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Production of Some Organic Compounds under Possible Primitive Earth Conditions[1]

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A mixture of gases, CH_4, NH_3, H_2O and H_2, which possibly made up the atmosphere of the Earth in its early stages, has been subjected to spark and silent discharges for times of the order of a week to determine which organic compounds would be synthesized. Several designs of apparatus and reasons for their construction are described. Analyses of the remaining gases were made and CO, CO_2, N_2 and the initial gases were found. A red compound that seems to be associated with the trace metals is formed, as well as yellow compounds probably polymers, which have acidic, basic and ampholytic properties. The mixture of compounds is separated into acidic, basic and ampholytic fractions with ion exchange resins. The amino acids are chromatographed on Dowex-50 and the acids on silica. Glycine, *d,l*-alanine, β-alanine, sarcosine, *d,l*-α-amino-*n*-butyric acid and α-aminoisobutyric acid have been identified by paper chromatography and by melting points of derivatives. Substantial quantities of several unidentified amino acids and small amounts of about 25 amino acids are produced, while glycolic, *d,l*-lactic, formic, acetic and propionic acids make up most of the acid fraction. Quantitative estimates of these compounds are given. Evidence is presented that polyhydroxy compounds of unknown composition are present. HCN and aldehydes are direct products of the discharge. Although there is insufficient evidence, the synthesis of the hydroxy and amino acids may be through the hydroxy and amino nitriles in the solution. The relation of these experiments to the formation of the Earth and the origin of life is briefly discussed.

Introduction

The formation of the solar system has been discussed by Urey[3] on the basis of a model that starts with a dust cloud containing the elements distributed in proportion to the cosmic abundances. Two of the principal conclusions of his work are that the planets formed at low temperatures ($<300°$) and that they had reducing atmospheres in their initial stages of formation. The reducing atmosphere would consist of methane, ammonia, water and hydrogen instead of the present oxidizing atmosphere of carbon dioxide, nitrogen, oxygen and water. The equilibria are such that in the presence of excess hydrogen most of the carbon would be as methane and most of the nitrogen would be as ammonia.[4]

That the Earth had a reducing atmosphere in its early stages was proposed by Oparin[5] about 20 years ago, and his arguments have been extended by Bernal[6] and Urey.[3,4] The general geochemical argument is that the ratio of hydrogen to oxygen in the universe is about a thousand to one, with the region of the Earth being rather anomalous. No one has shown any mechanism which would form oxygen in the region of the Earth but not in the region beyond Mars before the planets were formed. The formation of oxidizing conditions on the planets Mercury, Venus, Earth and Mars after the formation of the planets is explained by the escape of hydrogen from these planets. Their atmospheres are at a high enough temperature and the gravitational field is weak enough so that hydrogen and helium are the principal elements that can escape into outer space from the atmosphere.[3] The escape of the strong reducing agent H_2 results in an oxidizing atmosphere. In the region beyond Mars, the combination of low temperature and planets of high gravitational field prohibits the escape of hydrogen from these atmospheres, and so they are still reducing.

While there have been many studies with ultraviolet light and electric discharges on various combinations of compounds, there have been none with methane, ammonia, water and hydrogen taken together. The only work that would have any bearing on the reducing atmosphere would be the experiments of Loeb[7] who obtained glycine by the action of a silent discharge on a mixture of carbon monoxide, ammonia and water.

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(1) Abstracted from a dissertation submitted to the University of Chicago in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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(3) H. C. Urey, "The Planets," Yale Univ. Press, New Haven, Conn., 1952.

(4) H. C. Urey, *Proc. Nat. Acad. Sci., U. S.*, **38**, 351 (1952).

(5) A. I. Oparin, "The Origin of Life," Dover Publications, Inc., New York, N. Y., 1953.

(6) J. D. Bernal, "The Physical Basis of Life," Rutledge, London, 1951; *Proc. Phys. Soc. (London)*, **62A**, 537 (1949); **62B**, 597 (1949).

(7) W. Loeb, *Ber.*, **46**, 690 (1913).

Because of the lack of work on the mixtures of gases that would make up a reducing atmosphere, it was felt that experiments should be carried out to see which organic compounds could be obtained. A preliminary report of this work has appeared,[8] and the present paper will describe further experiments and discuss their relation to the formation of organic compounds on the primitive Earth.

Construction of Apparatus

In order to determine which organic compounds would be present in the oceans of the Earth with a reducing atmosphere, it would be necessary to duplicate all the conditions that would be present on the Earth. Since this is not feasible, an attempt was made to duplicate some of the conditions.

As a basis for discussion the following model of the Earth is postulated: 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°. Urey[3,9] has discussed the reasons for the low-temperature formation of the Earth, and these will not be reviewed here. Organic compounds formed by ultraviolet light and lightning accumulated in the ocean, and the simple compounds formed by these processes reacted with each other, forming more complex compounds. If volcanos are assumed to have been present, the local hot spots synthesized other simple organic compounds and these again accumulated in the ocean and reacted with the compounds formed by the other processes. Reactions catalyzed by inorganic ions, surfaces of oxides and metals formed additional compounds in the ocean. Formation of colloids and other complex aggregates of matter also took place. The complex compounds in the ocean could in turn have been concentrated by evaporation, been subject to pyrolysis, high pressure, ionizing radiations and other forms of energy. Compounds formed by these processes were in turn washed back into the ocean.

As long as the conditions did not destroy the organic compounds and as long as the compounds formed by the various sources of energy got into the ocean, the system with the greatest variety of conditions present would be the most advantageous for forming complex organic compounds.

The sources of energy for the production of the initial compounds were ultraviolet light, electric discharge and heat. Although the level of radioactivity was higher than at present, the energy available was still quite small. Cosmic rays might be a significant source of energy, if for any reasons their intensity was much higher in the past than at present, but it is doubtful that the energy available would compare to that from the sun. At present the Earth receives 1.6, 85, 570 cal./cm.^{2}/yr. for wave lengths below 1500, 2000 and 2500 Å.,[4] respectively. The present energy from lightning[10] is 0.9 cal./cm.^{2}/yr. Thus there would be more energy available from ultraviolet light than from electric discharge if the conditions were the same as at present, and, therefore, ultraviolet light would be the first source of energy to study. However, methane, ammonia, water and hydrogen do not begin to absorb appreciably until 1450, 2400, 1850 and 900 Å., respectively, and carbon monoxide which might be present does not absorb appreciably until 1545 Å.[11] As a result, with the exception of ammonia, it would be necessary to work in the region of 1000 to 2000 Å. to get appreciable absorption. Since one should work with a spectral distribution that approximates the sun, quartz would be unsatisfactory as a window, because quartz absorbs appreciably below 2000 Å. It would therefore be necessary to use fluorite windows to get adequate transmission. There is also the difficulty that the window might become coated with polymer formed by the ultraviolet light, and so an arrangement would be needed to keep the free radicals away from the window. This might be accomplished by means of a stream of gas blowing the free radicals away from the window, but in any case the experimental technique would be difficult.

It is possible that ultraviolet light would not be the principal source of organic compounds. Most of the reactions due to the ultraviolet light would take place in the upper atmosphere and the compounds formed might not get into the oceans before being decomposed by the ultraviolet light. The question is whether the mean life of the compound for destruction is greater or less than the mean life for transport to the ocean. No answer to this question can be given at present. Lightning discharges would take place close to the surface of the Earth, and therefore the organic compounds formed would reach the oceans with less difficulty.

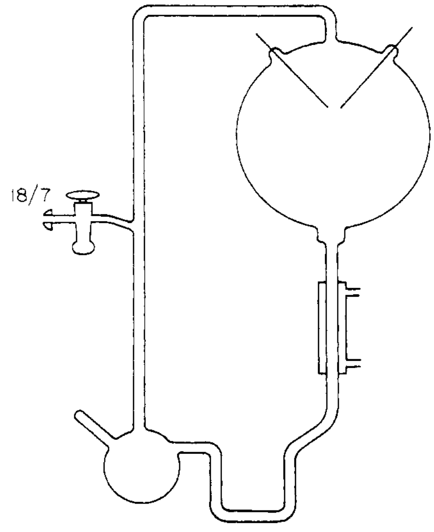

Fig. 1.—Apparatus no. 1.

The flow of the gases past a discharge in a one-pass arrangement is the usual method of operating with electric discharges. However, if we assume oceans to be present on the Earth in its early stages of formation, a single pass system would be a poor approximation to the conditions on the Earth. A better approximation to these conditions is shown in Fig. 1. The apparatus was made of Pyrex except for the tungsten electrodes. The water in the small flask is boiled to promote circulation and to bring water to the vicinity of the spark so that oxygenated organic compounds can be formed. The products of the discharge are condensed and flow into the flask through the U-tube, which prevents circulation of the water in the wrong direction.

In this apparatus most of the heat was lost through the five-liter flask and most of the water was condensed before reaching the condenser. The temperature was about 70–80°. Several runs were made with the flask covered by an inch coat of asbestos to prevent cooling of the gases in the large flask, and no changes in the products were observed. When the apparatus was filled with 10 cm. pressure of hydrogen, 20 cm. of methane and 20 cm. of ammonia, the total pressure in the apparatus varied from 60 to 80 cm. depending on the rate of boiling.

Figure 2 shows a second design of the apparatus. The gases are rapidly circulated past the discharge by means of the aspirator. The condenser, although not of efficient design, cooled the gases to about 35° and the water which was condensed flowed into the boiling flask through the U-tube. The rate of circulation of the gases could be determined by observing the flow of the water droplets in the tube between the condenser and the aspirator. The flow rate was about 3 cm.^{3}/sec., and the gas in the five-liter flask on the average was circulated in a half-hour. With the same mixture of gases as those used in apparatus no. 1 the pressure varied between 80 and 100 cm.

The spark discharge was a high frequency tesla coil that was designed for vacuum leak detection. Because of heating, coils that contain paraffin or other low melting substances were unsatisfactory. Central Scientific Co. #80721 spark coil proved to be quite satisfactory when used

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(8) S. L. Miller, *Science*, **117**, 528 (1953).

(9) H. C. Urey, *Proc. Roy. Soc.* (*London*), **A219**, 281 (1953).

(10) B. F. J. Schonland, "Atmospheric Electricity," Methuen, London, 1953, pp. 42, 63.

(11) W. A. Noyes, Jr., and P. A. Leighton, "The Photochemistry of Gases," Reinhold Publ. Corp., New York, N. Y., 1941.

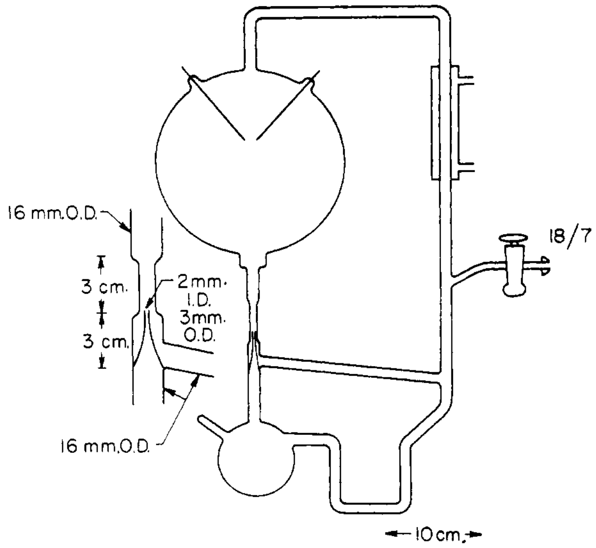

Fig. 2.—Apparatus no. 2.

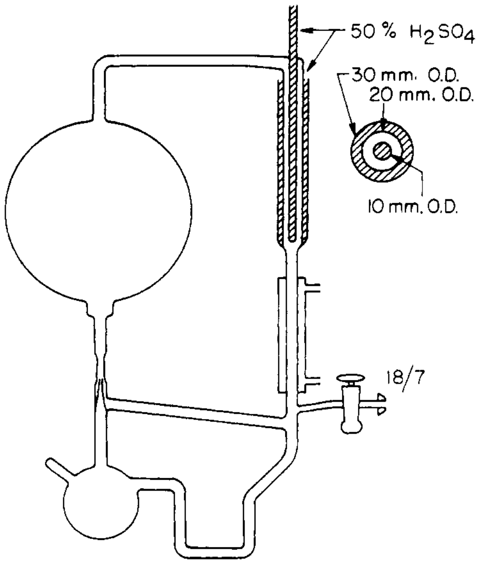

Fig. 3.—Apparatus no. 3.

continuously, although it was not designed for continuous operation. This spark had a high frequency voltage of 60,000 volts. Occasional cleaning of the spark gap was necessary to maintain a strong spark. Because of the success of this spark, no other models of spark coils were used.

The power consumption on the primary side of the spark coil was about 20 watts. Assuming that the coil operated with an efficiency of 50%, the power expended in the gases was 10 watts. Sparking for a week consumed 1.5×10^3 kcal. Since about a gram of organic compounds was synthesized during this time, this is not an efficient process from the standpoint of energy consumption, but by renewing the gases more frequently, greater efficiencies might be obtained.

A third apparatus, shown in Fig. 3, used a silent electric discharge instead of a spark. The aspirator arrangement was used because circulation of the gases would be very poor if the discharge were placed at any point in apparatus no. 1. The power source for the silent discharge was a 15,000-volt transformer. The power consumption on the primary side of the transformer was 120 watts. Assuming an efficiency of 80%, the power delivered to the discharge was 96 watts. A weeks run consumed 1.4×10^4 kcal. Thus the power consumption was greater than in the case of the spark. In addition the production of organic compounds is about one-third that of the spark discharge. The primary cause of the low yield may have been the formation of a steady state reaction mixture of gases in which the production of organic compounds was quite low, but if the hydrogen in the mixture were removed more organic compounds might be formed.

An attempt was made to simulate a lightning discharge by building up a large quantity of charge on a condenser until the spark jumped the gap between the electrodes. The discharge was of high intensity and produced such a large pressure of gases in the apparatus that it would not discharge. Very few organic compounds were produced, and this discharge was not investigated further.

A discharge formed by connecting the 15,000-volt transformer across the electrodes in apparatus no. 2 was tried, and considerable heat was generated. The voltage was reduced as low as possible while still maintaining the spark across the gap. An examination of the products showed a small quantity of glycine but no other ninhydrin-active compounds. The small production of compounds may have been due to the high temperature of the spark, which caused decomposition of any primary organic compounds that were formed.

The choice of temperature and pressure was made on the following basis: The total pressure had to be kept below 1.5 atmospheres because of the danger of breaking the glass. The methane and ammonia were taken in equal amounts so that appreciable quantities of carbon and nitrogen would react in the spark. The uncertainty of the relative amounts of methane and ammonia on the primitive Earth and the uncertainty of the size and temperature of the oceans which would dissolve much of the ammonia make any other choice of relative amounts of gases just as arbitrary.

Hydrogen was added because the experiment was an attempt to simulate a reducing atmosphere. However, hydrogen is produced as the organic compounds form, so that except for the initial stages of the sparking the reducing atmosphere could be simulated without an initial pressure of hydrogen. Several runs were made in the spark discharge apparatus in which no hydrogen was added initially, and the results were the same as when hydrogen was in the initial mixture of gases. Since it was probable that the low yield in the silent discharge was due to the steady state mixture of gases in which the concentration of hydrogen was lower than in the spark discharge, no hydrogen was added to the initial mixture of gases in the silent electric discharge.

The water was boiled and the apparatus kept hot for two reasons. The motion of the steam caused circulation of the gases in apparatus no. 1 and the steam pressure operated the aspirator in apparatus no. 2 and no. 3 which circulated the gases more efficiently. The formation of organic compounds containing oxygen would be aided by the water vapor near the spark.

It would seem also that if the vapor pressure of water were low longer times would be required to form the organic compounds containing oxygen, *i.e.*, the rate of production of these compounds would be roughly proportional to the vapor pressure of the water, so that even at 0° the rate of production would be slower by only two orders of magnitude.

In the first several experiments the apparatus was arranged as in Fig. 1 except that the electrodes and the condenser were interchanged. This resulted in a vapor pressure of the water of about 2.5 cm. A considerable quantity of hydrocarbons was formed and only a very small quantity of amino acids. It would seem then that with a lower pressure of water, longer chain organic compounds might be formed, although somewhat more slowly than with higher pressures of water vapor.

After each run the apparatus was washed out by shaking with hot concentrated sodium hydroxide to remove the silica scale that had formed, hot dichromate–sulfuric acid cleaning solution to oxidize any remaining organic material, followed by boiling water in the sealed apparatus for 24 hours to leach the sides of the apparatus. Fresh water was then added to the flask and the side arm sealed off.

The apparatus was pumped down to about 20 mm. pressure, at which the water would begin to boil. After several minutes of boiling to make certain that all the dissolved gases in the water had been removed, the apparatus was flushed several times with hydrogen, pumping out each time until the water boiled. Then hydrogen was added to the desired pressure, followed by adding methane to the desired pressure. Ammonia then was added rapidly to the required pressure. The pressure was observed to fall slowly as the ammonia dissolved in the water. This method is at best approximate, and so in several runs the desired amount of gas was measured in a flask of known volume and a Toepler pump was used to pump it into the apparatus.

The purity of the ammonia was 99.95% and the purity of the hydrogen 99.8% with the impurity consisting of moisture. The purity of the methane was 99.0% with ethane, nitrogen and carbon dioxide as impurities. The gases were used without further purification except that in several runs they were washed in a gas bubbler containing a sintered disk prior to admission to the system.

After the system was filled with gases and the stopcock closed, the water was brought to a boil and the sparking started. The spark in apparatus no. 1 and no. 2 would jump the gap if the ends of the electrodes were 10 mm. apart or less. The spark was purple. In addition to sparking between the ends of the electrodes, small brush discharges occurred along the electrode. At the tungsten–Pyrex seal the glass was curved around the electrode as shown in Figs. 1 and 2. Some sparking occurred between the tungsten and the glass, and deposits of organic material sometimes were found on the glass at this point. At no time did the spark puncture the glass.

About 3 or 4 mg. of yellow-brown organic polymer formed on the electrodes. Although part of this polymer could be dislodged by shaking water in the apparatus, most of it could be removed only by oxidation with hot dichromate–sulfuric acid. By collecting the dislodged polymer from several runs enough was obtained for a microanalysis which gave C, 37.40; H, 5.01; N, 7.54; ash, 42.18. This represents an average composition of $C_{10}H_{16\cdot0}N_{1\cdot73}O_{1\cdot58}$ when corrected for the rather large ash content, which was silica that washed down with the polymer. The sharp corners of the electrodes became rounded after use because of sputtering of the tungsten, and this tungsten was found among the trace elements present in the suspended silica described later.

With the silent electric discharge there was some sparking across the gap when the primary voltage was between 120 and 135 volts. This could be avoided by operating between 80 and 110 volts. After two weeks of operation (a new charge of gas being added after the first week), the interior of the discharge tube had become coated with organic material. No sparking occurred and little current seemed to be passing through the gap. After extensive cleaning with the dichromate–sulfuric acid the apparatus resumed satisfactory operation.

After a day of sparking the solution became yellow and some colloidal silica had formed in the boiling flask. The ammonia is sufficiently basic to etch the Pyrex. Blank runs without the spark gave equal quantities of silica. By the end of the week there was about 1.5 g. of silica in the flask and the yellow color had been largely obscured by the white silica. A small amount of red color formed, mostly on the silica in apparatus no. 1, but there was no red color formed in apparatus no. 2 and no. 3.

Analysis of Products

Gas analyses were performed on a Burrell build-up model gas analyzer. The ammonia was absorbed into 50% H_2SO_4, the H_2 and CO determined by oxidation over CuO, and the CH_4 by oxidation with a palladium catalyst and N_2 by difference. Because of the uncertainty of the NH_3 due to its solubility in the solution, the values are reported as percentages of the sample after the NH_3 had been absorbed into the H_2SO_4. Carbon dioxide could be detected in the solution by precipitation with barium hydroxide. Quantitative measurements were not made, but the amount present in a run was about 100 mg. Table I gives the analyses for the three apparatuses.

TABLE I

GAS ANALYSES

	Apparatus 1[a]	Apparatus 2[a]	Apparatus 3[b]
H_2, %	74.6	76.3	50.6
CO, %	10.0	5.8	1.2
CH_4, %	10.4	9.5	39.5
N_2, %	5.0	8.4	8.7
NH_3,[c] %	8.6	10.5	3.7
C as org. compds.[d] %	53	58	22

[a] Initial pressure of H_2, CH_4 and NH_3 was 10, 20 and 20 cm., respectively; final pressure in cooled apparatus was 50 cm. [b] Initial pressure of both CH_4 and NH_3 was 20 cm.; final pressure in cooled apparatus was 40 cm. [c] Percentage NH_3 calculated with the formula $100P_{NH_3}/(P_{H_2} + P_{CO} + P_{CH_4} + P_{N_2})$. [d] Calcd. from 100(initial P_{CH_4} − final $P_{CO + CH_4}$)/(initial P_{CH_4}).

From these values one sees that the spark discharge was more efficient in forming organic compounds than the silent electric discharge, and that the apparatus that rapidly circulated the gases was only slightly more efficient than the slower circulating apparatus. If one considers the equilibrium conditions for the reaction

$$CH_4 + H_2O = CO + 3H_2$$

the pressures observed correspond to 900°K. for the final mixture in the spark discharge and to about 750°K. for the final mixture in the silent discharge. The equilibrium pressure of carbon dioxide at this temperature would be about one atmosphere. Although there is some carbon dioxide produced, the amount is far from the equilibrium pressure.

Wendt and Evans[12] report a steady state formed with the reaction $2CO + 2H_2 = CH_4 + CO_2$ in the corona discharge corresponding to an equilibrium temperature of 900–950°K. Lunt[13] reports a steady state formed with the reaction $CO_2 + H_2 = CO + H_2O$ corresponding to temperatures of 800–1200°K. These values are in approximate agreement with the temperatures calculated for this system.

The residual gases from the cold apparatus were pumped off, care being taken to pump the noxious gases out of the room. The solution was removed from the apparatus after blowing a hole in the side arm of the small flask. Attempts to remove the silica by filtration or with low speed centrifuges were unsuccessful. An International Equipment Co. centrifuge with a 6-inch head and operated at 5000 r.p.m. brought down most of the silica, which formed a cake at the bottom of the centrifuge tube. The silica was washed with water and centrifuged again to recover as much of the organic compounds as possible. It was then dried in a vacuum desiccator over P_2O_5 and a micro-analysis using potassium dichromate as fusing agent showed the presence of 0.49% carbon and 0.73% nitrogen. The total silica from this run was 1.5 g., and so 7.3 mg. of carbon and 11 mg. of nitrogen were lost by retention on the silica.

The color of the solution from all three types of apparatus was yellow, and the ultraviolet absorption was very intense and contained no peaks above 230 mμ. The yellow is due to the tail of the absorption. The red color was found mostly with the silica and settled as a red layer at the bottom of the silica in the centrifuge tube. Only a small amount of red color was found in apparatus no. 1 compared with the first apparatus (apparatus A) used in this research which has been described previously.[8] The color was reproducible in apparatus A although after about 5 runs the amount of colored material was somewhat less. The use of tap distilled water instead of water redistilled from alkaline permanganate did not change the yield of red color.

The red layer of about 10% of the silica was separated from the white layer and a trace element analysis of each fraction was done.[14] Silicon, boron and aluminum were major constituents. The average of Hg, Zr, Mn, Mg, Fe, Pb, Co, Cu and Ti from the red and white layers of the silica from apparatus A was 0.02 and 0.006%, respectively. The average of these elements from the red and white layers from apparatus no. 1 was 0.007 and 0.001%, respectively. Tungsten was 0.1 and 0.5% of the red silica from apparatus A and no. 1, respectively, and no tungsten was detected in the white layer. Therefore it will be argued that the red color is either associated with one or more trace metals, or is adsorbed on the same particles of silica as the trace metals. Since the amount of red color produced is greater in apparatus A, which has the greatest amount of trace metals, the red color is either complexed with the metals or its synthesis is catalyzed by the metals. Consistent with this explanation was the fact that the color production became less after continued use of the apparatus, which would be explained by the leaching of the metals from the glass.

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[12] G. I. Wendt and G. M. Evans, THIS JOURNAL, **50**, 2610 (1928).

[13] R. W. Lunt, *Proc. Roy. Soc.* (*London*), **108A**, 172 (1925).

[14] The analysis was performed by A. Tompkins of the Institute for the Study of Metals.

A small amount of the red color could be extracted from the silica by continuous extraction with *n*-butyl alcohol. The absorption spectrum showed peaks at 390 and 540 mμ. Not enough material could be obtained for a chemical analysis. Prof. H. Klüver of the Biology Department kindly examined the solution to see if the red color were a porphyrin, or if there were any porphyrins present. Using very sensitive fluorescent techniques he was able to find none.

The yellow compounds had various degrees of acidity and basicity because different fractions of these compounds were adsorbed by cation but not anion resins, anion but not cation resins, and both cation and anion resins. A large fraction of the colored material was found in the acidic amino acid fraction. Some of this could not be removed from the weakly basic and strongly basic ion exchange columns even after extensive elution with HCl. The amount of material absorbed on the strongly acidic resin, Dowex-50, could not be estimated because this resin is black.

Organic solvents did not extract any appreciable quantity of the color. Because the amino acids follow along with these compounds and because there is leakage of organic material from the ion exchange resins, micro-analyses of the material were not performed. The amino acids were shown to be present by evaporating the solution to dryness *in vacuo* and running two-dimensional paper chromatograms. This procedure reveals substances other than amino acids and is not quantitative. In addition, to reveal substances in low concentration requires overloading the paper with respect to the glycine and alanine.

A separation procedure involving steam distillations and ether extractions from the mixture of compounds made basic with barium hydroxide and acid with sulfuric acid, followed by separations on various ion exchange resins, was tried. This procedure gave trouble because of the elevated temperatures involved and the losses by adsorption on the barium sulfate.

The procedure adopted is shown in Fig. 4. This procedure follows that of Carsten,[15] which desalts the amino acids in urine by use of a cation resin followed by an anion resin, and then performs the chromatographic separations of the amino acids on Dowex-50. This procedure was modified by absorption of the dicarboxylic amino acids on a weakly basic anion exchange resin in the acetate form at a *p*H of 5.0.[16] The use of weakly basic resins to absorb the acids follows the procedure of Haas and Stadtman.[17] Dowex-50, Dowex-2 and Amberlite IR 4B are ion exchange resins of the strongly acidic, strongly basic and weakly basic types, respectively. The Dowex-2 column at C in Fig. 4 contained 46 cc. of resin. The Dowex-50 columns at A, J and K contained 146, 11 and 512 cc., respectively. The IR 4B columns at E and H contained 12 and 46 cc., respectively.

Table II gives the weights of the various fractions. The evaporations were carried out in a flash still with the temperature always below 40°, and the fractions were dried over NaOH and $CaCl_2$ in a vacuum desiccator. The weights given in the table should be taken only as an upper limit, since the fractions were not dried by heating. There is leakage from the organic ion exchange resins which probably was not significant in the case of the Dowex resins, but may have been significant with the IR 4B. Runs 1, 2 and 3 were run in apparatus 1, 2 and 3, respectively. Run 3 is a combination of four runs and the carbon value is for the sum of the four runs. These data were not obtained for run 2 because the separation was by extraction and steam distillations, followed by adsorption on Dowex-2, Amberlite IRC 50 (a weakly acidic resin), and then chromatographing the amino acids on Dowex-50.

TABLE II

WEIGHTS OF COMPOUNDS OBTAINED IN THE SEPARATION

Fraction	Place in Fig. 4	Run 1, mg.	Run 3, mg.
Amino acids and bases	B	352	603
Amino acids (HCl salts)	D	307	442
Amino acids[a]	D_a	218	315
Neutral and basic amino acids	E	181	281
Acidic amino acids	F	85	43
Bases	C	58	218
Sodium salts of volatile acids	L	355	474
Non-volatile acids and part of boric acid	J		974
Loses on IR 4B (D–E–F)		41	118
Loses on Dowex 2 (B–D_a–C)		76	70
Carbon placed in apparatus as CH_4		710	4200
Carbon less carbon of residual gases		376	924

[a] D_a is calculated from D assuming average mol. wt. of 90.

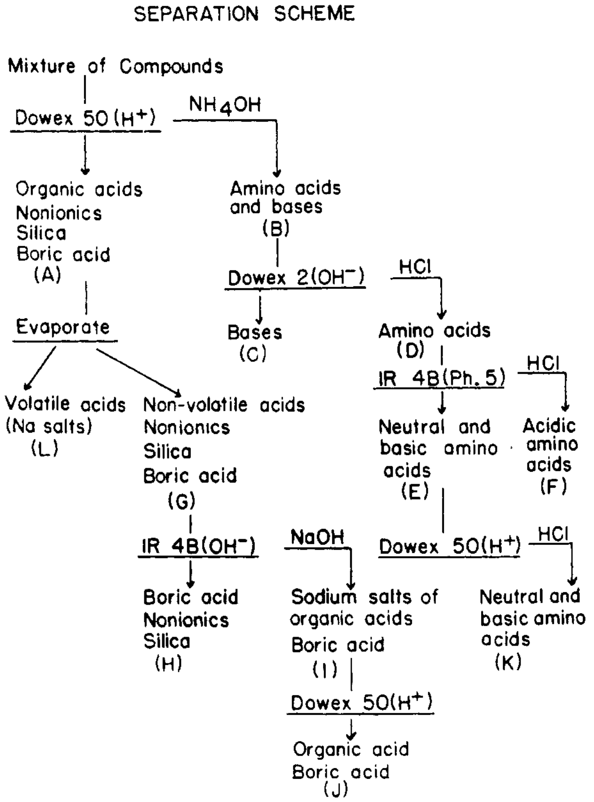

Fig. 4.

Some of the silica that was not centrifuged down was found at A in Fig. 4. Only small quantities of ninhydrin-reactive compounds were present in fraction A. There was a significant test for carbonyls in this fraction using the 2,4-dinitrophenylhydrazine procedure of Lappin and Clark.[18] In the step to obtain the acids, the Dowex-50 was washed with five bed volumes of ion free distilled water because it has been reported that organic acids are retained by cation resins unless washed thoroughly with water.[19]

The volatile acids in the effluent at A were separated from the non-volatile acids by evaporating the effluent under reduced pressure, the volatiles being evaporated into NaOH to prevent any loss. The sodium salts of the volatile acids were then evaporated down and dried over $CaCl_2$. The HCN and CO_2 in the initial solution was found in this fraction, and the HCN interfered to some extent with the chromatography of the organic acids. Therefore the cyanide was removed by precipitation with silver nitrate. Care was taken not to add an excess of Ag^+ which would oxidize the formate to CO_2.

The non-volatile acids were contaminated on the average by 0.3 g. of boric acid which was leached from the Pyrex of the apparatus by the ammonia. It was shown to be boric acid by the characteristic green flame of methyl borate,

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[15] M. E. Carsten, THIS JOURNAL, **74**, 5954 (1952).

[16] C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **195**, 669 (1952).

[17] V. A. Haas and E. R. Stadtman, *Ind. Eng. Chem.*, **41**, 983 (1949).

[18] G. R. Lappin and L. C. Clark, *Anal. Chem.*, **23**, 541 (1951).

[19] O. Samuelson, "Ion Exchangers in Analytical Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1953, p. 21.

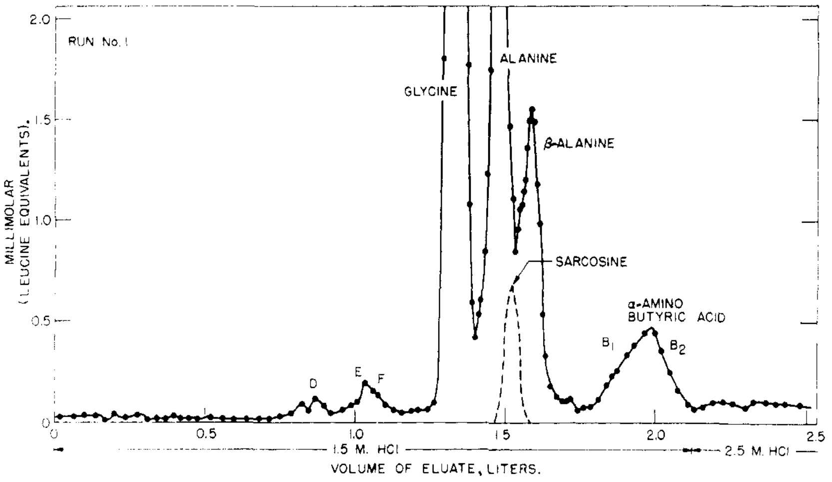

Fig. 5.—Chromatography of amino acids from run 1.

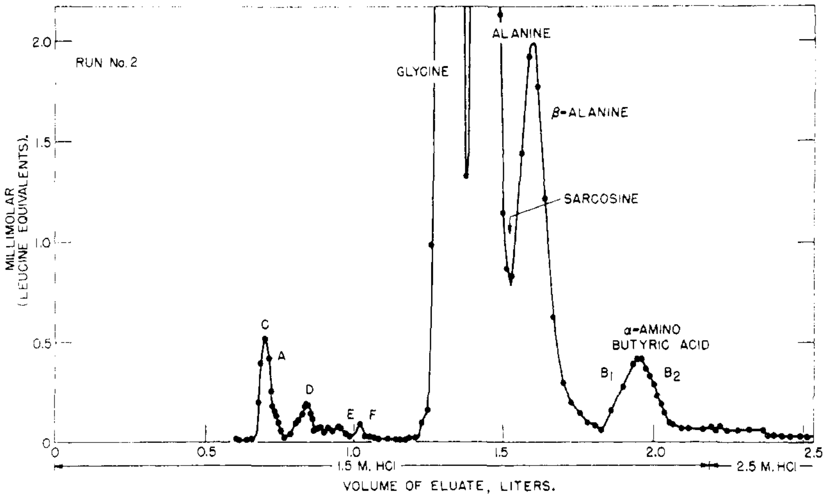

Fig. 6.—Chromatography of amino acids from run 2.

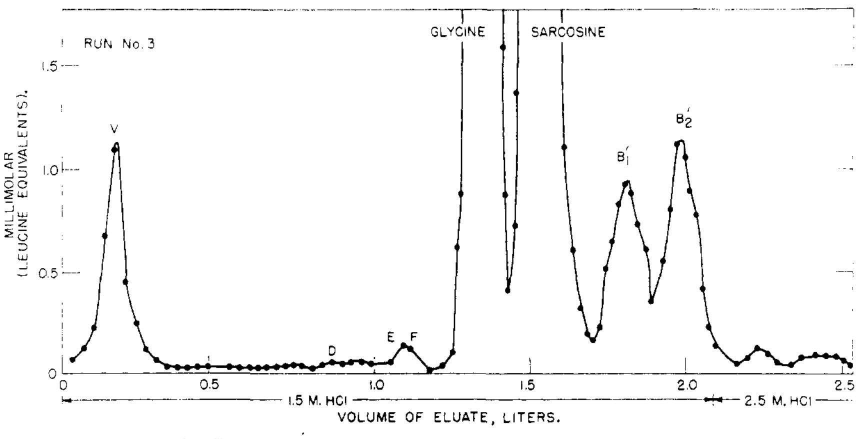

Fig. 7.—Chromatography of amino acids from run 3.

and by its equivalent weight of 62 when titrated with NaOH in the presence of excess glycerol to a phenol red end-point. An attempt was made to remove the boric acid by absorbing the organic acids on IR 4B in the hydroxide form, while boric acid and silicic acid would not be retained ordinarily since their ionization constants are less than 10^{-9}. However, after elution of the organic acids with NaOH and converting them to the free acids by passing them through a column of Dowex-50(H^+), a considerable fraction of the boric acid was found to be in this fraction. Therefore, some of the boric acid must have been retained on the IR 4B column. This retention is attributed to the presence of polyhydroxy compounds which make boric acid a strong acid. Organic extraction of the acids was not tried because there were acids present with distribution coefficients unfavorable for extraction.

Only a small amount of basic amino acids was found in run 2 when these amino acids were absorbed on a weakly acidic resin (Amberlite IRC 50). A weakly acidic resin at a *p*H of 4.7 will take up the basic amino acids but not the acidic or neutral amino acids.[20] The basic amino acids were eluted from the column with HCl and, after desalting, paper chromatograms were run. No amino acids were present in large amounts, but several amino acids were present in very small quantities. Because this procedure separated out only minor constituents of the mixture, and also introduced NaCl into the sample, a weakly acidic column was not used at point D or E in Fig. 4.

This procedure as shown in Fig. 4 resulted in the loss of volatile organic compounds that were not ionic. Volatile bases were lost at B when this fraction was evaporated to dryness, and bases stronger than ammonia were lost on the initial Dowex-50 column. Rather than elute these bases off with sodium hydroxide, these strong bases could be obtained together with the volatile amines by placing the unknown solution on a strongly basic resin in the hydroxide form. The boric acid, silicic acid, organic and amino acids and most of the polyhydroxy compounds would be adsorbed on this column, and the bases and non-ionic organic compounds would appear in the effluent.

The ferric chloride test on a sample of the unknown that had been concentrated to a small volume gave a red color but no violet color. The red color could have been due to acetic acid or other components in the mixture. A biuret test was performed on a concentrated solution of the unknown and a green color was obtained. Since this may have been due to the superposition of the biuret color on the yellow of the unknown, the color was read on a Beckman spectrophotometer with the unknown solution as comparison to cancel out the yellow color. Only the blue color due to the amino acids was observed. Because the test is not known to be specific for peptides, and because of the inconclusive test, little can be said about the presence of polypeptides in the solution, except that they are not present in large amounts.

Separation of Fraction E

The amino acids in fraction E were separated by the procedure of Moore and Stein[21] in which the amino acids are

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[20] J. C. Winters and R. Kunin, *Ind. Eng. Chem.*, **41**, 460 (1949).

[21] S. Moore and W. H. Stein, *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 179 (1950).

eluted from Dowex-50 in the H^+ form. The procedure was changed only in that the 2.5 M HCl was added after the α-aminobutyric acid had passed into the effluent instead of after the glutamic acid. The estimation of the amino acid concentration was by the colorimetric procedure of Moore and Stein.[22] Their procedure was altered by heating for 30 minutes instead of 15 minutes to develop further the color of the non-α-amino acids.

Figures 5, 6 and 7 show the elution diagrams of the amino acids produced in the three different apparatuses. The major peaks are labeled, and the paper chromatography of the amino acids in these peaks is shown in Fig. 8. The ion exchange columns were not exactly the same size, so the glycine peak was placed at 1.33 liters of HCl for ease of comparison. The other volumes were obtained by multiplying the volume at a given tube by the correction 1.33/(liters of eluant at glycine peak). The chromatography beyond 2.5 liters is not shown in the figures because no compounds could be detected with the quantitative ninhydrin test.

There seems to be only one compound in the glycine peak. The glycine was identified by paper chromatography and by preparing the *p*-toluenesulfonate,[23] m.p. 147.0–148.0.°[24] A mixed m.p. with an authentic sample of glycine *p*-toluenesulfonate, m.p. 146.5–147.5°, was not depressed.

The alanine was identified by paper chromatography and by the *p*-toluenesulfonate, m.p. 140.0–140.5°. The m.p. of an authentic sample of *d,l*-alanine *p*-toluenesulfonate was 140.0–141.0°, and there was no depression of the mixed melting point. *l*-Alanine *p*-toluenesulfonate melts at 133°,[25] showing the alanine produced in this system is the racemic form.

The alanine was checked directly for optical rotation. A solution of 80 mg. of the alanine produced in these experiments in 20 ml. of 6 M HCl gave a rotation of 0.000 ± 0.003 degree in a 2-dm. tube. Since the specific rotation of $d(-)$-alanine in 6 M HCl is $-14.5°$,[26] the rotation of the alanine would have been 0.12° if it had been either pure enantiomorph. If there had been rotation, it would have been ascribed to the action of living organisms, for there is no possibility of an asymmetric synthesis in this system.

The alanine peak contains sarcosine (N-methylglycine). The dotted line in Fig. 5 is an estimate of the position and height of the peak. It is seen to lie on the right side of the alanine peak and extends slightly into the left side of the β-alanine peak.

In run 3 the second large peak is mostly sarcosine and contains some alanine and some β-alanine. The sarcosine was identified by paper chromatography and by preparing the phenylhydantoin from phenyl isocyanate,[27] m.p. 109.8–110.2°. A mixed m.p. with an authentic sample of 1-methyl-3-phenylhydantoin, m.p. 109.5–110.3°, was not depressed.[28] Also present in the alanine peak is a small amount of α-aminoisobutyric acid which was identified by paper chromatography (it appears in the same spot as α-amino-*n*-butyric acid). This compound was separated from the alanine by elution from a column of Dowex-50 with a 0.2 M ammonium formate buffer (*p*H 3.2, 40% ethanol).[16] The phenyl hydantoin melted at 170.0–171.0°, and the mixed m.p. with an authentic sample of 3-phenyl-5,5-dimethylhydantoin, m.p. 170.0–171.5°, was not depressed.

The β-alanine was identified by paper chromatography and by preparing the *p*-toluenesulfonate, m.p. 121.0–122.0°. A mixed m.p. with an authentic sample of β-alanine *p*-toluenesulfonate, m.p. 121.5–122.5°, was not depressed (lit. 119.5–121°).[29]

The peak labeled α-aminobutyric acid is quite broad, indicating that there are probably several compounds present. The principal amino acid is d,l-α-amino-*n*-butyric acid, which was identified by paper chromatography and by preparing the phenylhydantoin, m.p. 123.0–124.0°. A mixed m.p. with an authentic sample of *d,l*-3-phenyl-5-ethylhydantoin, m.p. 123.5–124.5°, was not depressed (lit. 126–127° dec.).[30] On the left side of this peak is a compound B_1 and on the right side compound B_2. These two compounds have similar paper chromatographic behavior; they require heat for appreciable development with ninhydrin and have a reddish-purple color similar to sarcosine. These compounds do not seem to be aminobutyric acids, and it may be that they are isomers of aminobutyric acid. This is suggested by the fact that sarcosine, which is isomeric with alanine, appears very close to alanine in the elution from Dowex-50. In addition the N-substituted amino acids have larger R_f values in phenol than the isomeric α-amino acid. N-Methylalanine and N-methyl-β-alanine are possibilities for these compounds.

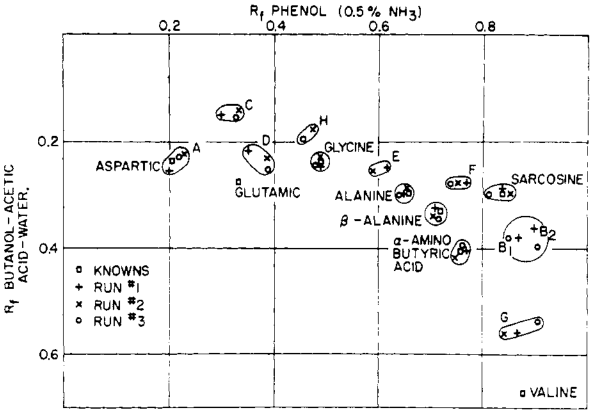

Fig. 8.—Paper chromatography of the amino acids.

In peak C,A of run 2 there are two principal compounds. There is about five times as much C as A in the C,A peak. Compound H may be the asymmetry on the right of the peak. These compounds were shown to be present in the acidic amino acid fraction in runs 1 and 3 by paper chromatography. Since the only amino acids reported to be taken up on ion exchange resins of the weakly basic type are dicarboxylic amino acids, these compounds are taken to be of this type. Compound A is tentatively identified as aspartic acid because its paper chromatographic behavior is identical with known aspartic acid, and it appears in the ion exchange eluant in the same spot that aspartic acid would. When the chromatograms are developed with ninhydrin in a solution of ethyl alcohol containing 25% glacial acetic acid and 3% collidine[31] instead of ninhydrin dissolved in butanol, compound A has the same green color as aspartic acid during the initial development of the color. The color turns purple after a day. Compound C was mistakenly identified as aspartic acid in the first report of this research.[8] The mistake in identification seems to have been due to inert salts in the unknown amino acids interfering with the flow of the solvents.

Peak D contains principally compound D. This amino acid is not dicarboxylic, because it is not retained on the weakly basic ion exchange resin but appears as the small peak D in run 1 and can be shown to be present in run 3 upon evaporation of the effluent in the region marked D and upon running paper chromatograms. Several other compounds were present in this peak, but to a smaller extent.

In run 1 there is about three times as much E as F, while in run 3 there is about three times as much F as E. In run 2 both these compounds are present but to a smaller extent. These compounds are not retained on ion exchange resins of the weakly basic type, and hence are not acidic amino acids.

The large peak V which appears after the passage of a quantity of acid equal to the interstitial volume of the resin in run 3 does not react with ninhydrin on paper. This may be due to compounds, probably polymers, that react with ninhydrin only under the reducing conditions of the quantitative ninhydrin procedure. Compound G, which ap-

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(22) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(23) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 182.

(24) All melting points are corrected.

(25) E. W. McChesney and W. K. Swann, Jr., THIS JOURNAL, **59**, 1116 (1937).

(26) M. S. Dunn, M. P. Stoddard, L. B. Rubin and R. C. Bovie, *J. Biol. Chem.*, **151**, 241 (1943).

(27) N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," T. Y. Crowell Co., New York, N. Y., 1947.

(28) Sarcosine is compound A of ref. 8.

(29) R. W. Holley and A. D. Holley, THIS JOURNAL, **71**, 2131 (1949).

(30) A. Mouneyrat, *Ber.*, **33**, 2395 (1900).

(31) A. L. Levy and D. Chung, *Anal. Chem.*, **25**, 396 (1953).

pears near valine in the paper chromatography, is present in all three runs. Paper chromatography shows that a considerable amount is present, but the amount is below the limits of detectability with the quantitative ninhydrin analysis of the Dowex-50 eluant.

In addition to the compounds described there were other compounds present in less than 0.1-mg. amounts that were revealed by paper chromatography. There were 20 compounds in run 1, 35 compounds in run 2, and 16 compounds in run 3. These compounds were not investigated further.

Table III shows the yields of the amino acids from the three runs. The values are corrected to the total yield for a single run, except for run 3 where the yield is for a combination of four runs. The values of the principal peaks are accurate to 10% and an error of 50% is likely in the estimated compounds. The color yield for β-alanine was 0.70 leucine equivalent and for sarcosine the color yield was 0.95 leucine equivalent. Compounds B_1 and B_2 were assumed to give the same color yield as sarcosine. The entries in Table III have been corrected for the color yield.

TABLE III

YIELDS OF AMINO ACIDS

Amino acid	Moles × 10^5	Mg.	Mole ratio (glycine = 1)	Yield, %
Run 1				
Glycine	63	47	1.00	2.1(4.0)
Alanine	34	30	0.54	1.7(3.2)
Sarcosine[b]	5	4	.08	0.3(0.5)
β-Alanine	15	13	.24	.8(1.5)
B_1[b]	1	1	.02	.07(0.13)
α-Aminobutyric acid[b]	5	5	.08	.3((0.6)
B_2[b]	3	3	.05	.2(.4)
Run 2				
Glycine	55	41	1.00	1.8(3.2)
Alanine	36	32	0.65	1.8(3.2)
Sarcosine[b]	2	2	.04	0.1(0.2)
β-Alanine	18	16	.33	1.0(1.8)
B_2[b]	0.1	0.1	.002	0.01(0.02)
α-Aminobutyric acid	3.0	3.0	.054	0.2(0.34)
B_1[b]	0.4	0.4	.007	0.03(.05)
Run 3				
Glycine	80	60	1.00	0.46(2.1)
Alanine[b]	9	8	0.11	.08(0.3)
Sarcosine	86	77	1.07	.74(3.4)
β-Alanine[b]	4	3	0.05	.03(0.1)
B_1	12.5	12.9	.16	.14(.63)
α-Aminobutyric acid[b]	1	1	.01	.01(0.05)
B_2	14.7	15.2	.18	.17(.76)

[a] The first figure is the yield based on the carbon placed in the system as methane, and the figure in parentheses is the yield based on the carbon laid down as organic compounds.
[b] Approximate values.

For run 1 the table lists 126 × 10^{-5} mole and 103 mg. of amino acids, or 149 mg. of amino acids as the hydrochlorides. Since 181 mg. of amino acids as the hydrochlorides was placed on the column, 32 mg. is not accounted for. Similarly in run 3, the table lists 207 × 10^{-5} mole and 177 mg. of amino acids, or 253 mg. of amino acids as the hydrochlorides. Since 281 mg. of amino acids as the hydrochlorides was placed on the column, this leaves 28 mg. not accounted for. The material not accounted for consists of compounds that are not ninhydrin reactive, compounds in small concentration, polymers, and water from incomplete drying of the sample before placing it on the column.

Fraction F. Acidic Amino Acids

Much of the weight recorded in Table III is due to the yellow polymer from the apparatus. Some of it may be from the resin, but no more than a few per cent. This fraction was tested by paper chromatography and the behavior is shown in Fig. 8. Compound C was present in greatest amount and compound A was also present in both runs 1 and 3. Compound H was present in runs 1 and 2 but was not observed in run 3.

Fraction C. Weak Bases

The bases were not analyzed except for an attempt to precipitate urea nitrate. Since no crystals were obtained it is concluded that there is not a large amount of urea in the sample.

Fraction J. Non-volatile Acids

The non-volatile acids were chromatographed using the silica gel procedure of Bulen, *et al.*[32] In this procedure the stationary phase is 0.5 *N* H_2SO_4 adsorbed on the silica and the mobile phase is various concentrations of butyl alcohol in chloroform, and the fractions are titrated with 0.01 *M* NaOH to a phenol red end-point. Two major peaks were present. One was identified as glycolic acid by the position of the peak in the chromatogram and by decarboxylation of this compound in 90% H_2SO_4 to yield formaldehyde, which gives a purple color with the specific reagent chromotropic acid.[33] The *p*-toluidide, prepared in the usual manner,[27] melted at 141.5–143.2°, and the mixed melting point with an authentic sample of glycolic *p*-toluidide, m.p. 141.5–143.0°, was not depressed. The other peak was identified as lactic acid by its position in the chromatogram and by decarboxylation of this compound in 80% H_2SO_4 to give acetaldehyde, which then reacts with *p*-hydroxybiphenyl to give a purple color.[34] The *p*-toluidide of this acid melted at 106.0–107.0°, and the mixed melting point with an authentic sample of *d,l*-lacto-*p*-toluidide, m.p. 106.0–107.0°, was not depressed.

There were several other small peaks, but these were not investigated. Between the two large peaks the titration was quite difficult because the end-point did not change sharply from yellow to red, but turned orange fairly sharply and gradually became red as more hydroxide was added. This is ascribed to the polyhydroxy complexes of the boric acid that were carried along with the acids.

Fraction L. Volatile Acids

The volatile acids were chromatographed using the same procedure as with the non-volatile acids. The principal acids present were formic, acetic and propionic. The higher aliphatic acids were only about 5% that of the propionic acid. The formic acid was identified by the position of the peak in the chromatogram, and by reducing the formic acid to formaldehyde with magnesium and treating the formaldehyde with chromotropic acid.[33] The anilide melted at 46.5–47.2°, and the mixed melting point with an authentic

TABLE IV

YIELDS OF ACIDS

Acid	Moles × 10^5	Mg.	Yield, %[a]
Run 1			
Formic	233	107	3.9(7.4)
Acetic	15.2	9.1	0.5(1.0)
Propionic	12.6	9.1	0.6(1.2)
Glycolic	56	42	1.9(3.5)
Lactic	39	35	1.8(3.4)
Run 3			
Formic	149	69	0.4(2.0)
Acetic	135	81	.7(3.5)
Propionic	19	14	.2(0.7)
Glycolic	28	21	.2(0.7)
Lactic	4.3	3.9	.03(0.2)

[a] The first figure is the yield based on the carbon placed in the system as methane, and the figure in parentheses is the yield based on the carbon laid down as organic compounds.

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(32) W. A. Bulen, J. E. Varner and R. C. Burrell, *Anal. Chem.*, **24**, 187 (1952).

(33) S. Dagley and A. Rodgers, *Biochem. et Biophys. Acta*, **12**, 591 (1933).

(34) F. Feigl, "Qualitative Analysis by Spot Tests," 3rd Edition, Elsevier Publishing Co., New York, N. Y., 1946, p. 400.

sample of formanilide, m.p. 46.2–47.0°, was not depressed. The recovery of formic acid on these columns was about 90%. The acetic acid was identified by the position of the peak in the chromatogram and by preparing the anilide, m.p. 113.0–113.5°. The mixed m.p. with an authentic sample of acetanilide, m.p. 113.5–114.0°, was not depressed.

Table IV shows the yields of the acids from runs 1 and 3. The glycolic and lactic acid are accurate to about 10%, while the aliphatic acids may be in greater error because of the HCN that interfered with the chromatography on silica.

Synthesis of Compounds Identified

There is the question of whether the compounds observed in this system were synthesized by microorganisms. To check this point, blank runs with the same gases but no spark were made, and the amino acid production was less than 10 micrograms. Since over 100 mg. was produced by the spark, the ratio is greater than 10^4. There is the possibility that microörganisms might synthesize the amino acids from the products of the discharge, so that the significant portion of the synthesis was still by microörganisms. To check this point the apparatus was cleaned in the usual manner, checked carefully for leaks, filled with water and the reduced gases, and the tube connecting the stopcock and the apparatus heated and drawn closed. The whole apparatus was placed in an autoclave for 18 hours at 25 lb. pressure (130°). The spark was then run for a week, and the apparatus was opened by cutting a side arm on the side of the apparatus opposite that from which the stopcock had been removed. The glass seal made before placing the apparatus in the autoclave was checked with a spark and found to be good. The production of amino acids was the same with this procedure as when the apparatus was not placed in the autoclave. In addition the apparatus was run at elevated temperatures, and ultraviolet light from the electric discharge irradiated parts of the apparatus. Most biological syntheses yield optically active compounds, and the alanine was shown to have no rotation. Furthermore, the organic compounds produced are among the simplest and do not represent the distribution one would expect if produced by living organisms. For these reasons it is stated with confidence that the organic compounds in this system were synthesized without the aid of microörganisms.

The next problem in attempting to understand the chemistry of the system is to determine which compounds are formed in the electric discharge, and which reactions are taking place in the solution phase of the system and the gas phase reactions not in the region of the spark. The following alternative hypotheses will be made for the synthesis of the products. 1. Formed in the electric discharge: HCN, amines, aldehydes, alcohols, most of the volatile acids, acrylonitrile, polymers, nitriles. Formed in solution: amino acids, hydroxy acids, part of aliphatic acids, polyhydroxy compounds, part of polymers. 2. All the products identified were formed in the gas phase of the system by free radicals and ions formed in the discharge.

In support of the first hypothesis, the previous work with electric discharges can be cited.[35] The production of HCN from mixtures of methane and ammonia,[36] mixtures of methane and nitrogen,[37] and mixtures of carbon monoxide, nitrogen and hydrogen[38] has been reported, as well as the production of amines from ethylene and ammonia,[39] nitriles and isonitriles from ethylene and nitrogen or ethylene and hydrogen cyanide.[40]

Formaldehyde has been produced from carbon monoxide and hydrogen,[41] and formaldehyde and acetaldehyde from methane and carbon dioxide.[42] Acetaldehyde from methane and carbon monoxide,[43] and acrolein from acetylene and carbon monoxide,[44] acids from carbon monoxide and water[45] and from methane and water[46] also have been reported.

Loeb's synthesis of glycine from carbon monoxide, ammonia and water in a silent discharge[7] is not evidence that amino acids can be formed directly in a discharge. The arrangement used, although not well described, probably had both a solution and a gas phase, and so the reactions to form the glycine may have taken place in the solution.

Polymers of varying composition have been produced by many workers.[35] Evidence that some of the polymer was formed in the gas phase was obtained by observing that a small amount of polymer of density less than water accumulated in the U-tube when the temperature of the gases near the discharge was reduced by a slower rate of boiling. This probably was due to the increased synthesis of polymers where the vapor pressure of the water was low, since a decrease of water vapor should cause a decrease in acid groups in the organic compounds and hence a decrease in solubility in the ammoniacal solution.

In order to determine a few of the products that were formed in the system directly from the discharge, a stopcock was placed at the base of the U-tube in apparatus no. 1, and a sample was withdrawn during the course of the run. The sample gave a positive Prussian Blue test for cyanide, and positive tests for carbonyls with 2,4-dinitrophenylhydrazine[18] and formaldehyde with chromatropic acid.[47]

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(35) A summary of these reactions has been given by G. Glockler and S. C. Lind, "Electrochemistry of Gases and Other Dielectrics," John Wiley and Sons, Inc., New York, N. Y., 1939. See also G. C. Akerlof and E. Wills, "Bibliography of Chemical Reactions in Electric Discharges," Office of Technical Services, Department of Commerce, Washington, D. C., 1951.

(36) K. Peters and H. Kuster, *Brennstoff-Chem.*, **10**, 108 (1929).

(37) O. Liebknecht, U. S. Patent 1,235,343; *C. A.*, **11**, 2564 (1917); A. Koenig and W. Hubbuch, *Z. Elektrochem.*, **28**, 202 (1922); E. Briner and A. Baerfuss, *Helv. Chim. Acta*, **2**, 663 (1919); E. Briner, J. Desbaillets and H. Paillard, *ibid.*, **21**, 115 (1938); E. Briner, J. Desbaillets and M. Wertheim, *ibid.*, **21**, 859 (1938).

(38) F. Fisher and K. Peters, *Brennstoff-Chem.*, **12**, 268 (1931); E. Briner and H. Hoefer, *Helv. Chim. Acta*, **23**, 826 (1940).

(39) L. Francesconi and A. Cirulo, *Gazz. chim. ital.*, **53**, 598 (1923).

(40) L. Francesconi and A. Cirulo, *ibid.*, **53**, 327 (1923).

(41) A. Koenig and R. Weinig, *C. A.*, **21**, 3834 (1927); W. Loeb, *Z. Elektrochem.*, **12**, 282 (1906); E. Ricard, U. S. Patent 2,205,542; *C. A.*, **34**, 7187 (1940); R. H. Sahasrabudhey and A. Kalyanasundaram, *Proc. Ind. Acad. Sci.*, **27A**, 366 (1948); R. H. Sahasrabudhey and S. M. Deshpande, *Proc. Ind. Acad. Sci.*, **31A**, 317 (1950).

(42) D. Finlayson and J. Plant, U. S. Patent 1,986,885; *C. A.*, **29**, 1201 (1935); Gutehoffnungshütte Oberhausen A.-G., British Patents 353,076 and 353,455; *C. A.*, **26**, 3446 (1932); German Patent 580,580; *C. A.*, **28**, 979 (1934).

(43) S. M. Losanitsch, *Ber.*, **44**, 312 (1911).

(44) J. N. Collie, *Proc. Chem. Soc.*, **21**, 201 (1905).

(45) A. Koenig and R. Weinig, *C. A.*, **21**, 3834 (1927).

(46) W. Loeb, *Ber.*, **41**, 87 (1908); S. Pawlikowski, *C. A.*, **44**, 2391 (1950).

(47) Reference 34, p. 395.

Because the boiling flask was at 100° and because the ammonia was in excess, alcohols, hydrocarbons, aldehydes, amines and HCN would be volatile, and because of the excess ammonia, the aliphatic acids would be much less volatile than either the acids or the ammonium salts of the acids would be. The amino acids, non-volatile acids and non-volatile bases would accumulate in the flask without loss except for those compounds that are hydrolyzed or destroyed by heat.

In the first hypothesis, the α-amino acids would be formed by the cyanohydrin synthesis

$$\mathrm{RCHO + NH_3 + HCN = RCH(NH_2)CN + H_2O}$$
$$\mathrm{RCH(NH_2)CN + 2H_2O = RCH(NH_2)COOH + NH_3}$$

Similarly the N-substituted amino acids would be synthesized by

$$\mathrm{RCHO + CH_3NH_2 + HCN = RCH(NHCH_3)CN + H_2O}$$
$$\mathrm{RCH(NHCH_3)CN + 2H_2O = RCH(NHCH_3)COOH + NH_3}$$

The hydroxy acids would be synthesized in a similar manner

$$\mathrm{RCHO + HCN = RCH(OH)CN}$$
$$\mathrm{RCH(OH)CN + 2H_2O = RCH(OH)COOH + NH_3}$$

The hydrolysis of these nitriles in ammonia is open to question. The usual procedure is to hydrolyze with hydrochloric acid or barium hydroxide after first isolating the nitrile. A method for hydrolysis with sodium hydroxide under a pressure of ammonia has been patented[48] as well as a method for hydrolysis of glycolnitrile under pressure at 180–225°.[49] Glycolic acid can be obtained in good yield by a steam distillation of a mixture of potassium cyanide and formaldehyde.[50]

The U-tube offers an opportunity for the hydrolysis of the nitriles. A solution of 25% KCN and 18% formaldehyde yields potassium glycolate, the potassium salts of glycine, iminodiacetic acid, nitrilotriacetic acid and ammonia upon standing at room temperature.[50,51] This type of reaction might occur to some extent in the U-tube, and the pressure of ammonia would suppress the formation of the iminodiacetic and nitrolotriacetic acids. It is possible that compound C might be iminodiacetic acid, since both are dicarboxylic amino acids.

If the synthesis of the hydroxy acids and amino acids is through the nitrile in spite of the problem of hydrolysis, then the rate of formation of the amino acids would be

$$\begin{aligned} \mathrm{d(R_iCH(NH_2)COOH)}/\mathrm{d}t &= k_i(\mathrm{R_iCH(NH_2)CN}) \\ &= k_iK_i(\mathrm{R_iCHO})(\mathrm{HCN})(\mathrm{NH_3}) \end{aligned}$$

and similarly for the hydroxy and methylamino acids. k_i is the rate constant for the hydrolysis of the aminonitrile, K_i is the equilibrium constant for the formation of the nitrile, i is the number of carbons in the alkyl chain of the aldehyde. This assumes that the slow step is the hydrolysis of the nitrile and that the nitrile is formed by a rapid and reversible equilibrium.[52,53]

The ratio of the hydroxy acid to the amino acid will be

$$h_i\mathrm{H}_i/k_iK_i(\mathrm{NH_3})$$

where h_i and H_i are the rate constant for hydrolysis and equilibrium constant, respectively, of the hydroxynitrile. If h_iH_i/k_iK_i is independent of i, then the ratio of the hydroxy acid to the amino acid should be independent of the aldehyde. In run 1, the mole ratio glycolic/glycine is 0.89 and the mole ratio lactic/alanine is 1.15. In run 3, the ratio glycolic/glycine is 0.35 and the ratio of lactic/alanine is 0.48. The agreement of the ratios is good considering the large number of assumptions. To explain the lower ratio in run 3, it would be necessary for the pressure of ammonia to be lower than in run 1, and similarly there would have to be a higher pressure of methylamine in run 3 than in run 1 to account for the large yield of sarcosine.

The acids might come from the hydrolysis of nitrile formed in the discharge, although the hydrolysis would be slower than with the hydroxy and amino nitriles. The oxidation of aldehydes also might form some of the acids. Because of the α-hydrogens on these aldehydes the oxidation probably would not be by a Cannizzaro reaction. A large fraction of the acids might be formed in the discharge directly.

The polyhydroxy compounds could be explained by condensations of formaldehyde[54] which generally are carried out in basic solution. Thus Loew[55] obtained a 75% yield of optically inactive hexose by allowing 4% aqueous formaldehyde to stand in saturated calcium hydroxide for several days. The reaction seems to be autocatalytic with the condensation going through glycolic aldehyde as an intermediate.[56]

The β-alanine would be synthesized by the reaction[57]

$$\mathrm{CH_2{=}CHCN + NH_3 = H_2NCH_2CH_2CN}$$
$$\mathrm{H_2NCH_2CH_2CN + 2H_2O = H_2NCH_2CH_2COOH + NH_3}$$

and by the reaction[58]

$$\mathrm{CH_2{=}CHCOOH + NH_3 = H_2NCH_2CH_2COOH}$$

The production of acrylonitrile has not been reported, but it is a reasonable compound to be formed in a mixture of acetylene or ethylene and hydrogen cyanide. The hydrolysis presents the same difficulties as with the hydrolysis of the α-aminonitriles. Acrylic acid has not been reported as being synthesized in electric discharges, but might be formed in the discharge in the same manner that the other aliphatic acids would be.

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[48] Ges. für Kohlentechnik, German Patent 656,838; *C. A.*, **32**, 4180 (1938).

[49] P. M. Kirk and J. H. Paden, U. S. Patent 2,364,538; *C. A.*, **39**, 3556 (1945).

[50] K. Polstorff and H. Meyer, *Ber.*, **45**, 1905 (1912).

[51] H. Franzen, *J. prakt. Chem.*, [2] **86**, 133 (1912).

[52] The equilibrium favors the formation of the hydroxynitrile and is rapidly attained in weakly basic solution.

[53] See W. F. Yates and R. K. Heider, THIS JOURNAL, **74**, 4153 (1952); W. J. Svirbely and J. F. Roth, *ibid.*, **75**, 3106 (1953).

[54] J. F. Walker, "Formaldehyde," Reinhold Publ. Corp., New York, N. Y., 1953, p. 165.

[55] O. Loew, *J. prakt. Chem.*, **34**, 51 (1886).

[56] W. Langenbeck, *Naturwissenschaften*, **30**, 30 (1942); E. Katzschmann, *Ber.*, **77B**, 579 (1944).

[57] S. R. Buc, J. H. Ford and C. E. Wise, THIS JOURNAL, **67**, 92 (1945); J. H. Ford, *Org. Syntheses*, **27**, 1 (1947).

[58] G. H. Carlson and C. N. Hotchkiss, British Patent 561,013; *C. A.*, **39**, 4891 (1945).

The aspartic acid might be synthesized by the amination of maleic or fumaric acids[59]

$$\text{HOOC—HC=CHCOOH} + \text{NH}_3 = \text{HOOC—CH}_2\text{CH(NH}_2\text{)COOH}$$

This reaction proceeds at a significant rate at 100°. Aspartic acid could also be synthesized by a cyanohydrin synthesis with the compound $OHCCH_2COOH$.

If one is to assume that the amino acids and hydroxy acids are formed in the discharge, according to hypothesis 2, then the production of a great many other products would be expected at the same time from the radicals and ions of the discharge. Many of these fragments would be unstable to hydrolysis and so should not be in the final solution of the products. However, one product that would not hydrolyze and might be formed easily would be oxalic acid, which was not found in large quantities in the acid fraction. In a similar manner urea might be expected in large quantities from the reaction of two NH_2 radicals and a carbon monoxide molecule although there would be loss by hydrolysis. Since a large quantity of urea or oxalic acid was not observed, this would tend to discount the formation of the more complex products such as the amino acids and hydroxy acids in the discharge.

However, the difficulty of explaining the hydrolysis of the various nitriles in the solution phase of the apparatus makes one unable to decide between the two hypotheses outlined. It is quite possible that both schemes are contributing to the synthesis of the products. Clearly, more experimental evidence is required, and further work is being done on these points.

Discussion

If these experiments are to any degree a representation of the reducing atmosphere of the Earth, then we see that not only would the formation of organic compounds be easy, but that most of the carbon on the surface of the Earth would have been in the form of organic compounds dissolved in the oceans. The atmosphere would still have had an excess of hydrogen, but instead of all the carbon in the atmosphere being methane, there would have been a significant amount of carbon monoxide. Part of the carbon would have been in the ocean as carbon dioxide, although the reducing character of the atmosphere would have tended to reduce much of the carbon dioxide back to methane. If the temperature of the oceans were less than 50° most of the ammonia would have been dissolved in the ocean instead of remaining in the atmosphere as in the apparatus here described. This assumes that the oceans were of a size comparable to that of the present ones. If the temperature were low, and as long as there was an excess of hydrogen, most of the nitrogen would have been in the form of ammonia instead of N_2. While much of the ammonia in the atmosphere might be decomposed into its elements by various processes, the ammonia in the ocean would not decompose. A steady state concentration of ammonia would have been set up in the atmosphere by various forms of energy, and since the ammonia that was formed in this steady state would dissolve in the ocean, the reaction of nitrogen with hydrogen to form ammonia would have been driven to completion.

These ideas are of course speculation, for we do not know that the Earth had a reducing atmosphere when it was formed. Most of the geological record has been altered in the four to five billion years since then, so that no direct evidence has yet been found. However, the experimental results reported here lend support to the argument that the Earth had a reducing atmosphere; for if it can be shown that the organic compounds that make up living systems cannot be synthesized in an oxidizing atmosphere, and if it can be shown that these organic compounds can be synthesized in a reducing atmosphere, then one conclusion is that the Earth had a reducing atmosphere in its early stages, and that life arose from the sea of organic compounds formed while the Earth had this atmosphere. This argument makes 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.

A literature review to support this argument will not be given here, but an examination of previous work with electric discharges[35] and photochemical reactions[11] will convince one that the argument has a good experimental basis. This reasoning, coupled with the independent argument that hydrogen is so abundant in the universe, places the assumption of a reducing atmosphere on sufficiently firm basis that it should be taken into account in future discussions of both the formation of the Earth and the origin of life.

A discussion of biological topics related to these experiments, such as the origin of optical activity in living organisms and the synthesis of other organic compounds that might make up the first living organisms, will be given in another paper.

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(59) J. Wolff, *Ann.*, **75**, 293 (1850); M. R. Engel, *Compt. rend.*, **104**, 1805 (1887); **106**, 1734 (1888); G. Stadnikoff, *Ber.*, **44**, 44 (1911); T. Enkvist, *ibid.*, **72**, 1927 (1939); Y. Tutiya, *J. Agr. Chem. Soc. Japan*, **17**, 706 (1941); *C. A.*, **36**, 4803 (1942).