nephrine in the venom gland of the tropical toad *Bufo marinus* turns over even more slowly than the epinephrine in the rat adrenal. From the biochemical standpoint the slow rate of synthesis of epinephrine in the adrenal gland and in the toad venom gland discourages the use of these tissues as sources of active enzymes involved in epinephrine synthesis. It is possible that the rate of turnover of adrenal nor-epinephrine may differ from that of epinephrine. This is now being investigated.

Details of the chemical and isotopic procedures will be presented in a subsequent paper.

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Comments and Communications

On Some Recent Experiments with Supercooled Water

IN a paper published in 1944 Rau (*1*) reported the supercooling of water to −72° C by the successive freezing and melting of water droplets in an apparatus which depended upon a dry-ice acetone mixture for cooling. This extremely low temperature prompted Cwilong (*2*) to repeat this work, but he was unable to confirm Rau's results.

During a program on ice physics conducted at the Commonwealth Engineering Company under the auspices of the United States Air Force (*3*) this experiment was repeated.

Using a dry-ice and acetone coolant, a drop of known size was frozen and melted with care to avoid evaporation. The drop was frozen a few times at the same temperature, frozen at a lower temperature, became elongated, and finally did not freeze when the temperature was lowered to −60° C. However, when the identical experiment was repeated using liquid nitrogen as the coolant, a freezing temperature could never be obtained which was lower than −6.5° C. The drop remained hemispherical throughout ten or more repeated freezings and did not elongate.

These data, therefore, indicate that the water in Rau's experiment was contaminated with acetone, as suggested by Cwilong, and that the determination of a −72° C nucleation temperature for pure water is in error. This conclusion is further substantiated by the work of Cohen and Van der Horst (*4*) who were able to obtain ice crystals of similar configuration to those obtained by Rau, when they froze dilute acetone-water solutions.

Recent experimental findings by Smith-Johannsen (*5*) have established that considerable supercooling of bulk water is possible if one takes care to cool the water drop in an apparatus where no air-solid interface below 0° C is in contact with the bulk water. In the Smith-Johannsen apparatus, where a water droplet is cooled on the central region of a plastic surface, which in turn rests upon the top of a cooling bar, nucleation has been found to begin at about −20° C. Using this apparatus we were able to obtain data similar to that reported by Smith-Johannsen and by modifying the apparatus to accommodate considerably larger volumes of water, we found that freezing temperatures of −35° C could frequently be obtained. These extremely low values, we feel, are due to heat-transfer effects within the bulk water and will be the subject of further investigation.

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A Substitute for Drawing Ink in the Preparation of Diagrams for Photographic Reproduction

IN seeking a substitute for India or drawing ink in the preparation of graphs, charts, line drawings, and similar items for photographic reproduction, it was found that the pencil "Mars Lumograph" (J. S. Staedtler, Inc.) was most satisfactory. Material prepared with this pencil photographed exceedingly well because of the flat, black line it produced. This was especially true if a high contrast film was used, such as Reprolith or Kodolith. These pencils come in varying degrees of hardness, but the one giving the best over-all results was the EXB grade. For filling in solid areas, as in histograms, however, the EX-EXB grade is recommended. Major advantages over ink are as follows: (1) neatness, in that erasures may be made without frayed or fuzzy lines; (2) increase in speed of preparation, because the need for inking the penciled drawing is eliminated; (3) ease in handling, because the amount of drawing equipment is reduced, and consequently less knowledge of drafting is required; and (4) the degree of blackness produced matches typewriting done with a fresh ribbon, and

all legends may therefore be typed. The only disadvantages noticed were that, in blacking in areas, a fine dust sometimes forms and must be blown off to prevent smudging; and occasionally the lines may have to be gone over twice, especially if a hard smooth-surfaced paper is used.

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Electrophoretic and Chromatographic Studies of Purified Human Profibrinolysin[1]

BY A method previously described (*1*) it has been possible to prepare a human profibrinolysin that appears to be 100% pure electrophoretically. The starting material for this preparation was pyrogenic lyophilized human plasma (supplied by the Office of Naval Research). The one deviation in our current procedure from the previous report is in the first step. Instead of taking the precipitate from dialysis, the starting material is the residue from an acetic acid precipitation at pH 5.2 ± 0.1. In the electrophoretic studies the homogeneity of the single component was tested by reversing the current. The isoelectric point for this single component is pH 6.1; this would indicate profibrinolysin to be a gamma globulin according to reference electrophoretic curves (*2*).

By quantitative chemical methods profibrinolysin was found to be 13.4% nitrogen (micro-Kjeldahl) and 2.03% carbohydrate (orcinol).

Two-dimensional paper chromatograms with phenol and butanol-acetic acid water as developing agents, indicated the following 17 amino acids to be present: alanine, arginine, aspartic, cystine, glutamic, glycine, histidine, hydroxyproline, isoleucine, leucine, methionine, proline, serine, threonine, tryptophane, tyrosine, and valine.

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Horizontal Migration Method of Paper Chromatography

THE method of horizontal migration, which is sometimes referred to as circular paper chromatography (*1*), is a distinct phase in the development of paper chromatography. The method of Rutter (*2*) as modified by Rao and Beri (*3*) involves the use of a circular filter paper on which a small rectangular "tail" is

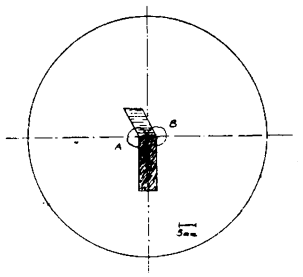

FIG. 1. *A*, the unknown substance (single or mixture); *B*, the known substance (single or mixture).

cut in such a way that its base lies on the diameter, and its sides are at equal distances from the center. The tail is folded back on its base so that it is perpendicular to the plane of the paper. The substance to be analyzed is introduced at the center as a microdrop. When the solvent rises up the tail and spreads as a halo on the horizontal filter paper, the substance migrates from its point of application and forms a ring and, if a mixture, separates into concentric circular zones. This method is more advantageous than the downward or the upward migration method in speed of development, ease of manipulation, and simplicity

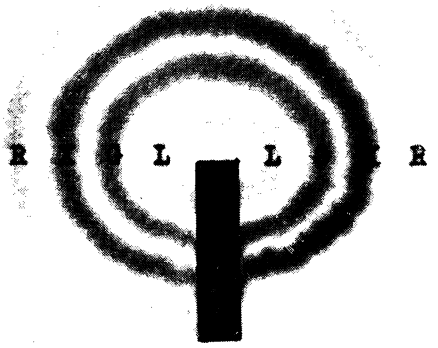

FIG. 2. R, rhamnose; X, xylose; G, galactose; L, lactose (with moist butanol as solvent).

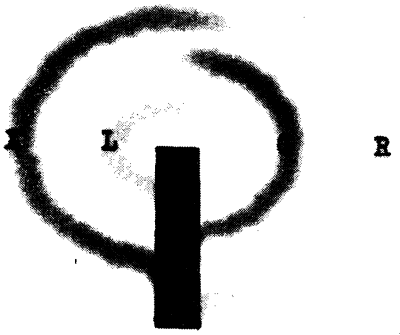

FIG. 3. X, xylose; L, lactose; G, galactose; R, rhamnose (with moist butanol as solvent).