A modified method for the separation of basic amino acids on the automatic amino acid analyzer*

The quantitative analysis of amino acids in biological preparations has been greatly simplified by the automatic ion-exchange chromatographic method of SPACKMAN, STEIN AND MOORE[1]. Frequently, free amino acids are encountered in urine, tissue homogenates and serum which are not well resolved by the 6 h procedure using the 15 cm column. These amino acids are readily separated and identified on the 50 cm column, but this method is time consuming (22 h) and uses large quantities of ninhydrin and eluting buffer.

KOMINZ[2] has reported an accelerated method for chromatography of basic free amino acids on the 50 cm column using a 0.70 *N* sodium citrate buffer pH 5.28. A system for analyzing basic amino acids in 80 min has also been developed by SPACKMAN[3]. Neither of these methods gives complete resolution of all the basic free amino acids which are found in urine, tissue homogenates and serum, and the second method requires several major modifications in the operation of the amino acid analyzer.

The present report describes a rapid method for analysis of basic amino acids which has been applied to serum, tissue homogenates, urine and protein hydrolysates. An analytical run takes $8^1/_3$ h and only requires the equipment and reagents regularly used with the Spinco model 120 amino acid analyzer. The method resolves 11 basic amino acids and the shortened operating time allows a 60 % saving in reagents.

A sample volume of 1–3 ml was applied to the 15 cm column which had been poured to a resin height of 21 cm with Spinco type 15 A resin. The eluting buffer was the 0.37 *N* sodium citrate, pH 4.26, and was pumped through at 30 ml/h. The column

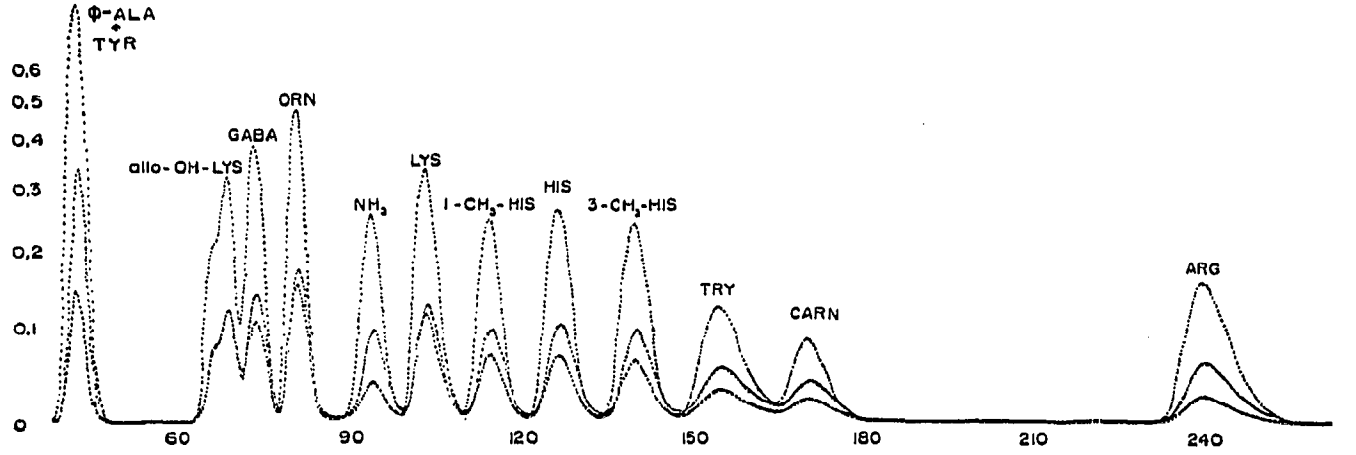

Fig. 1. Chromatogram of a synthetic amino acid mixture containing 0.5 μmole of each amino acid. The optical density is recorded on the ordinate and the effluent volume on the abscissa. The following abbreviations are used: ø-ala, L-phenylalanine; Tyr, L-tyrosine; allo-OH-Lys, DL-allo-hydroxy-lysine; GABA, γ-aminobutyric acid; Orn, L-ornithine; NH_3, ammonia; Lys, L-lysine; 1-CH_3-His, L-1-methylhistidine; His, L-histidine; 3-CH_3-His, L-3-methylhistidine; Try, L-tryptophan; Carn, L-carnosine; Arg, L-arginine.

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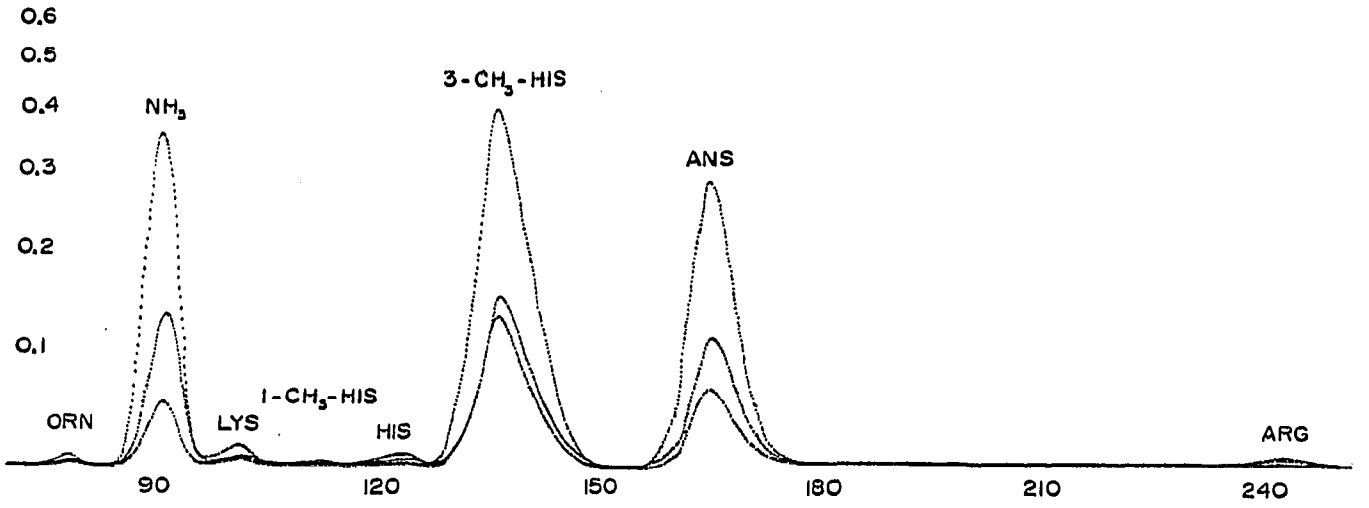

Fig. 2. Chromatogram of the free amino acids in a homogenate of rabbit skeletal muscle. The units are the same as Fig. 1. Ans represents L-anserine.

temperature at the beginning of elution was 30° and was increased to 50° at 4 h. Ninhydrin was added at 30 min at 15 ml/h.

The ability of this method to resolve 11 ninhydrin-reactive compounds in a synthetic mixture is illustrated in Fig. 1. The resolution was nearly equal to that obtained with the 50 cm column and the sharp, narrow peaks allowed accurate identification and quantitation. Fig. 2 shows the analysis of the free amino acids in a homogenate of rabbit skeletal muscle. The location of the peaks of 15 ninhydrin-reactive compounds is summarized in Table I.

TABLE I

LOCATIONS OF NINHYDRIN REACTIVE PEAKS

*Compound**	*Effluent to peak in ml*
L-Tyrosine, L-phenylalanine	44
D-Galactosamine	50 (shoulder on tyr-phe)
DL-Allo-δ-hydroxylysine**	68
γ-Aminobutyric acid	73
L-Ornithine	80
Ammonia	92
L-Lysine	102
L-1-Methylhistidine	114
L-Histidine	125
L-3-Methylhistidine	138
L-Tryptophan	158
L-Anserine	165
L-Carnosine	170
Creatinine	178
L-Arginine	241

* Tyrosine, phenylalanine, ammonia, lysine, histidine and arginine were contained in Spinco Type 1 amino acid calibration mixture, Lot No. CM 108. The other compounds were purchased from the California Corporation for Biochemical Research, Los Angeles 63, California.

** The shoulder on the ascending limb of the curve is believed to represent an impurity in the reagent standard.

This method of chromatographic analysis of basic amino acids has been used in this laboratory for several months and has allowed substantial savings in both time and reagents.

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Chromatographic separation of histamine and some metabolites on cellulose phosphate paper*

The metabolism of histamine (M) in animals has been reviewed recently[1]. The main products of metabolism are 1-methyl-4-(β-aminoethyl)-imidazole (MeM), imidazole-4(5)-acetic acid (ImAA), imidazoleacetic acid riboside (ImAA-riboside), 1-methyl-imidazole-4-acetic acid (MeImAA), and acetyl-histamine (AcM). Quantitative analyses of ^{14}C-labelled histamine metabolites have been performed by crystallization of the different products as their respective picrates or *p*-iodophenyl-sulfonyl (pipsyl) salts[2,3]. These methods, however, are quite cumbersome and time-consuming.

Paper chromatography has been used by SCHAYER AND KARJALA[2] to separate radioactive metabolites of histamine. Three radioactive peaks were found using a solvent system composed of 1-butanol–ethanol–ammonia (80:10:30, v/v). The first peak contained ImAA-riboside, the second MeImAA and ImAA, and the third M, MeM and AcM. Good separation of histamine, histidine and acetyl-histamine can be obtained using Whatman No. 1 filter paper with isopropanol–0.25 *N* NH_4OH (3:1)[4]. In this system MeM can not be separated from M nor ImAA from MeImAA[5]. Thus, until now, it has not been possible to separate all of the above mentioned metabolites of histamine by paper chromatography.

In a preliminary effort to separate the different known metabolites of histamine by chromatography, a variety of papers were tried, Schleicher and Schüll papers No. 5984D, 2040A, blue R589D, white R589, 2043A, 2041, red R589, 598, 2043-D, 602 extra dense, 576, green R589, black R589D, 470 and 470A. Also, different solvents (as recommended by BLOCK *et al.*[6]) and ionophoresis at different pH values were evaluated. None of these techniques proved successful. Amberlite ion exchange resin loaded papers (Sa-2, Wa-2, Sb-2 and Wb-2) and Whatman cellulose ion exchangers (P-20, CM-50, DE-20 and ET-20) were tried next. Best results were achieved with the cation exchanger, cellulose phosphate paper (P-20). These results are reported below.

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