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Note

Simple method for the determination of diaminopimelic acid in rumen liquor hydrolysates

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The diaminopimelic acid (DAP) concentration can be used as a measure of bacterial growth in total rumen contents. Previously, DAP has been estimated by methods[1] which failed to determine simultaneously all other amino acids present in the sample. The present paper describes a modified system for the separation and quantitation of DAP and all other amino acids present in hydrolysates from sheep rumen contents based on the two column ion-exchange chromatography technique of Spackman *et al.*[2].

MATERIALS AND METHODS

The analyses were performed on a TSM amino acid analyser equipped with C-3 (8% cross-linked) cation-exchange resin (Technicon, Tarrytown, NY, U.S.A.) using two columns: column A for the basic amino acids and column B for the acidic and neutral acids, with resin bed sizes of 9.5 × 0.4 cm I.D. and 23.5 × 0.5 cm I.D., respectively. Three different buffers were used in the new system (Table I). An aliquot (50 μl or 100 μl) of the hydrolysate sample was applied to both columns. Column A was run first with buffer 1 for 36 min. Fractionation on column B was then effected for 24 min with buffer 2 and 24 min with buffer 3.

Before each estimation the columns were washed for 4 min each with 0.2 *N* sodium hydroxide regeneration mixture followed by a 6-min equilibration period with the appropriate starting buffers (buffers 1 and 2) being pumped through the

TABLE I

COMPOSITION AND pH OF THE SODIUM CITRATE BUFFERS

	Buffer		
	1	*2*	*3*
Sodium ion concentration (*N*)	0.405	0.2	0.2
Citrate concentration (*N*)	0.1	0.1	0.1
Methyl cellosolve (%, v/v)	0	6.0	0
Thiodiglycol (%, v/v)	0.1	0.1	0.1
pH ± 0.01	5.25	3.25	4.10

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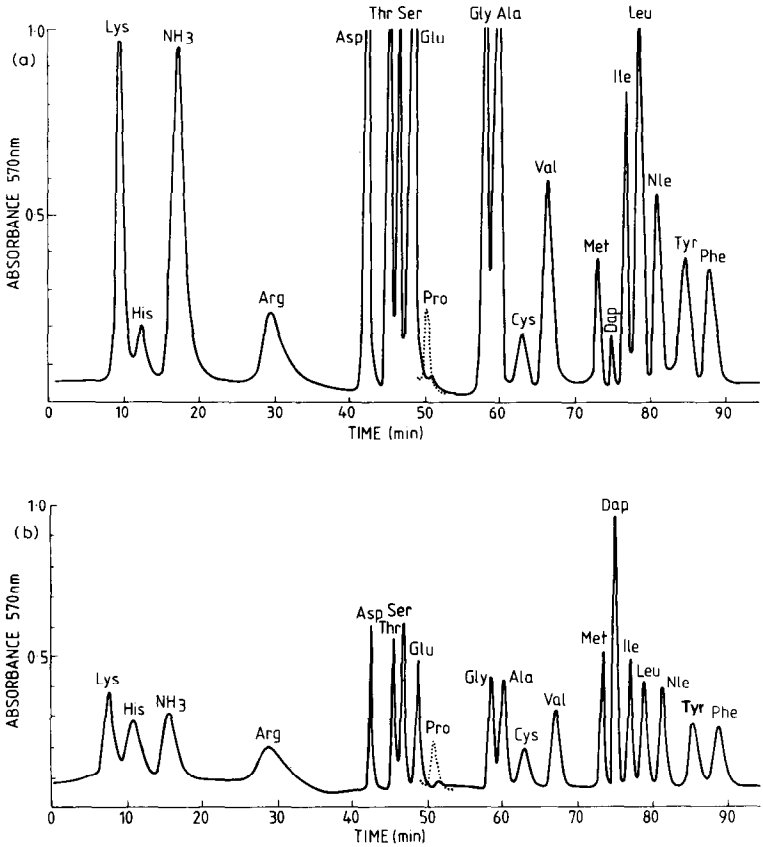

Fig. 1. (a) Elution profile of a 100-μl hydrolysate sample containing 50 nmoles internal norleucine standard. (b) External standard chromatogram containing 25 nmoles of each amino acid per 50 μl of sample.

respective columns. The analytical system was then washed for 4 min with solutions of 0.3% Brij-35 (30%) and 50% methyl cellosolve introduced simultaneously.

Both columns were run at 60°C with a flow-rate of 30 ml/h. Column pressure did not exceed 500 p.s.i. With each 100-μl test sample, 50 nmoles of norleucine were used as an internal standard.

External standards consisted of 25 nmoles of each amino acid per 50 μl sample (Pierce Hydrolysate Standard).

RESULTS AND DISCUSSION

It is possible by manipulation of pH, column temperature, methyl cellosolve concentration and resin bed length to shift amino acid peaks by varying degrees according to their sensitivities to these parameters.

By testing pH values ranging from 3.80 to 4.30, it was found that a resolution of all amino acids close to DAP could be effected at pH 4.10, when combined with a column temperature of 60°C. Lower or higher temperatures (between 30°C and 65°C) failed to resolve the overlap of a number of amino acids especially methionine

and isoleucine. Methyl cellosolve concentration affected DAP elution in relation to valine. A 6.0% (v/v) solution was the most efficient in the concentration range of 2.0 to 6.5% (v/v) for the optimal resolution of these two amino acids.

DAP was eluted after 76 min. Elution diagrams for standard and test samples are given in Fig. 1a and b. The DAP peak was resolved clearly with a low norleucine equivalent of 0.70 ± 0.01. The method allowed measurements of between 2 and 50 nmoles of DAP.

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