Journal of Chromatography, 97 (1974) 284–285

CHROM. 7544

Note

An improved simple method for chromatography of blood amino acids

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(Received April 1st, 1974)

In developing a biochemical screening programme for neonates and children it is necessary to study amino acids in blood as well as in urine. A number of methods have been developed using whole blood and serum. Scriver *et al.*[1] used serum which had been collected in capillary tubes. Efron *et al.*[2] used whole blood collected on filter paper which was subsequently autoclaved to denature the haemoglobin. In a method developed by Plochl[3] the amino acids were eluted from filter paper discs and the solvent containing amino acids was then chromatographed.

A much simpler technique has been described by Culley[4] which uses the same filter paper soaked in whole blood but which avoids the extra work involved in either eluting or autoclaving. The discs of filter paper are punched out as before and attached to the chromatographic plate by means of a glass rod and the amino acids are eluted by an initial run in an ammonia–ethanol–water (18:1:1) solvent. After drying, the plate is chromatographed in the normal way. This procedure gives a similar pattern to that obtained by spotting serum straight on to the plate.

A modification to the spotting technique which involves streaking the samples over a distance of 2 cm along the origin gives better separation into discrete bands and has been adopted for the analysis of urine and serum. It was felt that this technique could be adapted for use with filter papers by cutting a strip from the paper instead of punching a disc.

The blood was collected on the filter paper used for the Guthrie test. Strips of 1 × 4 mm were cut out using a modified Gelman gel punch. The punch was modified by inserting an extra centre plate between the two blades, thus separating the blades by 4 mm instead of 2 mm. The strips were placed on the chromatographic plates at the origin and secured by placing a glass plate on top of them. The edge of the glass plate was kept a little above the level of the solvent in the tank to ensure that there was no capillary action between the two plates. The plate was developed until the front had moved about 5 cm. It was found that the mobile phase ethanol–ammo-

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nium hydroxide–water (18:1:1) was quite adequate. The glass plate was removed and the chromatographic plate dried in a current of warm air to ensure that the ammonia had been removed completely. The plate was then chromatographed in the solvent[5]: butanol–acetone–acetic acid–water (7:7:2:4). After a run of 14 cm the plate was removed, dried and re-chromatographed in the same solvent with 7.5 ml of 7% ninhydrin in butanol–acetone (1:1) added per 100 ml of solvent.

Using this method it was found that discrete bands were obtained. Fig. 1 shows on the left the separation with discs as described by Culley[4] and on the right with the technique described. It is felt that with no extra labour whole blood can be separated into discrete bands which are similar to those obtained by streaking serum and are considerably easier to interpret than spots.

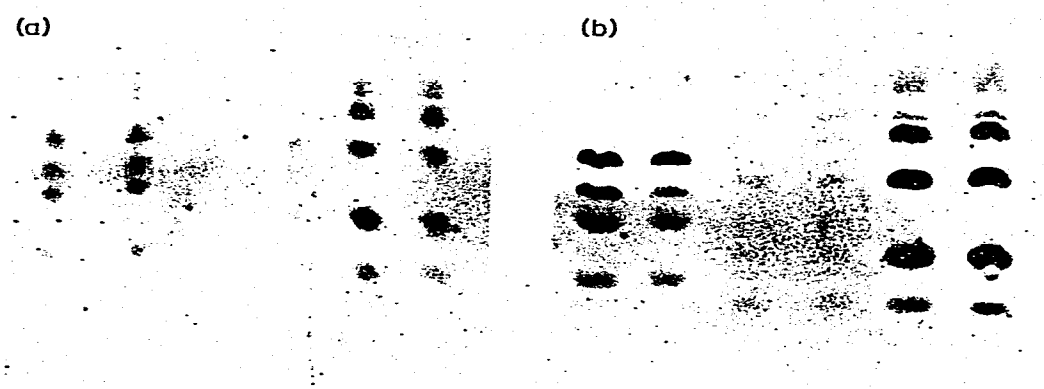

Fig. 1. Thin-layer chromatograms of blood amino acids with discs (a) and with strips (b).

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