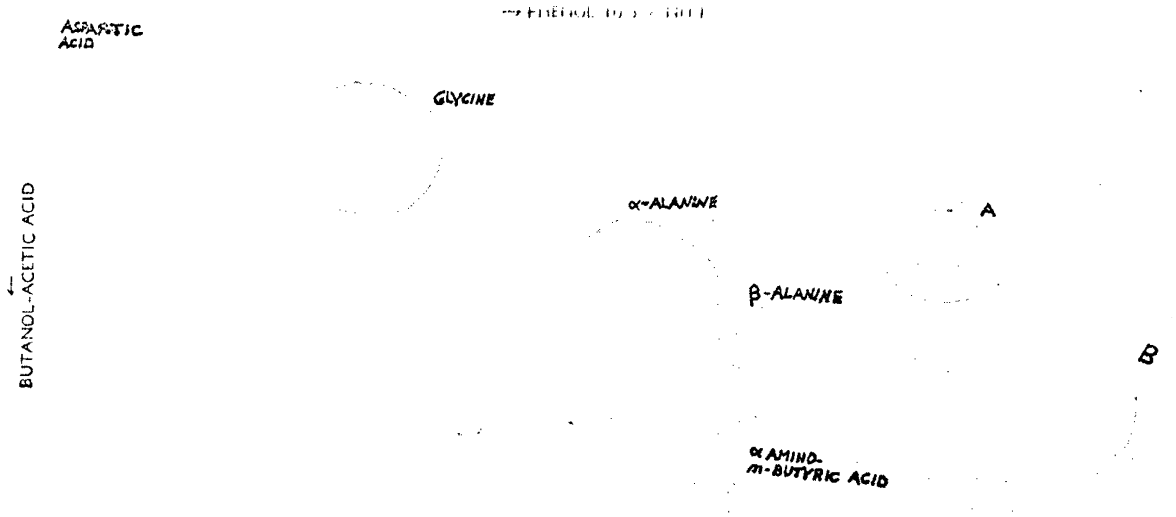

FIG. 2.

ing to remove the acids, neutralizing with $Ba(OH)_2$, 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 $HgCl_2$, $Ba(OH)_2$, H_2SO_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, α-alanine and β-alanine are identified. The identification of the aspartic acid and α-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-

[1] The author is indebted to Robert Puckett, of this laboratory, for technical assistance in preparing this apparatus.

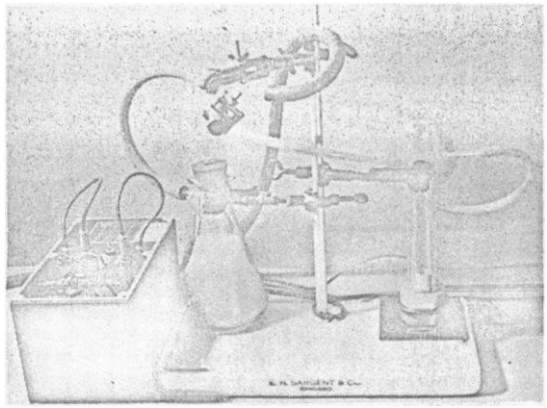

FIG. 1. Vacuum microsublimation apparatus in operation. The vapor trap in the vacuum line, consisting of a vacuum flask partially filled with glass beads, aids in preventing turbulence in the vacuum hood.

bottom test tubes, 15 mm in diameter. These may be cut down to any height, depending on the capacity desired (10 mm in height is equivalent to about 1 ml in capacity). Commercially available 5-ml beakers also may be used.

The biological fluid, tissue extract, or solution is concentrated by evaporation to 1 or 2 ml and then transferred to a microbeaker. The beaker is placed on the heating block of the melting point apparatus and heat applied. The ground-glass lip of the vacuum hood is then placed over the beaker on the block. Vacuum is applied to assist in drying the residue.

When the residue is completely dry the vacuum hood is removed, and the microbeaker is encased in aluminum foil. This prevents sublimation from occurring on the walls by keeping the wall temperature the same as that on the bottom. A microscope cover slip is now placed over the mouth of the microbeaker, and the vacuum hood is placed over this assembly on the heating block. The hood is pressed down on the block and vacuum slowly applied through a screw-type pinch clamp in the vacuum line (Fig. 1). Caution must be exercised in applying the vacuum to prevent turbulence, which may displace the cover slip. The temperature of the block is gradually raised by means of a voltage regulator until crystals of sublimation are seen forming on the cover slip. This temperature is maintained for a few moments to ensure quantitative separation. The vacuum is slowly released by means of the pinch clamp, and the vacuum hood is removed. The microbeaker is removed from the block and the cover slip inverted and transferred to the block. Another cover slip is placed on top, and the melting point of the sublimate determined. For sharper definition in determining the melting point the heating block may be placed on the stage of a dissecting microscope and the crystals thus magnified during the melting point determination (Fig. 2).

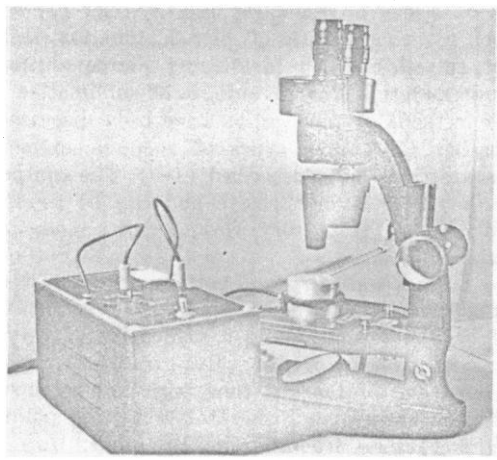

FIG. 2. Micromelting point apparatus with heating block mounted on stage of dissecting microscope. Contrast between crystals and background is enhanced by coating the heating block surface with a black, heat-stable substance such as ceramic ink.

By employing this apparatus the author has been successful in quantitatively recovering 4 μg Amytal from 2.5 ml of whole blood.

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The Effect of Carbon Monoxide on Respiration in the Sweet Potato, *Ipomoea batatas* Lan.[1]

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Since the addition of certain *ortho*-diphenolic substances (catechol, chlorogenic acid, etc.) to thin slices of roots of sweet potatoes increases the rate of oxygen consumption and carbon dioxide evolution, it has been suggested that a polyphenol oxidase is the terminal oxidase in sweet potato respiration (*1, 2*). In line with this, Walter (*3*) reported that sweet potato homogenates showed considerable phenol oxidase activity, but little or no cytochrome oxidase activity. This has been interpreted as further evidence for the possible operation of the phenol oxidase as a terminal oxidase, since the apparently insignificant amounts of cytochrome oxidase that were found could hardly play a major respiratory role in this tissue (*4*).

Recently, however, the author was able to demonstrate a very active cytochrome oxidase in sweet potatoes by a spectrophotometric method (*5*). It was also shown that crude sweet potato homogenates are not

[1] The author wishes to thank Albert W. Frenkel, of the University of Minnesota, for his helpful comments.

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