Screening for Aminoacidopathies with Prepared Cellulose Layers on Aluminium Foil

THE recent report by Bremer, Nützenadel and Bickel[1] of the use of prepared thin-layer plates of cellulose for screening of plasma for inborn errors of amino-acid metabolism prompts us to report our experience with prepared cellulose layers on aluminium foil (E. Merck, Darmstadt, Germany) for screening of both plasma and urine. Thin-layer chromatography of amino-acids on cellulose is faster, and has greater sensitivity and resolution, than paper chromatography[2,3] without sample preparation. Additionally, the flexible aluminium foils can be dipped in the location reagent instead of being sprayed, and appropriate sections cut out for testing with specific reagents.

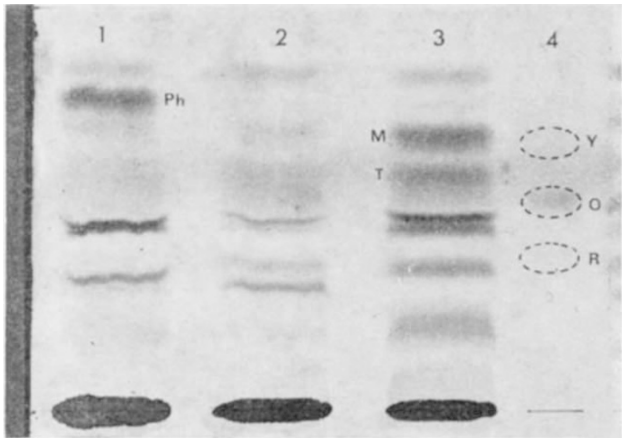

Fig. 1. Plasma amino-acids. 1, Phenylketonuria (Ph, phenylalanine); 2, normal plasma; 3, tyrosinosis (T, tyrosine; M, methionine); 4, marker (Y, yellow; O, orange; R, red).

We cut against the "grain" two plates (10 × 20 cm) from the 20 cm square layers supplied. (Solvent flow with the grain is 20 per cent faster than against with no differences in separation.) Samples (5 μl.) of plasma were applied as a band (2 cm) to duplicate sheets together with a marker solution (2 μl. of the alcoholic fraction obtained by shaking Quink black ink (1 ml.) with butanol (8 ml.)) and dried with a cold fan; volumes of urine (2–12 μl.) depending on creatinine concentration (Table 1) were applied as bands (1·5 cm) and dried with warm air. Chromatograms were developed twice (7·5 cm rise, 50 min at 25° C) with *n*-butanol–acetone–acetic acid–water (35 : 35 : 10 : 20) and dried with warm air between runs. After chromatography the urine sheet and one of the plasma sheets were dipped in ninhydrin (0·5 per cent in acetone) and the amino-acids visualized at 40° C (hot plate) for 30 min. A permanent record could be obtained by overspraying with aqueous nickel sulphate (7 per cent) and, when dry, coating with a vinyl lacquer (aerosol). The second plasma sheet was cut in two along a line drawn between the red and orange markers.

Table 1. RELATING URINARY CREATININE CONCENTRATION TO VOLUME APPLIED TO CHROMATOGRAM

(Simplified from Woolf[4])

Creatinine (mg/100 ml.)	0–15	16–25	26–50	51–100	101–200	201+
Volume of urine (μl.)	12	10	8	6	4	2

The lower portion was dipped in *o*-phthalaldehyde (2 per cent in acetone) and heated at 40° C for 10 min to locate histidine and glycine. The upper portion was dipped in isatin (1 per cent acetone) and heated for 5 min at 100° C (oven) to locate proline (Figs. 1 and 2).

In our experience, specific location reagents are necessary to detect with certainty the moderate elevation in plasma concentration found in some cases of histidinaemia, hyperprolinaemia and hyperglycinaemia, but ninhydrin alone is satisfactory for urinary amino-acid chromatograms, where the concentrations of the characteristic amino-acids are usually considerably elevated.

In general, we have found that a reagent concentration approximately three times greater than that recommended for paper chromatograms is required to obtain optimum colour development when dipping thin-layer chromatograms, and both Ehrlich and Sakaguchi reagents can be applied in this way, but both Pauly's reagent (diazotized sulphanilic acid) and Folin's β-naphthoquinone-4-sulphonate reagents are best applied by spraying. The useful iodo-platinate reagent for sulphur amino-acids cannot be used on aluminium layers because of the formation of platinum black and evolution of hydrogen; the reagent, however, can be used on glass plates to detect cystine, homocystine, methionine, etc. Homocystinuria is thus best detected by the nitroprusside reaction on urine.

We emphasize that this is a valuable screening method of great use to the busy clinical laboratory, but that deviations from the normal pattern should be investigated by such methods as two-way thin-layer chromatography, electrochromatography or ion exchange resins. By this technique, we have detected the appropriate abnormalities in samples from authentic cases of phenylketonuria, tyrosinosis, histidinaemia[7], hyperglycinaemia, hyperprolinaemia, hyperalaninaemia (lacticacidaemia), maple syrup urine disease, cystinuria, Hartnup disease and various generalized aminoaciduria such as cystinosis.

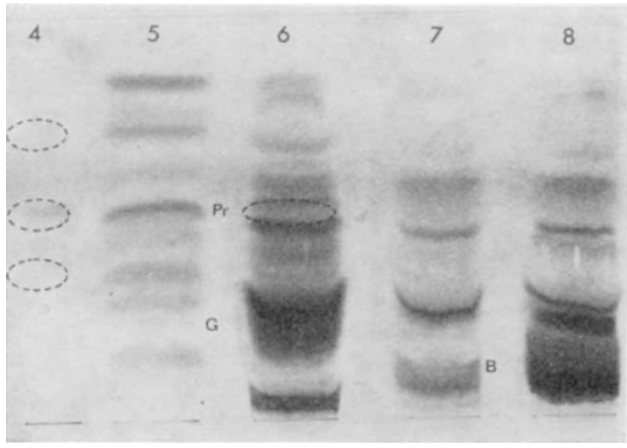

Fig. 2. Urine amino-acids. 4, Marker; 5, standard mixture; 6, hyperprolinaemia (Pr, proline; G, glycine); 7, normal urine; 8, cystinuria (B, basic amino-acids and cystine).

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