

tubes for extracting the colour. After 1 hr, the density of colours are measured at 509 nm in a spectrophotometer, using methanol as a blank.

To obtain NE/E ratio, the extinction of the solution containing non-essential amino acids is kept as the numerator and that for the essential amino acids (Band 1 and 2) as the denominator.

RESULTS

A typical chromatogram of the plasma amino acids is shown in (Fig. 3). The bands are distinct and a good separation of amino acids has been achieved. There is a close correspondence between the Rf values of different groups of amino acids as determined by the present technic and those obtained by Whitehead^{1,9}. The values found for the ratio of NE/E amino acids of approximately 1000 plasma samples chromatographed in our laboratory ranged between 0.7 and 2.1 (mean = 1.3, SD = 0.51) in normal children.

COMMENTS

The present method based on paper chromatography offers a rapid determination of the plasma amino acid ratio which has been first suggested as an indicator of protein nutrition status. Satisfactory results comparable to those obtained by Whitehead¹ and Pardaman² were achieved.

In the method described in this paper, following steps, i.e., the evaporation of the supernatant and the staining of the chromatogram in the alcoholic copper nitrate solution, used by Whitehead¹ have been eliminated. Moreover, it is not needed to apply the whole mixture of the protein precipitate and the ethanol on both faces of the paper as described by Saini¹⁰. Only the mixture is centrifuged and the whole clear supernatant is applied on one face of the paper.

The method is rapid and does not require considerably more working time.

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nutrition.

Simple and rapid paper chromatographic method suggested by Whitehead¹ and Pardaman² is suitable for the determination of this ratio.

The present paper reports a modification of Whitehead¹ and Pardaman² technics employing the same solvent system. In this method a clear supernatant is used without evaporation.

MATERIALS

Absolute ethanol (reagent grade).

Chromatography paper.-Whatman No. 3MM filter paper (25x27 cm).

Ninhydrin, 0.2% W/V in acetone.-0.5 g

ninhydrin (indane-1 :2 :3-trione hydrate) in 250 ml. acetone.

About 50 ml. is needed for each batch.

Methanol (reagent grade).

Chromatography paper.- Whatman No. 3 MM filter paper (25x27 cm).

Centrifuge tube, 10 ml.

Chromatography apparatus.- Shandon Unikit tank.

METHOD

Blood samples are taken in heparinized or oxalated tubes. The plasma is separated by centrifugation. If the plasma cannot be analyzed immediately it should be stored at 5°C. Plasma measuring 0.25 ml. and 0.35 ml. ethyl alcohol are mixed in a centrifuge tube and shaken vigorously. The protein precipitate is separated by centrifugation. The whole clear supernatant is applied as a rectangular spot (5x1 cm.) to the chromatography paper (Whatman No. 3 MM). Ordinarily three samples are applied on each paper (Fig. 1). Two edges of the paper are brought together to form a cylinder with the tongued clips.

The chromatogram is run in the solvent. A satisfactory separation of the plasma amino acid is achieved when the solvent front has ascended about 20 cm.

The papers are removed from the tank and air dried. They are stained with ninhydrin solution and then dried. For full development of the colour of the bands, the papers are kept in an oven at 90-100°C for a min. The separation of amino acids as obtained in this method is shown in (Fig. 2,3). Amino acid band No. 1,2 and 3 are used for quantitation. Band 1 represents leucine and isoleucine, band 2 valine and methionine as the two major spots in the upper half of the chromatogram and band 3 the non-essential amino acids, glycine, glutamine, serine and taurine. These compounds are selected for analysis, outlined and cut out. The spots containing essential amino acids (band 1 and 2) are cut into small strips in a suitable tube. Band 3 is similarly treated. Eight milliliters of methanol is added to each of the two

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FIG. 1
DIAGRAM ILLUSTRATING ONE-WAY CHROMATOGRAPHY

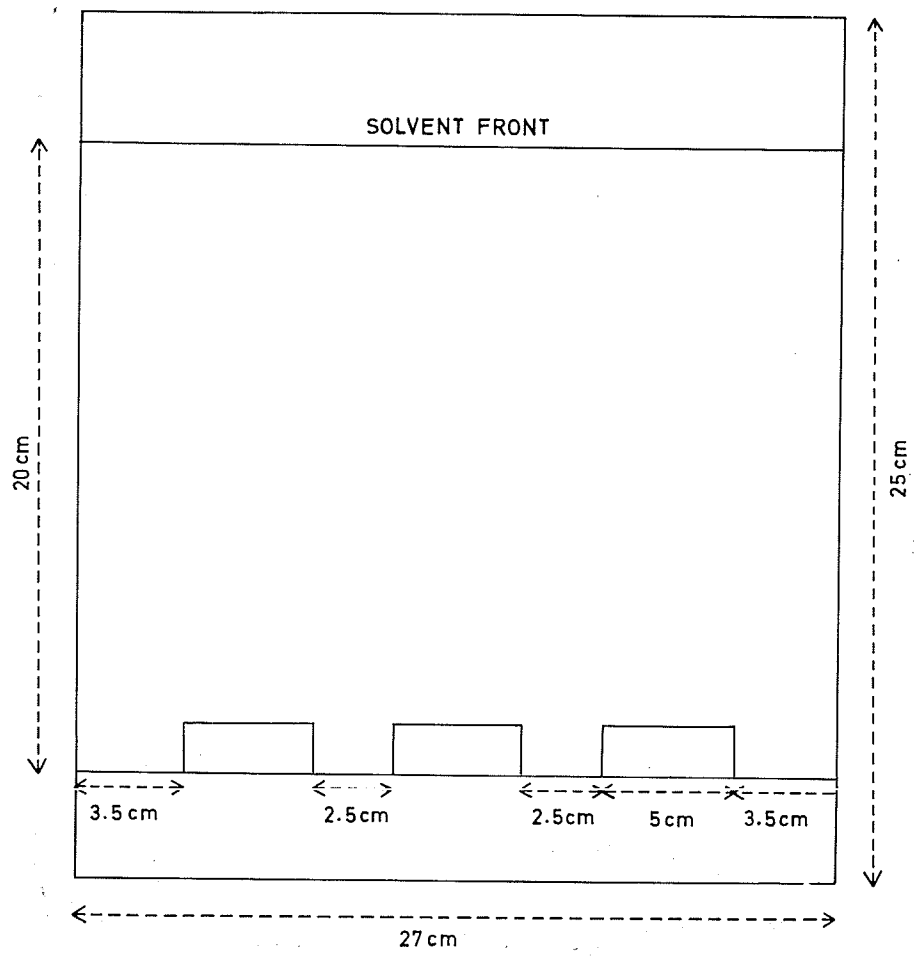


FIG. 2
SCHEMATIC SEPARATION OF AMINO ACIDS
BY UNIDIMENSIONAL CHROMATOGRAPHY

