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Note

Thin-layer chromatography of amino acids in blood*

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From studies such as that of Scriver *et al.*[1] it has become apparent that hereditary metabolic disorders constitute a significant part of the patient caseload in pediatric hospitals. Although prenatal diagnosis can often be obtained in high-risk subjects, for adequate diagnosis of these metabolic disorders before the onset of clinical disease it is necessary to screen the entire population at risk, that is all newborn infants.

Most states and provinces have mandatory screening programmes for phenylketonuria, but the cost of a specific screening programme for each of the disorders of amino acid metabolism would be prohibitive and unjustified in view of their rarity, so a simple non-specific screening programme to detect as many abnormalities as possible is desirable. In several states and provinces, this testing is performed by thin-layer or paper chromatography[2–9], and we believe that our technique offers advantages over those previously described.

The method described is a modification of that of Culley[10] for the screening of metabolic errors involving amino acid metabolism. Our technique is more rapid and sensitive than paper chromatography, and the sample required is a small filter paper disc impregnated with dried blood which is processed without deproteinisation. The discs are applied manually, but an automated device such as that used for the Guthrie test[11] could be used in processing large numbers of samples.

EXPERIMENTAL

Materials

We have obtained optimum results with chromatography on 20 cm × 40 cm glass plates and a mixture of cellulose** (18 g), silica gel G** (12 g) and distilled water (130 ml), as used in our urine screening programme. The elution solvent was 98% ethanol–28% ammonia–distilled water (18:1:1) and the separation solvent *n*-butanol–acetic acid–water (13:3:5). The color reagent was 0.2% ninhydrin in 98% ethanol (95 ml) and 1% cadmium acetate in acetic acid (5 ml).

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** Machery, Nagel and Co., supplied by Brinkmann, Westbury, N.Y., U.S.A.

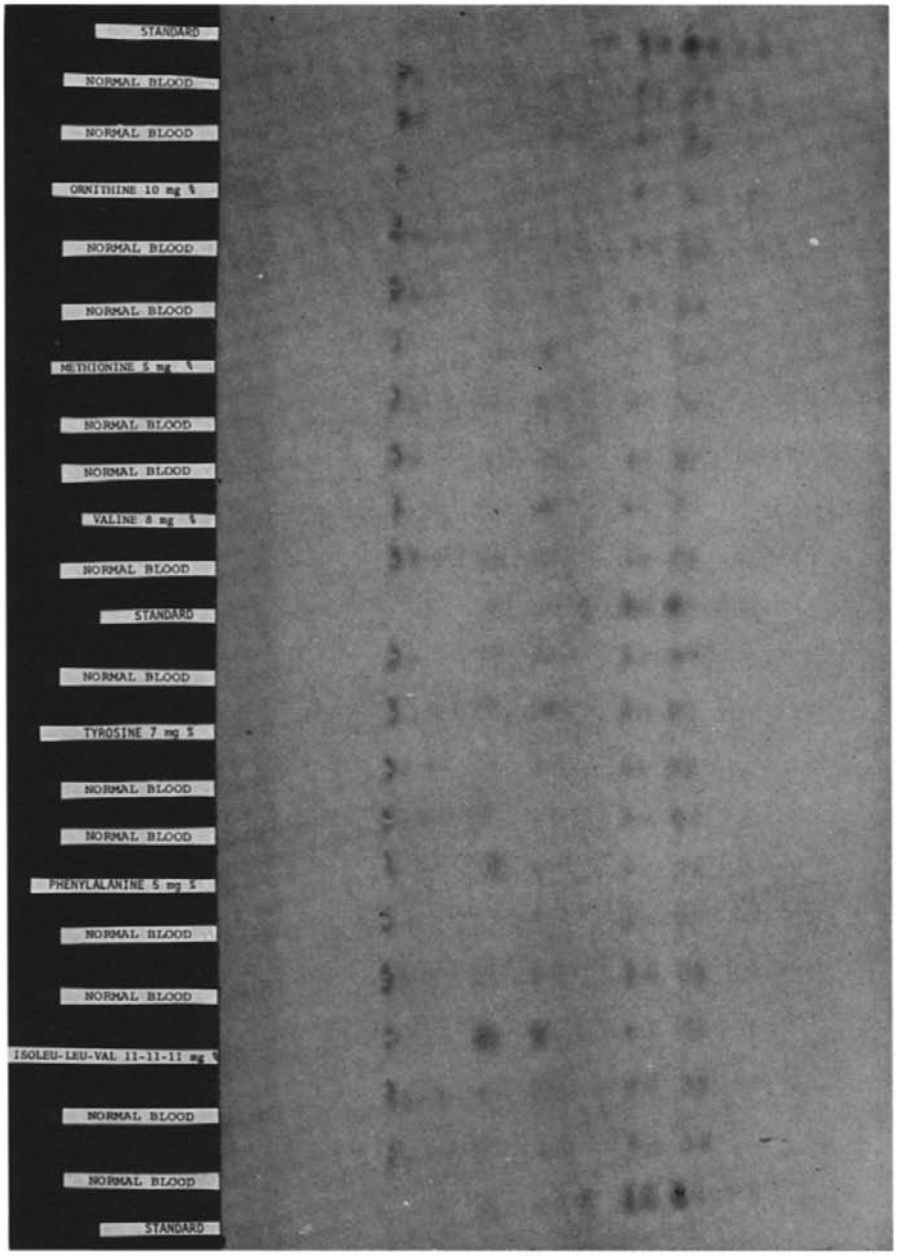

Fig. 1. Three standard discs, six abnormal blood discs and thirteen normal blood discs on a 20 × 40 cm plate.

Method

A strip of double-gummed ½-in. Scotch® tape is applied lengthwise on a 20 × 38 cm plain glass plate 2 cm from the edge. Using a hand leather punch, discs of blood are prepared from the sample filter paper and are applied to the gummed tape at 2-cm intervals; again, the automatic punch[11] could be used here.

Fig. 2. This 20 × 20 cm plate shows four discs from phenylketonuric patients and two discs from Maple Syrup urine disease patients, with two normal discs for reference. Phenylalanine and branched-chain amino acids can be readily detected at moderate elevations.

Elution

The glass plate with the disc is then lowered on to a thin-layer chromatography (TLC) plate and held in place with clips; the two plates are placed in the eluting solvent which is allowed to migrate about 4 cm; the plain glass plate with the paper disc is then removed; and the TLC plate is dried for 5 min at 70° in a forced air circulation oven.

Chromatography

Separation of the amino acids is accomplished with a double migration in the separating solvent. The ammonia used for the elution gives a strong yellow background to the TLC plate; this can be eliminated by heating the plate for 10 min at 100° between and after migration. After spraying the color reagent, the plate is heated at 100° for 20 min to develop the colors.

DISCUSSION

With this technique 500 samples can be processed and the results read in a single working day. Using an automatic spreading device* enough plates for 2000 samples and standards can be prepared in 2 h, but with fewer samples a simpler and less expensive manual spreader can be used. The cost of materials and reagents is less than ten cents per sample; thus the technique compares very favourably with paper chromatography in terms of cost. With the use of a semi-automatic punch and disc distributor, a very large work load can be accomodated with minimal technical support, and the positive retention of the discs by the gummed strip ensures that the discs cannot be lost or misplaced. The Scotch tape appears to be inert to the solvents used, and shows no effect on migration or staining of the amino acids.

With the use of standards (paper discs impregnated with amino acid solutions) alterations in the blood amino acid pattern can be readily seen, and the sensitivity is adequate for screening of newborns. The collection of blood on filter paper has been shown to be simple and quantitative, and the amino acid content of the dried blood remains stable for long periods. Paper impregnated with urine can be used instead of blood although the variability of the amino acid content is obviously greater.

The illustrations show adequate separation of the amino acids and sensitivity is such that moderate increases in amino acids can be readily detected (Figs. 1 and 2).

In summary, we feel that this method is more rapid and gives better resolution than paper chromatography for the amino acids in blood or in urine. By simple modifications the method could be used in chromatography of other compounds of biological interest such as carbohydrates. In particular, we feel that the method is particularly amenable to semi-automated processing in large-scale surveys.

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