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ties of the compounds manometrically (5). In the other, the material is applied to the paper along 8 cm of the base line rather than as a spot and, after resolution, areas 8 × 5 cm containing the various compounds are cut from the paper and rolled in shell vials. Ten anesthetized houseflies are then introduced into each vial, and the toxicity of the compounds is characterized by rate of knockdown and 24-hr mortality.

The paper chromatographic method is useful in studying the metabolism of phosphorus insecticides in plants, mammals, and insects. With it, for example, we have been able to demonstrate the conversion of parathion and its methyl analog to the corresponding phosphates by an enzyme system found in *Periplaneta americana* (L.) (2). Further studies are in progress. The method has also been of value in studying the action of heat on purified parathion and methyl parathion and in isolating the compounds formed and in studying their biological properties (1).

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## A Production of Amino Acids Under Possible Primitive Earth Conditions

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The idea that the organic compounds that serve as the basis of life were formed when the earth had an atmosphere of methane, ammonia, water, and hydrogen instead of carbon dioxide, nitrogen, oxygen, and water was suggested by Oparin (1) and has been given emphasis recently by Urey (2) and Bernal (3).

In order to test this hypothesis, an apparatus was built to circulate CH<sub>4</sub>, NH<sub>3</sub>, H<sub>2</sub>O, and H<sub>2</sub> past an electric discharge. The resulting mixture has been tested for amino acids by paper chromatography. Electrical discharge was used to form free radicals instead of ultraviolet light, because quartz absorbs wavelengths short enough to cause photo-dissociation of the gases. Electrical discharge may have played a significant role in the formation of compounds in the primitive atmosphere.

The apparatus used is shown in Fig. 1. Water is boiled in the flask, mixes with the gases in the 5-l flask, circulates past the electrodes, condenses and empties back into the boiling flask. The U-tube prevents circulation in the opposite direction. The acids

<sup>1</sup> National Science Foundation Fellow, 1952-53.

<sup>2</sup> Thanks are due Harold C. Urey for many helpful suggestions and guidance in the course of this investigation.

and amino acids formed in the discharge, not being volatile, accumulate in the water phase. The circulation of the gases is quite slow, but this seems to be an asset, because production was less in a different apparatus with an aspirator arrangement to promote circulation. The discharge, a small corona, was provided by an induction coil designed for detection of leaks in vacuum apparatus.

The experimental procedure was to seal off the opening in the boiling flask after adding 200 ml of water, evacuate the air, add 10 cm pressure of H<sub>2</sub>, 20 cm of CH<sub>4</sub>, and 20 cm of NH<sub>3</sub>. The water in the flask was boiled, and the discharge was run continuously for a week.

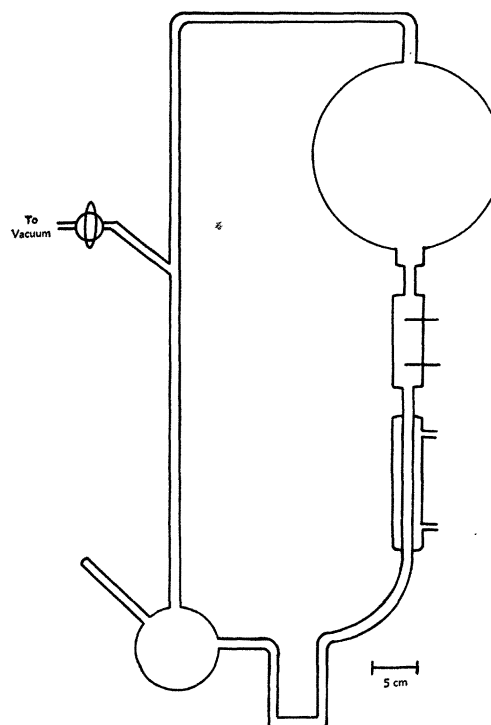


FIG. 1.

During the run the water in the flask became noticeably pink after the first day, and by the end of the week the solution was deep red and turbid. Most of the turbidity was due to colloidal silica from the glass. The red color is due to organic compounds adsorbed on the silica. Also present are yellow organic compounds, of which only a small fraction can be extracted with ether, and which form a continuous streak tapering off at the bottom on a one-dimensional chromatogram run in butanol-acetic acid. These substances are being investigated further.

At the end of the run the solution in the boiling flask was removed and 1 ml of saturated HgCl<sub>2</sub> was added to prevent the growth of living organisms. The ampholytes were separated from the rest of the constituents by adding Ba(OH)<sub>2</sub> and evaporating *in vacuo* to remove amines, adding H<sub>2</sub>SO<sub>4</sub> and evaporat-

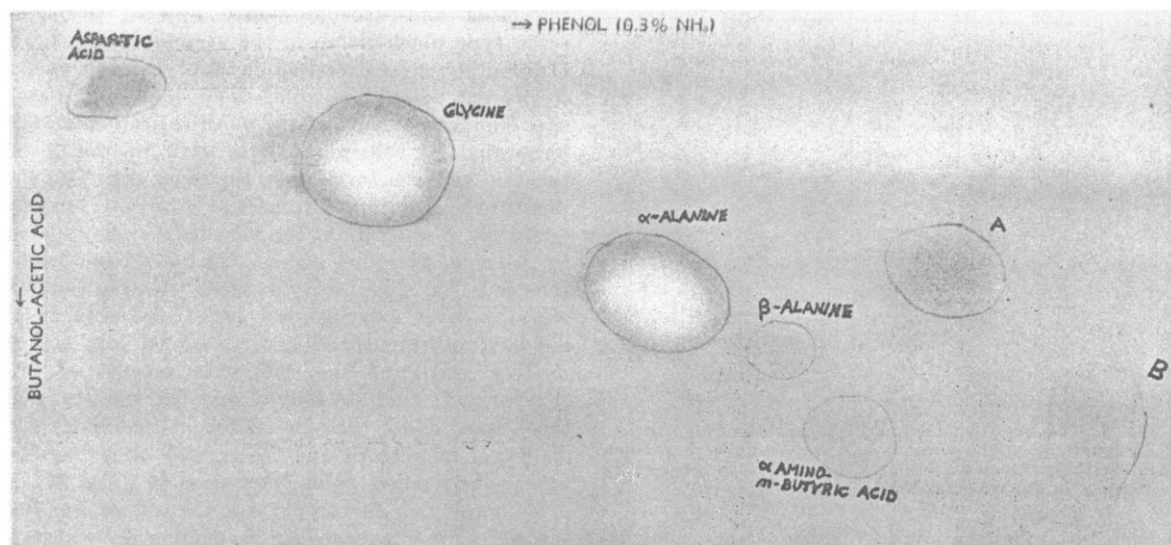


FIG. 2.

ing to remove the acids, neutralizing with  $\text{Ba}(\text{OH})_2$ , filtering and concentrating *in vacuo*.

The amino acids are not due to living organisms because their growth would be prevented by the boiling water during the run, and by the  $\text{HgCl}_2$ ,  $\text{Ba}(\text{OH})_2$ ,  $\text{H}_2\text{SO}_4$  during the analysis.

In Fig. 2 is shown a paper chromatogram run in *n*-butanol-acetic acid-water mixture followed by water-saturated phenol, and spraying with ninhydrin. Identification of an amino acid was made when the  $R_f$  value (the ratio of the distance traveled by the amino acid to the distance traveled by the solvent front), the shape, and the color of the spot were the same on a known, unknown, and mixture of the known and unknown; and when consistent results were obtained with chromatograms using phenol and 77% ethanol.

On this basis glycine,  $\alpha$ -alanine and  $\beta$ -alanine are identified. The identification of the aspartic acid and  $\alpha$ -amino-*n*-butyric acid is less certain because the spots are quite weak. The spots marked A and B are unidentified as yet, but may be beta and gamma amino acids. These are the main amino acids present, and others are undoubtedly present but in smaller amounts. It is estimated that the total yield of amino acids was in the milligram range.

In this apparatus an attempt was made to duplicate a primitive atmosphere of the earth, and not to obtain the optimum conditions for the formation of amino acids. Although in this case the total yield was small for the energy expended; it is possible that, with more efficient apparatus (such as mixing of the free radicals in a flow system, use of higher hydrocarbons from natural gas or petroleum, carbon dioxide, etc., and optimum ratios of gases), this type of process would be a way of commercially producing amino acids.

A more complete analysis of the amino acids and other products of the discharge is now being performed and will be reported in detail shortly.

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## A Vacuum Microsublimation Apparatus

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The analytical biochemist is frequently confronted with the task of isolating microquantities of substances in a chemically pure state from small quantities of tissues or biological fluids. Kofler (1) edited a book covering the use of microsublimation, melting point, eutectics, etc., in identifying microquantities of organic material. The advantages of sublimation over other methods of purification have been discussed by Hubacher (2). Many types of vacuum sublimation apparatus have been described (1-3). The equipment described here is inexpensive and can be assembled readily by any laboratory worker with a modicum of glassblowing skill.

To a thick-walled, round-bottom, Pyrex test tube, 30 × 200 mm, is attached a glass side arm about one in. from the bottom. Using a suspension of very fine emery in glycerin or fine valve-grinding compound, the open end of the test tube is ground against the aluminum block of a Fisher-Johns melting point apparatus (Fisher Scientific Co., St. Louis, Mo.) until it makes a vacuum-tight seal when dry. This is the vacuum hood. Microbeakers are prepared from flat-

<sup>1</sup> The author is indebted to Robert Puckett, of this laboratory, for technical assistance in preparing this apparatus.