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Note

Separation of amino acids from untreated urine on thin layers of silica gel

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Thin-layer chromatographic and other methods for the screening of amino-acidopathies have been reviewed[1] and separations of amino acids from untreated urine on thin layers have also been reported[2–5]. In some of these methods, distortion due to contaminants is avoided by using, for example, small volumes. The approach in the twin-layer technique[3] has been considered to be ideal[1] for the analysis of amino acids for diagnosis of aminoacidopathies, probably because untreated samples can be used, there is no limit to the size of the sample employed and two-dimensional separations can be carried out. The method described in this paper has all of the above advantages and is also simpler. The main significance of the present procedure, however, lies in the approach to the problem of chromatographing untreated samples, which depends on certain previously reported postulates[6].

EXPERIMENTAL

Preparation of plates

A 20-g amount of silica gel G (nach Stahl) was suspended in 50 ml of water and the suspension was spread over clean glass plates (20 × 20 cm), the spreader being adjusted to yield layers 0.5 mm thick. The plates were air dried and were not activated.

Application of the sample

A 40-μl volume of untreated urine and 10 μl of a solution of an amino acid mixture (containing 1 mg each of alanine, arginine, glutamic acid, histidine, leucine, lysine, methionine, phenylalanine, taurine, threonine and tyrosine per millilitre of 0.01 *N* hydrochloric acid containing 20% of isopropanol) was applied on one corner of the plate as a round spot at a distance of 1.5 cm from the two adjacent edges.

Runs in the first direction

The plate was subjected to two consecutive runs (10 cm and 18 cm) in the solvent system isopropanol–isopentanol–ammonia (12:3:5) (solvent A) or pyridine–isopropanol–isopentanol–ammonia (8:5:2:5) (solvent B). After these runs, the plate was placed under a fan for about 1 h so as to drive off ammonia.

Runs in the second direction

The solvent system isopropanol–*n*-butanol–isopentanol–formic acid–water

(5:3:2:1.5:2) (solvent C) was used for two consecutive runs (10 cm and 18 cm) at right-angles to the direction of the previous runs. The plate was dried for 30 min under a fan and sprayed with a 0.2% solution of ninhydrin in acetone in order to stain the amino acid spots.

RESULTS AND DISCUSSION

The most significant aspect of the present method is the use of solvent systems A and B, in which sodium chloride and urea (in amounts corresponding to urine volumes of even 100 μl) move very compactly and therefore do not distort even those amino acid spots which migrate close to them[6].

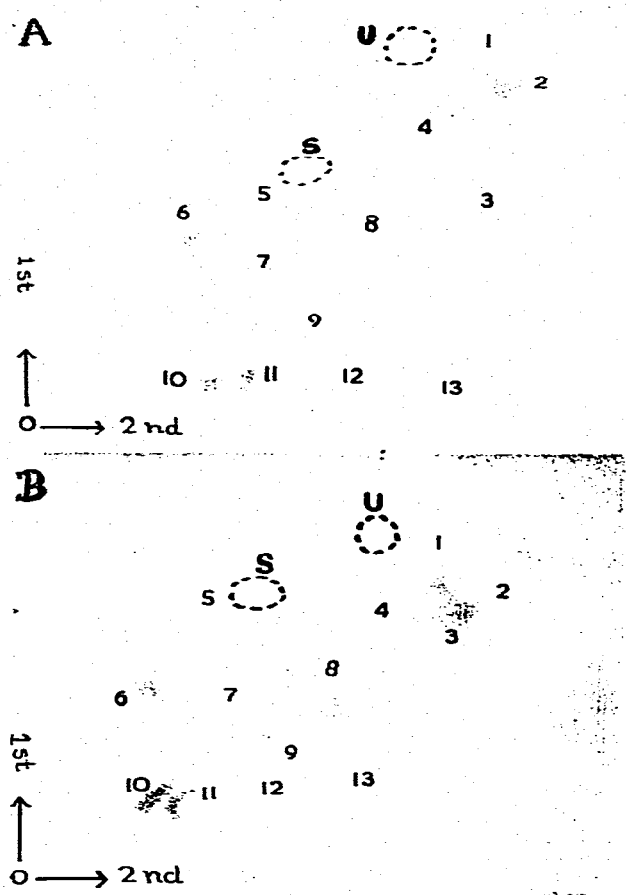

Fig. 1. Two-dimensional chromatograms of amino acids from untreated urine on thin layers of silica gel. For both chromatograms, 40 μl of urine and a standard amino acid mixture was spotted for chromatography. In the first direction, two consecutive runs (10 cm and 18 cm) were conducted in solvent A for chromatogram A and in solvent B for chromatogram B. In the second direction, both chromatograms were subjected to two consecutive runs (10 cm and 18 cm) in solvent C. Amino acids: 1 = phenylalanine; 2 = leucine; 3 = tyrosine; 4 = methionine; 5 = taurine; 6 = histidine; 7 = glutamine; 8 = threonine; 9 = glycine; 10 = lysine; 11 = arginine; 12 = alanine (elongated spot); 13 = glutamic acid; U = urea; S = salt.

In solvent A, the spot of sodium chloride (R_F 0.43) overlaps the spots of methionine (R_F 0.45) and tyrosine (R_F 0.41) but does not distort them. In solvent B, the spot of sodium chloride (R_F 0.40) overlaps the spots of phenylalanine (R_F 0.43), leucine (R_F 0.42), methionine (R_F 0.41) and tyrosine (R_F 0.39) but does not distort them. It is also interesting that in both solvents A and B, urea (R_F 0.55 in solvent A and 0.53 in solvent B) moves beyond the positions of all of the amino acids. In solvent C (in the second direction), phenylalanine (R_F 0.68), leucine (R_F 0.71), methionine (R_F 0.64) and tyrosine (R_F 0.63) move far away from sodium chloride (R_F 0.38) (Figs. 1A and 1B).

It is difficult to choose between solvents A and B as the most suitable for use in the first direction. Using solvent A in the first direction, the separation of the group consisting of leucine, phenylalanine, methionine and tyrosine is better but the separation of the group consisting of glycine, glutamine, taurine and threonine is poorer (Figs. 1A and 1B).

The procedure has also been used successfully for urine volumes as high as 80–100 μl. In such instances solvent A is superior to solvent B.

A 60-μl volume of plasma–alcohol[7] or a similar blood–alcohol mixture can also be conveniently chromatographed one-dimensionally for amino acids using solvent A on silica gel plates.

Solvents A and B also give excellent results in the one-dimensional separation of sugars from untreated urine, from volumes corresponding to 100 μg of creatinine, on silica gel layers.

In general, better separations of amino acids have been obtained on cellulose than on silica gel layers[1]. In the present method, however, good separations are possible only on silica gel layers. This result agrees well with the fact that salts migrate diffusely on cellulose layers in solvents A and B.

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