Citric fermentation. Only citric acid was identified although oxalic acid frequently appears during the process. These two acids, the R_F values of which are very similar, can be distinguished by elution with pyridine and acetic anhydride according to FURT AND HERMANN[5], a red colour being obtained for citric acid and gas evolution for oxalic acid.

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Chromatography of neutral amino acids on columns of cellulose powder

In a previous paper[1] we described the fractionation of peptides in columns of cellulose powder using a volatile solvent, *viz.*, a mixture of ethyl alcohol and water. In spite of the great number of existing methods for the separation of amino acids we thought it would be interesting to apply this system to the fractionation of these acids.

Two sizes of columns were used in this work, smaller ones of 31 × 0.9 cm and larger ones of 100 × 0.9 cm. The columns were prepared by pouring into a glass tube a slurry obtained by suspending 1 part of cellulose powder (Whatman standard grade) in 3 parts of a mixture of ethyl alcohol and water of the appropriate concentration. After the cellulose powder had sedimented a pressure of 15 lbs. per sq. in. was applied. The columns were then washed under pressure with about 2 l of the ethyl alcohol–water mixture. These columns can be used many times, provided they are washed between runs with about 1 l of absolute ethyl alcohol and then equilibrated with the solvent to be used for the chromatography.

The columns were mounted on an automatic fraction collector and the flow was adjusted to 2.5 ml/h; 0.75 ml fractions were collected. Alternate fractions were analysed by a modification of the ninhydrin method of TROLL AND CANNAN[2] and by one-dimensional paper chromatography.

The mixture of amino acids to be separated was dissolved in 1 ml of the appropriate solvent, applied to the top of the columns and washed down with two 1 ml portions of the same solvent.

One of the mixtures used in these separations contained phenylalanine, glycine, leucine, proline, threonine, alanine, methionine, serine and valine. A simple mixture

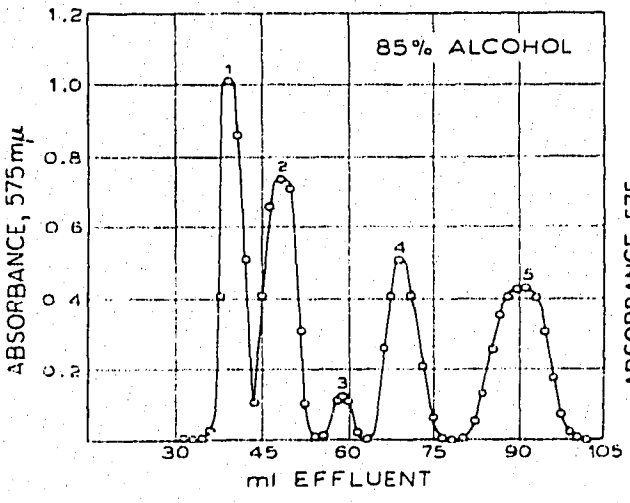

Fig. 1. Chromatographic separation of a mixture containing 1 μmole of each of the following amino acids: DL-leucine (1), DL-phenylalanine (2), L-proline (3), DL-threonine (4) and glycine (5). Eluant: mixture of ethyl alcohol (85%) and water. Column dimensions: 30 × 0.9 cm. Flow rate: 2.5 ml/h. Fractions of 0.75 ml each.

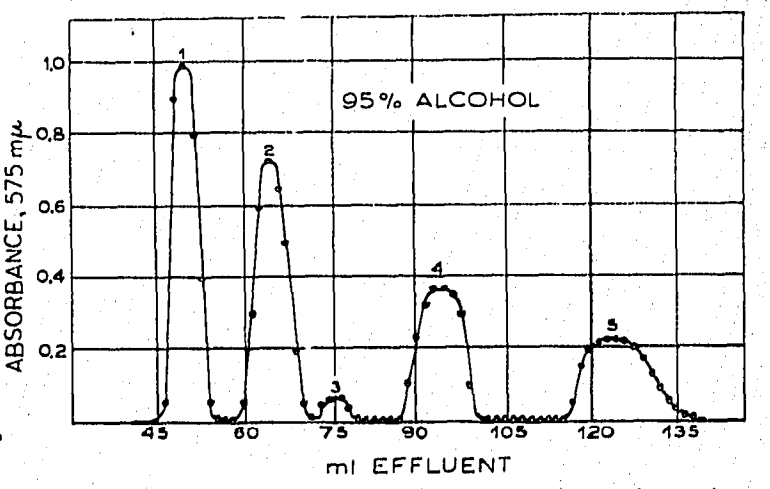

Fig. 2. Chromatographic separation of a mixture containing 1 μmole of each of the following amino acids: DL-leucine (1), DL-phenylalanine (2), L-proline (3), DL-threonine (4) and glycine (5). Eluant: mixture of ethyl alcohol (95%) and water. Column dimensions: 30 × 0.9 cm. Flow rate: 2.5 ml/h. Fractions of 0.75 ml each.

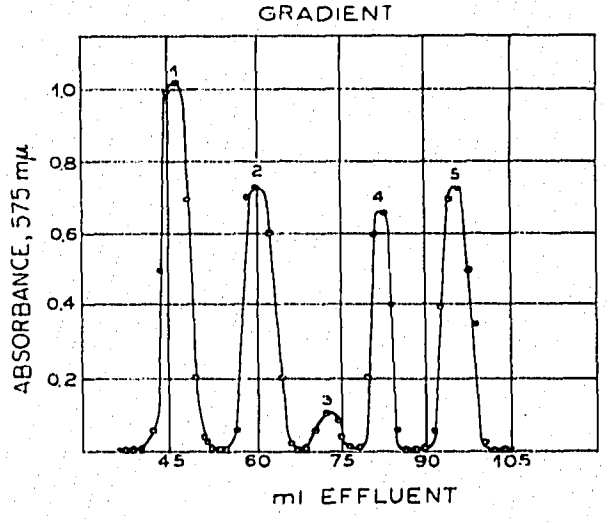

Fig. 3. Chromatographic separation of a mixture containing 1 μmole of each of the following amino acids: DL-leucine (1), DL-phenylalanine (2), L-proline (3), DL-threonine (4) and glycine. Gradient elution. Column dimensions: 30 × 0.9 cm. Flow rate: 2.5 ml/h. Fractions of 0.75 ml each.

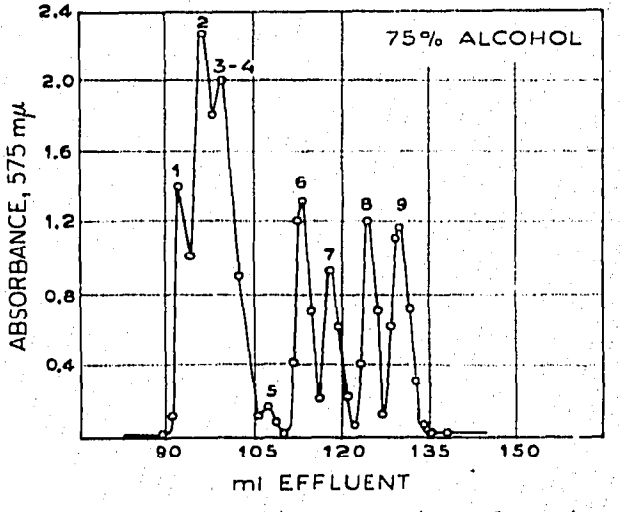

Fig. 4. Chromatographic separation of a mixture containing 0.7 μmole of each of the following amino acids: DL-leucine (1), DL-valine (2), DL-phenylalanine (3), DL-methionine (4), L-proline (5), DL-alanine (6), DL-threonine (7), DL-phenylalanine (3), DL-methionine (4), DL-serine (8) and glycine (9). Eluant: mixture of ethyl alcohol (75%) and water. Column dimensions: 100 × 0.9 cm. Flow rate: 2.5 ml/h. Fractions of 0.75 ml each.

containing only phenylalanine, glycine, leucine, proline and threonine was also used.

When a concentration gradient was used, this was obtained by the automatic addition of a 40% ethyl alcohol to 325 ml of 95% ethyl alcohol.

In Figs. 1 and 2 the fractionation of the mixture containing five amino acids, using 85 and 95% ethyl alcohol, is shown. In the first case all the fractions came out sharply, but the separation of the leucine and phenylalanine peaks is not complete. Using 95% alcohol all the five amino acids are separated with pronounced loss of

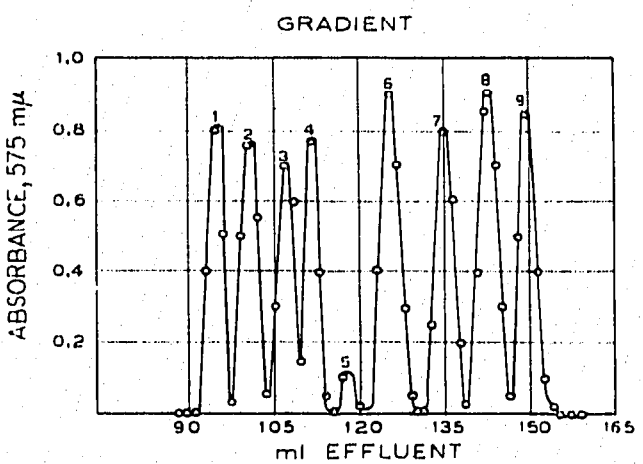

Fig. 5. Chromatographic separation of a mixture containing 0.7 μmole of each of the following amino acids: DL-leucine (1), DL-valine (2), DL-phenylalanine (3), DL-methionine (4), L-proline (5), DL-alanine (6), DL-threonine (7), DL-serine (8) and glycine (9). Gradient elution. Column dimensions: 100 × 0.9 cm. Flow rate: 3 ml/h. Fractions of 0.75 ml each.

sharpness for the peaks of threonine and glycine. Complete separation and sharpness of all peaks can, however, be obtained by using gradient elution as shown in Fig. 3.

With the mixture of nine amino acids good separation could not be achieved by employing a single concentration of alcohol. The best results were observed in this case when the solvent was 75% alcohol (Fig. 4), but this is still much inferior to the separation obtained when gradient elution was used (Fig. 5).

The method presented in this paper may prove valuable for preparative work where the volatility of the solvent is an advantage.

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