}^{-2}\text{yr}^{-1}$  (Rense, 1953). This line is absorbed by  $\text{CH}_4$ ,  $\text{H}_2\text{O}$ ,  $\text{NH}_3$  and  $\text{CO}$ .

Hydrogen atoms from the photolysis of  $\text{CH}_4$ ,  $\text{NH}_3$  and  $\text{H}_2\text{O}$  would react with  $\text{CO}$  to give formaldehyde (Caress and Rideal, 1928; Taylor, 1926). Carbon monoxide activated by wave lengths of less than 1545 Å. reacts with  $\text{H}_2$  to give formaldehyde and glyoxal (Groth, 1937). Hydroxyl radicals would react with hydrocarbons to give aldehydes (Milas, Stahl, and Dayton, 1949). If any O atoms should be formed by photolysis of water or  $\text{CO}$ , they would react rapidly with  $\text{H}_2$  to give  $\text{H}_2\text{O}$  and with hydrocarbons to give aldehydes.†

Active nitrogen, probably N atoms in the  $^4\text{S}$  state (Jackson and Schiff, 1955) reacts with methane and other hydrocarbons to give hydrogen cyanide in good yield (Winkler and Schiff, 1953). Photodissociation of  $\text{N}_2$  ( $<1100$  Å.) or  $\text{NH}$  radicals gives N atoms.  $\text{NH}$  and  $\text{NH}_2$  radicals from the photolysis of

\* The hydrogen cyanide concentration in the silent discharge case is too low to account for the yield of amino acids unless the hydrolysis of the nitriles is more rapid than in Run 1. Hydrogen peroxide catalysis is a possibility. The ratios of products in the various runs is strong evidence that the products were formed by the same mechanism.

† If aldehydes were synthesized from the Lyman  $\alpha$  radiation with a quantum yield of 1.0, the yield for the earth would be  $2 \times 10^{13}$  mols  $\text{yr}^{-1}$ . If the aldehydes were dissolved in the present oceans this would give a solution of  $3 \times 10^{-8}$  M. Of course, the efficiency of the Lyman  $\alpha$  radiation would not have been 100 per cent, but the oceans probably would have been smaller in volume, and the electrical discharges and high-temperature reactions would contribute to the production of aldehyde.

ammonia might react with hydrocarbons to give hydrogen cyanide, but this has not yet been demonstrated.

The reactions outlined above show that aldehydes and hydrogen cyanide would be produced photochemically, and there probably are other photochemical reactions that would also give these compounds.\*

Infrared radiation by the polyatomic molecules of the reducing atmosphere probably would result in a cool atmosphere and ocean rather than the boiling temperatures used in these experiments or the molten earth proposed by some workers. However, if there were any local areas of high temperature, hydrogen cyanide would be formed (Migrdichian, 1947), and aldehydes might be synthesized from hydrocarbons and carbon monoxide by reactions analogous to the Fischer-Tropsch or hydroformalation reactions (Storch, Golumbic, and Anderson, 1951).

If conditions on the earth were cool, then the hydrolysis of the nitriles would still take place, but more slowly than in these experiments. The Strecker synthesis of amino acids will work at much lower concentrations of aldehyde and hydrogen cyanide than obtained in these experiments. At very low concentrations, however, the Strecker synthesis will not operate. The rate of synthesis of amino acid is given by

$$-d(\text{HCN})/dt = kK(\text{NH}_3)(\text{RCHO})(\text{HCN}) \quad (4)$$

The  $kK(\text{RCHO})$  means the sum of this term over the different aldehydes. The hydrolysis of hydrogen cyanide to formic acid is a competing reaction with the rate

$$-d(\text{HCN})/dt = r(\text{HCN}) \quad (5)$$

where  $r$  is the rate constant for the hydrolysis of hydrogen cyanide. Thus, if the concentrations of aldehydes are so low that  $kK(\text{NH}_3)(\text{RCHO})/r \ll 1$ , then cyanide will not be available for the Strecker synthesis because of hydrolysis to formic acid. It is necessary to know the values of  $K$ ,  $k$ ,  $H$ ,  $h$ , and  $r$ , their  $p\text{H}$ , and their temperature dependence for a quantitative treatment of this problem.

From a qualitative standpoint it can be seen that the Strecker synthesis will operate in very dilute solutions. The  $H$  for acetaldehyde at 25° C. is  $1.4 \times 10^4$  (Yates, 1952) and the  $K$  is probably greater. The experiments reported here indicate that  $h$ ,  $k$ , and  $r$  are of the same order of magnitude. Thus, if the hydrolysis of the nitriles in the hydrosphere is by the same mechanism as in these experiments (probably  $\text{OH}^-$  attack on the carbon of the nitrile) then  $(\text{RCHO})$  can be as low as  $10^{-4}$ – $10^{-5}$  M, and the Strecker synthesis will still operate. If the value of  $k$  relative to  $r$  (and  $h$ ) is increased by catalytic hydrolysis (for example,  $\text{SH}^-$ ,  $\text{HPO}_4^{2-}$ ), then the concentration of aldehydes could be much lower.

\* Some preliminary experiments performed at Brookhaven National Laboratory, Upton, N. Y., showed that the 1850 Å. mercury line can synthesize amino acids from  $\text{CH}_4$ ,  $\text{NH}_3$  and  $\text{H}_2\text{O}$ . Only  $\text{NH}_3$  and  $\text{H}_2\text{O}$  absorb this line, but apparently the radical reactions formed the active carbon intermediates. Formaldehyde was detected. The yield of amino acids was very low.

The ratio of hydroxy acid to amino acid is given by EQUATION 1. If the concentration of ammonia is very low and (RCHO) and (HCN) are sufficiently high, then hydroxy acid rather than amino acid or formic acid will be synthesized.

There are competing reactions that the aldehyde can undergo instead of a Strecker synthesis. The aldehydes can be reduced or oxidized, the latter being important if any oxygen were present. The most important competing reaction would be aldol condensations. These condensations would give biologically important products such as trioses, tetroses, pentoses, and hexoses. The rate of these condensations relative to the Strecker synthesis would not depend markedly on the concentrations of aldehydes, since the aldol condensations would be second-order reactions. Therefore, the competing reactions of the aldehydes would not predominate at low concentrations.

In the above discussion the composition of the primitive atmosphere of our planet has been assumed to be reducing. The general geochemical argument for the reducing atmosphere given by Oparin and by Urey is that the ratio of hydrogen to oxygen in the universe is about 1000:1, the earth being rather anomalous in this respect. No one has demonstrated any mechanism that, before the planets were formed, would produce oxygen in the region of the earth, but not in the region beyond Mars. The formation of oxidizing conditions on Mercury, Venus, Earth, and Mars after their formation is explained by the escape of hydrogen from these planets. Their atmospheres are sufficiently hot and their gravitational fields sufficiently weak to allow hydrogen to escape into outer space from the atmosphere. The escape of the strong reducing agent,  $H_2$ , results in an oxidizing atmosphere. In the region beyond Mars the planets have low temperatures and high gravitational fields. These conditions prohibit the escape of hydrogen from their atmospheres, as a result of which they are still reducing.

A second argument for the existence of a reducing atmosphere on the primitive earth is based on the assumption that, for life to arise, there must be present, first, a large number of organic compounds similar to those that would make up the first organism. Therefore, if it can be demonstrated that the organic compounds that make up living systems *cannot* be synthesized under oxidizing conditions, and if it can be shown that these organic compounds *can* be synthesized under reducing conditions, then one conclusion would be that the earth had a reducing atmosphere in its early stages and that life arose from the sea of organic compounds that was formed while the earth had such an atmosphere.

From a review of the literature on electrical discharges (Glockler and Lind, 1939) and ultraviolet light (Noyes and Leighton, 1941), from the results of the experiments described in this paper, and from the first part of this discussion, one can see that organic compounds can be synthesized easily under reducing conditions.

There have been many attempts to synthesize organic compounds under oxidizing conditions, usually from carbon dioxide and water, and these attempts almost always have failed. A review of such attempts, in which ul-

traviolet light was used (Rabinowitch, 1945), shows that success was claimed by some workers, but when their experiments were repeated in other laboratories or when contaminating reducing agents were removed, no organic compounds were synthesized. The action of electrical discharges on carbon dioxide and water also has resulted in failure (Wilde, Zwolinski, and Parlin, 1953). Of course, if a strong reducing agent such as Na or Mg is used, organic compounds can be formed, but these reducing agents would not be present on the earth with either a reducing or an oxidizing atmosphere. High-energy radiations on ammonium carbonate solutions might give organic compounds, but the presence of ammonia would imply reducing conditions.

There has been one successful synthesis of organic compounds from carbon dioxide and water. This was done with 40 million electron-volt helium ions from a 60-inch cyclotron (Garrison *et al.*, 1951; Garrison and Rollefson, 1952). Formic acid was obtained in small yield and, if ferrous iron was added to the solution (as a reducing agent), then a small yield of formaldehyde was obtained in addition to formic acid. In view of the absence of a strong source of high-energy particles, the small yields, and the very simple organic compounds synthesized, it would seem that instead of showing that organic compounds can be synthesized, this experiment is an excellent demonstration that organic compounds cannot be synthesized effectively under oxidizing conditions.

If any organic compounds should be synthesized under oxidizing conditions, however difficult this may be, then the question of their stability would arise. In the presence of molecular oxygen the organic compounds would be oxidized rather rapidly, especially in the presence of light (Palit and Dhar, 1930). An important reaction of the oxygen would be the oxidative deamination of the amino acids. This reaction is catalyzed by blood charcoal and, probably, by many iron compounds (Warburg, 1949). The oxidative deamination is a significant reaction even in the absence of catalysts (Abelson, 1956b). Oxygen also would attack aromatic compounds such as the purines and pyrimidines, especially in the presence of light. These arguments make a strong case that free oxygen must have been absent when the organic compounds were formed as well as during the development of heterotrophic organisms. Shortly after the appearance of oxygen on the earth the autotrophic organisms must have developed, for otherwise the nutrients would have been exhausted rapidly.

If the Strecker synthesis was the principal synthesis of amino acids on the primitive earth, then ammonia must have been present in the ocean, even though  $N_2$  could have been the principal nitrogen species in the atmosphere.\* This implies that the earth must have been rather reducing, with a pressure of  $N_2$  of at least  $10^{-3}$  atmospheres, unless one is to assume that the amino acids were formed in limited areas that contained reducing conditions.

\* Ammonia is quite soluble in water. The vapor pressure in atmospheres is given by  $p_{NH_3} = \alpha[(NH_4OH) + (NH_4^+)]$ , where the concentrations of  $NH_4OH$  and  $NH_4^+$  are in mols/liter. For  $25^\circ C$ ,  $\alpha$  is  $9.3 \times 10^{-5}$  at  $pH = 7$ ,  $8.8 \times 10^{-4}$  at  $pH = 8$ , and  $6.1 \times 10^{-3}$  at  $pH = 9$ . Thus, unless the temperature of the ocean was rather high ( $70-100^\circ C$ ), most of the ammonia would be in the ocean. The cyanide could be formed from the  $N_2$  in the atmosphere (as happened in Run 6).

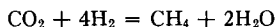
This argument would not be valid if there are other reasonable syntheses of amino acids. One possibility would be the reductive amination of any  $\alpha$ -keto acids present in the ocean, although decarboxylation of the keto acid would be a competing reaction. Another reaction would be synthesis of amino acids from  $\alpha$ -keto aldehydes and ammonia catalyzed by mercaptans (Wieland, Franz, and Pfeiderer, 1955; Wieland and Jaenicke, 1955). A possible source of the  $\alpha$ -keto aldehydes would be from the oxidation of polyhydroxyl compounds obtained from aldehyde condensations. These two syntheses require ammonia, however. It is very difficult to see how an amino group can be synthesized directly from  $N_2$  by any reasonable process except under reducing conditions. Reasonable syntheses of amino acids involving hydroxylamine, nitrites, or nitrates would require strong reducing agents to convert the nitrogen to an amino group. A direct synthesis of the amino acids in an electrical discharge, if possible, probably would require reducing conditions.

On the basis of primarily geochemical arguments, Rubey (1955) has contended that the primitive earth had an atmosphere of carbon dioxide, nitrogen, carbon monoxide, and water. This atmosphere would come mainly from the interior of the earth rather than be composed of the residual gases of the cosmic dust cloud. Abelson (1956a) has examined the action of a spark discharge on this mixture of gases and found that good yields of amino acids could be obtained as long as some hydrogen was present.\* Conversely, when no hydrogen was present, no amino acids were obtained. The production of amino acids was more rapid if  $CO_2$ ,  $H_2O$ , and  $NH_3$  (instead of  $N_2$ ) were used. The mechanism of the reaction was not investigated, but it may well be a Strecker synthesis as in the case of methane, ammonia, and water.

Because of the presence of hydrogen in the gas mixtures used by Abelson, the mixtures were reducing, although not nearly to the same degree as the mixture of methane, ammonia, water, and hydrogen. Therefore, the argument that reducing conditions are necessary to synthesize organic compounds is not altered, but whether the atmosphere was strongly reducing or only weakly so cannot be decided on the basis of ability to synthesize organic compounds.

As hydrogen escapes into outer space from a strongly reducing atmosphere, such an atmosphere would become less reducing and, finally, become oxidizing. Thus, the atmosphere proposed by Urey would be converted in the course of time to that proposed by Rubey. The principal question involves the relative lengths of time that the earth had these respective atmospheres. This question is not critical to the problem of spontaneous generation, since organic compounds can be synthesized in both proposed atmospheres (provided that the one suggested by Rubey contains some hydrogen), and since the organic compounds would be similar in both cases.

\* The equilibrium constant for the reaction



is  $7 \times 10^{21}$  at  $25^\circ C.$ , so that the mixture of gases used by Abelson is thermodynamically unstable. Whether this equilibrium would be attained on the earth is not predicted by thermodynamics, but carbon dioxide in the presence of hydrogen can be reduced to carbon monoxide and methane in electrical discharges and probably by ultraviolet light.

## SUMMARY

(1) Experiments are described showing that electrical discharges in a mixture of methane, ammonia, hydrogen, and water will produce amino, hydroxy, and aliphatic acids. This mixture of gases has been proposed as the composition of the earth's atmosphere in the early stages of formation.

(2) The same compounds (but in different yields) are formed with both spark and silent discharges; when methane, nitrogen, water, and hydrogen are sparked; and when ferrous ammonium sulfate is added to the system.

(3) Hydrogen cyanide and aldehydes are direct products of the electrical discharge. These compounds react to give amino- and hydroxynitriles, which are then hydrolyzed to the corresponding acid (Strecker synthesis) in the aqueous phase of the system.

(4) Arguments are presented to show that the same types of compounds would be synthesized if the earth had a reducing atmosphere. Both ultraviolet light and electrical discharges would produce aldehydes and hydrogen cyanide, and therefore would contribute to the amount of amino acids in the ocean.

(5) It is probable that the Strecker synthesis will operate at the low concentrations of aldehydes and hydrogen cyanide that might be expected in the oceans of the primitive earth.

(6) It is pointed out that organic compounds would not be synthesized on the earth if oxidizing conditions were present. Therefore, if one assumes that amino acids (and other organic compounds) must be present for life to arise, then the atmosphere of the earth must have been reducing. In particular, ammonia must have been present in the oceans for the synthesis of amino acids. This implies that the partial pressure of hydrogen was at least  $10^{-3}$  atmospheres.

*References*

- ABELSON, P. H. 1956a. Amino acids formed in "primitive atmospheres." *Science*. **124**: 935.
- ABELSON, P. H. 1956b. Private communication.
- BULEN, W. A., J. E. VARNER & R. C. BURRELL. 1952. Separation of organic acids from plant tissues. *Anal. Chem.* **24**: 187.
- CARESS, A. & E. K. RIDEAL. 1928. The chemical reactions of carbon monoxide and hydrogen after collision with electrons. *Proc. Roy. Soc. London*. **A120**: 370.
- GARRISON, W. M., D. C. MORRISON, J. G. HAMILTON, A. A. BENSON & M. CALVIN. 1951. Reduction of carbon dioxide in aqueous solutions by ionizing radiation. *Science*. **114**: 416.
- GARRISON, W. M. & G. K. ROLLEFSON. 1952. Radiation chemistry of aqueous solutions containing both ferrous ion and carbon dioxide. *Discussions Faraday Soc.* **12**: 155.
- GLOCKLER, G. & S. C. LIND. 1939. *Electrochemistry of Gases and other Dielectrics*. Wiley & Sons. New York, N. Y.
- GROTH, W. 1937. Photochemische Untersuchungen im Schumann-ultraviolet. IV. *Z. physik. Chem.* **B37**: 315.
- JACKSON, D. S. & H. I. SCHIFF. 1955. Mass spectrometric investigation of active nitrogen. *J. Chem. Phys.* **23**: 2333.
- LAPPIN, G. R. & L. C. CLARK. 1951. Colorimetric method for determination of traces of carbonyl compounds. *Anal. Chem.* **23**: 541.
- MIGRICHIAN, V. 1947. *The Chemistry of Organic Cyanogen Compounds*. :5. Reinhold. New York, N. Y.
- MILAS, N. A., L. E. STAHL & B. B. DAYTON. 1949. Reactions of hydroxyl radicals with organic compounds. *J. Am. Chem. Soc.* **71**: 1448.

- MILLER, S. L. 1953. A production of amino acids under possible primitive earth conditions. *Science*. **117**: 528.
- MILLER, S. L. 1955. Production of some organic compounds under possible primitive earth conditions. *J. Am. Chem. Soc.* **77**: 2351.
- MILLER, S. L. 1957. The mechanism of synthesis of amino acids by electric discharges. *Biochim. et Biophys. Acta.* **23**: 48D.
- NEIDIG, B. A. & W. C. HESS. 1952. Simultaneous estimation of threonine and serine. *Anal. Chem.* **24**: 1627.
- NOYES, W. A., JR. & P. A. LEIGHTON. 1941. *The Photochemistry of Gases*. Reinhold. New York, N. Y.
- OPARIN, A. I. 1938. *The Origin of Life*. Macmillan. (Republished by Dover, 1953.) New York, N. Y.
- PALIT, C. C. & N. R. DHAR. 1930. Photochemical oxidation by air. *J. Phys. Chem.* **34**: 993.
- RABINOWITCH, E. I. 1945. *Photosynthesis*. **1**: 81. Interscience. New York, N. Y.
- RENSE, W. A. 1953. Intensity of the Lyman-alpha line in the solar spectrum. *Phys. Rev.* **91**: 299.
- RUBEY, W. W. 1955. Development of the hydrosphere and atmosphere with special reference to the probable composition of the early atmosphere. *Geol. Soc. Am. Spec. Papers.* **62**: 631.
- STEIN, W. H. & S. MOORE. 1949. Chromatographic determination of the amino acid composition of proteins. *Cold Spring Harbor Symposia Quant. Biol.* **14**: 179.
- STORCH, H. H., N. GOLUMBI & R. B. ANDERSON. 1951. *The Fischer-Tropsch and Related Syntheses*. Wiley & Sons. New York, N. Y.
- TAYLOR, H. S. 1926. Photosensitization and the mechanism of chemical reactions. *Trans. Faraday Soc.* **21**: 560.
- UREY, H. C. 1952a. *The Planets*. Yale Univ. Press. New Haven, Conn.
- UREY, H. C. 1952b. On the early chemical history of the earth and the origin of life. *Proc. Natl. Acad. Sci. U. S.* **38**: 351.
- WALL, J. S. 1953. Simultaneous separation of purines, pyrimidines, amino acids and other nitrogenous compounds. *Anal. Chem.* **25**: 950.
- WARBURG, O. 1949. *Heavy Metal Prosthetic Groups and Enzyme Action*. :38. Oxford Univ. Press. Oxford, England.
- WIELAND, T., J. FRANZ & G. PFLEIDERER. 1955. Über die Bildung von Aminosäuren aus  $\alpha$ -Keto-aldehyden. *Chem. Ber.* **88**: 641.
- WIELAND, T. & F. JAENICKE. 1955. Der Mechanismus der oxydo-reduktiven Aminierung von  $\alpha$ -Keto-aldehyden. *Chem. Ber.* **88**: 1967.
- WILDE, K. A., B. T. ZWOLINSKI & R. B. PARLIN. 1953. The reaction occurring in  $\text{CO}_2\text{-H}_2\text{O}$  mixtures in a high-frequency electric arc. *Science*. **118**: 43.
- WINKLER, C. A. & H. I. SCHIFF. 1953. Reactions of active nitrogen. *Discussions Faraday Soc.* **14**: 63.
- YATES, W. F. & R. L. HEIDER. 1952. The dissociation of lactonitrile in aqueous solution. *J. Am. Chem. Soc.* **74**: 4153.

#### *Discussion of the Paper*

PHILIP H. ABELSON (*Geophysical Laboratory, Carnegie Institution of Washington, D. C.*): Stanley Miller's contributions in his study of the synthesis of amino acids from mixtures of methane, ammonia, and water have been important and significant. However, there has been a tendency for others to overinterpret his findings. I have heard thoughtful scientists conclude that, because amino acids could be synthesized from such a mixture, the early atmosphere of the earth must have been composed of methane, ammonia, and water.

To explore this suggestion, the effects of electrical discharges on twenty different gaseous mixtures have been studied with equipment similar to that employed by Miller. Various proportions of methane, ammonia, and water; carbon dioxide, nitrogen, and water; carbon dioxide, nitrogen, hydrogen, and water; methane, nitrogen, and water; carbon dioxide, ammonia, hydrogen, and water; and carbon monoxide, nitrogen, hydrogen, and water were tested.

Amino acids, including glycine, sarcosine, alanine, and  $\beta$ -alanine, were synthesized with these compositions, with two notable exceptions. When proportions of methane and ammonia were high with respect to water vapor, and when the mixture of carbon dioxide, nitrogen, and water was employed, amino acids were not produced. In general, the atomic proportions of the components in the other mixtures were chosen to be proportional to those found in glycine; namely, 2-C, 1-N, 2-O, and 5-H. However, it was found that wide variations in carbon-nitrogen ratio could be tolerated.

A tremendous volume of geologic evidence<sup>1</sup> exists concerning the past history of the earth. W. W. Rubey has summarized that part of it that bears on the evolution of the sea and the atmosphere. He concludes that the ocean and air were formed as products of the degassing of the interior of the earth. Evidence for volcanic activity is found in the earliest rocks. Gases associated with present-day eruptions include water, carbon dioxide, nitrogen, carbon monoxide, hydrogen, and sulfur. Condensation and absorption of such a mixture lead to an atmosphere composed of carbon monoxide, nitrogen, hydrogen, and small amounts of water and carbon dioxide.

Further adjustments of composition would occur under the influence of ultraviolet light and in the presence of a reducing crust. Energy associated with electrical discharges is very small in comparison with the energy present in visible and ultraviolet radiation. Later experiments may well show that the major synthetic mechanisms probably involved ultraviolet light.

These investigations will have lasting significance concerning the origin of life insofar as they are related to what, on geologic grounds, was the real primitive world. Ideally, the laboratory should predict or explain compounds that one day will be found in early sedimentary rocks.

#### *Reference*

1. RUBEY, W. W. 1951. Geologic history of sea water. *Bull. Geol. Soc. Am.* **62**: 1111-1148.