A method for the improved resolution of basic amino acids on the automatic amino acid analyzer

In the course of performing analyses of basic amino acids on the 15 cm column developed with pH 5.28, 0.35 *N* sodium citrate buffer at 50° by the method of SPACKMAN, STEIN AND MOORE[1], difficulties are frequently encountered. These include skewing and spreading of peaks resulting from surface irregularities or poor resins, and marked build-up of pressure due to disintegration of the fine resin particles. In addition, traces of unusual amino acids are sometimes present in protein hydrolyzates which cannot be resolved from the four main constituents.

Because of these problems, a new procedure has been developed which gives greater resolution and greater sensitivity. The sample is loaded on the 50 cm (instead of 15 cm) column and developed with pH 5.28, 0.70 *N* (instead of 0.35 *N*) sodium citrate buffer at 50°. As in the original method, the ninhydrin is not started for 30 min to allow the acidic and most of the neutral amino acids to pass through. Tyrosine and phenylalanine emerge as discrete peaks preceding lysine. Fig. 1 illustrates the appearance of a protein hydrolyzate which has been analyzed by this procedure. Six hours are required for the determination.

The degree of resolution obtained with the 50 cm column at 30°–50° using pH 4.26, 0.38 *N* sodium citrate buffer[1] would not be expected under the present conditions. Fig. 2 illustrates the resolution obtainable with a synthetic mixture of basic amino acids and other slow-moving compounds. It demonstrates that a quick preliminary screening of physiological fluids is possible, and that in some case this 6 h procedure may be used in place of the 22 h 30°–50° pH 4.26 method. It also demonstrates that

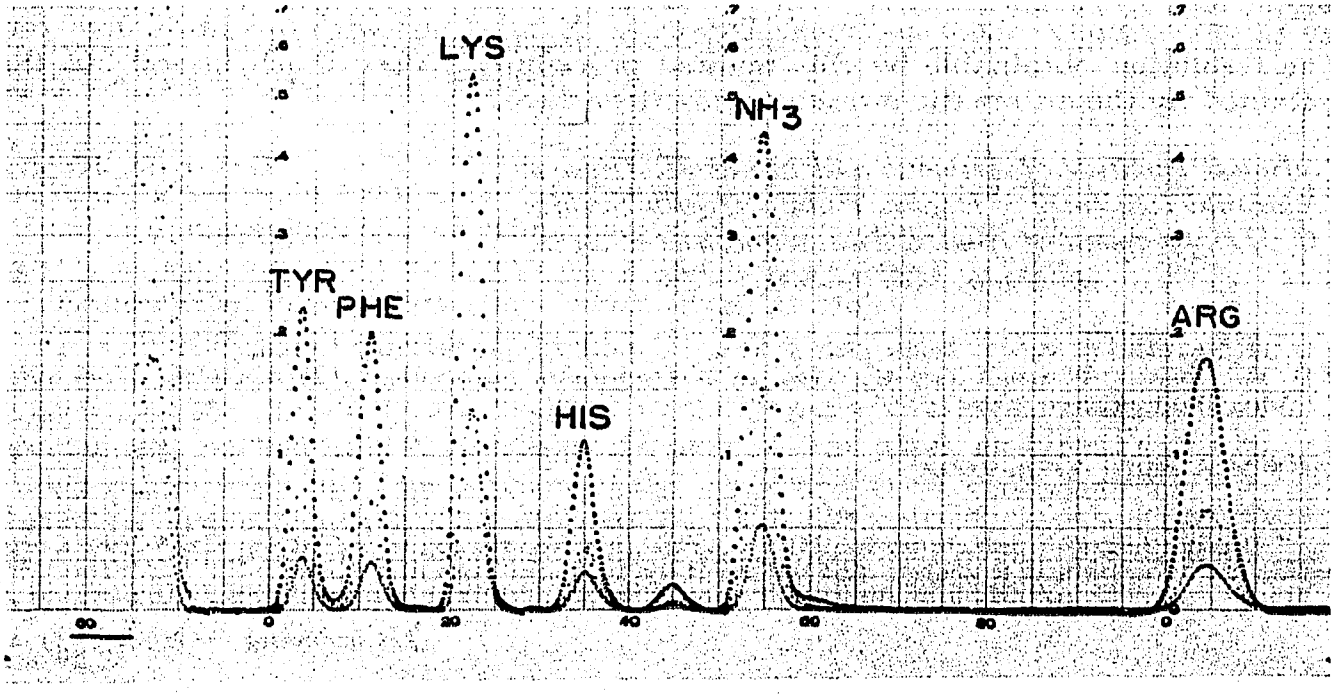

Fig. 1. Chromatographic analysis of hydrolyzate of adult *Phormia* actin, on 50 cm column developed with pH 5.28, 0.70 *N* sodium citrate at 50°. A small peak is present between histidine and ammonia which is probably 1-methylhistidine, resulting from contamination of the protein with a small amount of anserine.

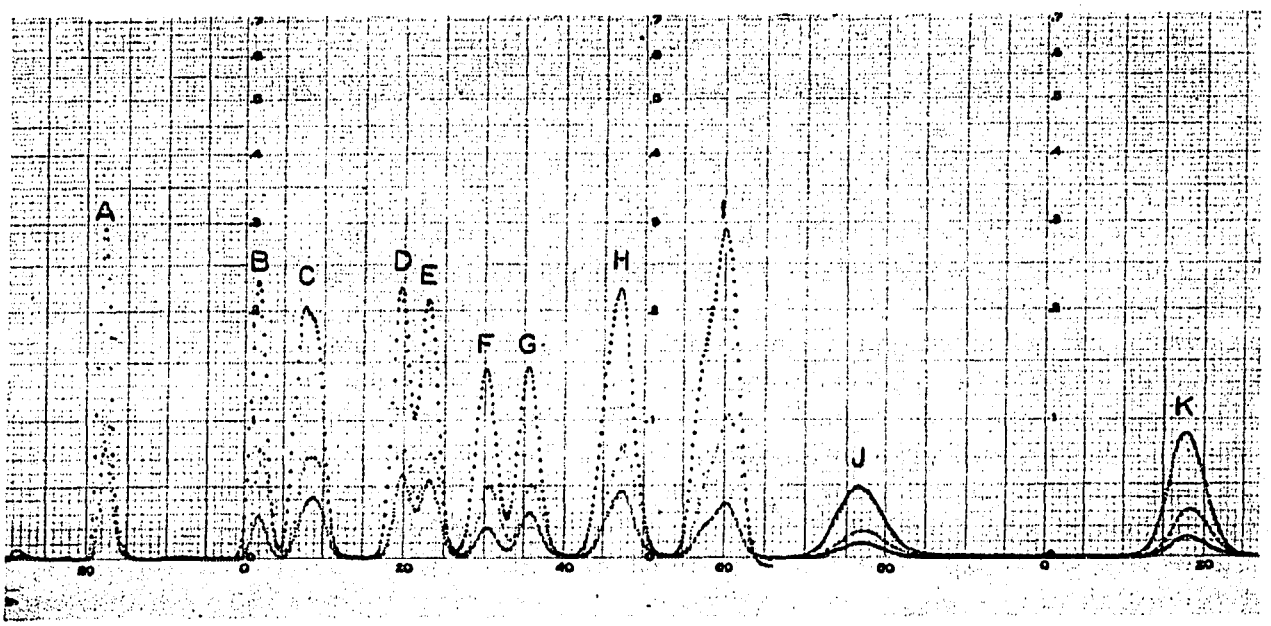

Fig. 2. Chromatographic analysis of mixture of basic amino acids and other slow-moving compounds, on 50 cm column developed with pH 5.28, 0.70 *N* sodium citrate at 50°. (A) α-aminobutyric acid; (B) glucosamine; (C) galactosamine and hydroxylysine; (D) ornithine; (E) lysine; (F) isomer of galactosamine*; (G) histidine; (H) 1- and 3-methyl-histidines; (I) ammonia, ethanolamine and carnosine; (J) tryptophan; (K) arginine.

some of the rarer compounds that may contaminate a protein hydrolyzate can be clearly resolved, such as the 1-methylhistidine present in the hydrolyzate of Fig. 1.

In addition to its usefulness with the automatic amino acid analyzer, the pH 5.28, 0.70 *N* sodium citrate buffer has been used successfully for a number of years[2] on 15 cm columns of 200–400 mesh Dowex 50 X 12 at 50° with 6–8 ml/h flow rate. The resolution obtainable by this manual procedure is very much inferior to that obtained by the automatic procedure described here.

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[1] D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.
[2] D. R. KOMINZ, F. SAAD AND K. LAKI, in *Conference on the Chemistry of Muscular Contraction*, Igaku Shoin, Tokyo, 1957, p. 66.

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* Present in D(+)-galactosamine HCl, C-grade, lot No. 104403 supplied by California Corporation for Biochemical Research, and obtained by hydrolysis of chondroitin.