Conclusion

A quick method of performing the Paul-Bunnell reaction is described which is as sensitive as the orthodox method. When read at two hours the rapid test appears to be definitely more sensitive than the orthodox method. When the rapid method is performed in the later stages of glandular fever a much higher titre is obtained than by the orthodox method. This might prove to be of diagnostic importance in such cases, particularly if they have not been investigated in the early stages. The validity of this point is being investigated further.

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A Simple Method for Desalting Biological Fluids for Paper Chromatography of Amino-acids

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Paper chromatography of biological fluids, especially urine, is difficult, owing to the presence of salts and other interfering substances, which prevent a satisfactory separation of the amino-acids in chromatograms.

Consden, Gordon, and Martin (1947) desalted the specimens electrolytically and used them for chromatography. Ion exchange resin was used by Phillips and Pollard (1953). Baliga, Krishnamurthy, Rajagopalan, and Giri (1955) used alcohol for desalting. A very simple and easy method for desalting urine and other biological fluids, which can be carried out in any ordinary clinical laboratory, is now described.

Procedure

Filtered urine, 25 ml., is evaporated to dryness in a dish over a water-bath. If proteins or phosphates are precipitated on heating, they are filtered off and the filtrate is evaporated to dryness over the water

FIG. 1.—Protein hydrolysate. 1, Aspartic acid; 2, glutamic acid; 3, serine; 4, glycine; 5, threonine; 6, alanine; 7, histidine; 8, lysine; 9, arginine; 10, tyrosine; 11, valine; 12, leucine and isoleucine.

FIG. 2.—Amino-acids in a specimen of normal urine.

FIG. 3.—Urine with protein hydrolysate extracted with butanol-phenol.

bath. The dish with the residue is left in a vacuum desiccator over concentrated sulphuric acid for 24 hours to dry the residue completely. The dry residue is then treated with 5 ml. of a 3:2 mixture (v/v) of redistilled phenol and butanol. The residue is stirred well with the mixture of phenol-butanol and is left over for about half an hour with occasional stirring and then centrifuged. The clear supernatant fluid will contain all the amino-acids concentrated five times but free from the other interfering substances, and is suitable for chromatography of the amino-acids. The required volume of phenol-butanol mixture is taken in a calibrated capillary tube and spotted on paper which is kept about 3 in. above an electric hot-plate. Small quantities are added from time to time, allow-

ing the spot to dry each time before fresh amounts are added. Any undue heating of the paper is avoided by frequently removing the hot-plate from below. Spotting ordinarily takes about 20 to 30 minutes, at the end of which a more or less fully dried small spot is obtained. The paper is immediately fixed in the chromatography chamber.

FIG. 4.—Amino-acids in gastric juice from a case of cancer of the stomach.

FIG. 5.—Single-dimension chromatogram of casein hydrolysate and urine with casein hydrolysate extracted with butanol-phenol.

Recovery experiments performed by adding protein hydrolysate to samples of urine showed that all the amino-acids were extracted in the same relative concentrations as in the original protein hydrolysate (Figs. 1, 3, 4, and 5).

Discussion

The desalting procedure of Consden *et al.* (1947) involved the loss of amino-acids (Stein and Moore, 1951; Baliga *et al.*, 1955). At the same time it required equipment beyond the scope of ordinary clinical biochemistry laboratories. The alcoholic desalting of Baliga *et al.* (1955) is suitable, but the alcohol extracts salts and interfering substances from the urine if the 95% concentration of alcohol is not rigorously maintained throughout the extraction. This required very skilful handling. Phenol-butanol extraction is easy and suitable as a routine procedure for the desalting of biological fluids for the chromatography of the amino-acids. This method has been employed

by us for the study of the amino-acid content of the gastric juice also. The two-dimensional chromatogram of the amino-acids in the gastric juice in a case of cancer of the stomach is shown in Fig. 4. Desalting with a phenol-butanol mixture is suggested for the routine testing of specimens of urine when amino-aciduria is suspected.

Summary

A rapid and simple method for desalting biological fluids for paper chromatography of the amino-acids is described.

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