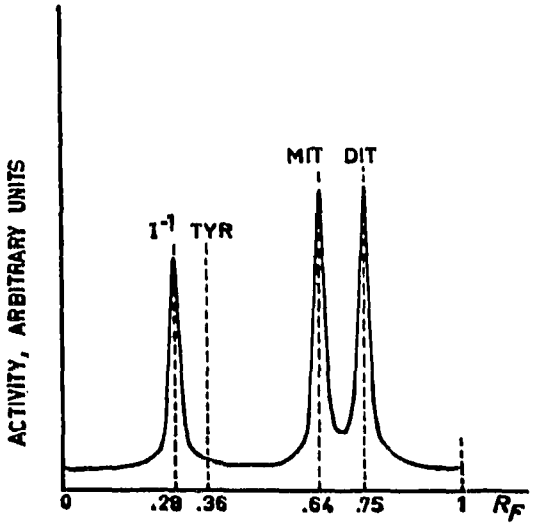

Fig. 1. Thin-layer radiochromatography of a mixture of MIT, DIT, I^-, eluted with *n*-butanol–acetic acid–water (4:1:5).

The yield was evaluated by counting of the supernatant.

When compared with separation methods on paper, thin-layer chromatography has the advantage of reducing the operation time, making this technique suitable for routine controls in the production of labelled MIT and DIT.

In addition, the higher adsorption capacity of silica gel in plate chromatography is very useful for preparative purposes.

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Thin-layer chromatography of metabolic derivatives of tryptophan

Paper chromatography is routinely used in our laboratory for the identification and determination of several metabolic derivatives of tryptophan of the so-called "via kynurenine", in urine of normal and pathological subjects. This method, which is suitable for quantitative analyses, is, however, time-consuming when used for qualitative purposes[1].

Therefore, an attempt was made to apply thin-layer chromatography (TLC) to the separation of tryptophan metabolites. The technique described here not only has the usual advantages of TLC, but several others as well, particularly as regards speed, in which it surpasses the previous method.

DIAMANTSTEIN AND EHRHART[2] were the first to apply TLC on silica gel to the fractionation of tryptophan, indole, indican, anthranilic and quinolinic acids. We

achieved more reproducible results than the above-mentioned authors by employing polyamide as an adsorbent for the chromatography of compounds derived from tryptophan.

Polyamide has already been used for the isolation of phenolic substances, since it has a high adsorption capacity and, moreover, the sorption processes are reversible, which is useful for analytical purposes. Thin layers of polyamide have been employed for the separation of flavanoids[3–5], polyhydroxyphenols and pro-anthocyanidins[6], antioxidants[7], gallic acid and its esters[8], and essential oils[9].

This new technique on thin layers of polyamide powder has some advantages, particularly as regards resolution power, in comparison with that on silica gel; polyamide powder is also undoubtedly preferable to other materials tried by us, such as dextran, neutral, basic or acid alumina and kieselguhr.

Table I reports the results obtained with polyamide as an adsorbent, of a rapid chromatographic separation of nine tryptophan derivatives. The best results are obtained when the mixture formic acid–methanol–water (2.5:37.5:60) is employed.

Substitution of acetic acid for formic acid, as well as the use of higher alcohols instead of methanol causes a decrease in the quality of fractionation.

TABLE I

R_F VALUES AND FLUORESCENCES

Adsorbent: polyamide (Woelm).
Solvent: 99% formic acid–methanol–water (2.5:37.5:60)

Compound	*Mean R_F*	*Fluorescence at 3655 Å*
Xanthurenic acid	0.17	blue-green
Xanthurenic acid 8-methyl ether	0.30	blue
Kynurenic acid	0.36	blue (turning yellowish)
3-Hydroxyanthranilic acid	0.50	blue-violet
N-α-Acetyl-3-hydroxykynurenine	0.68	yellow-green
o-Aminohippuric acid	0.73	violet
N-α-Acetylkynurenine	0.78	azure
3-Hydroxykynurenine	0.86	yellow-green
Kynurenine	0.90	azure

When methyl, ethyl, propyl, isopropyl, butyl and isobutyl alcohols and ethylene and propylene glycols mixed with water in different ratios are employed negative results are obtained. Mixtures such as pyridine–*n*-butanol–water, triethanolamine–*n*-butanol–water, pyridine–isopropanol–water, ammonia–isopropanol–water, pyridine–methanol, and ammonia–methanol in various ratios give front and spot splitting and have a detrimental effect on the compounds chromatographed. Large and overlapping spots result when 0.1, 1, 3 and 5 % aqueous solutions of NaCl, KCl, Na_2HPO_4, NaH_2PO_4, $NaNO_3$, Na_2SO_4 and CH_3COONa are used.

When urine is chromatographed directly no R_F variation is observed. By running standard solutions containing pure compounds on the same chromatoplate the spots can be identified and at the same time a rough quantitative comparison between known and unknown samples can be made.

Since, however, the volumes of urine to be spotted are very small (0.001–0.05 ml), the method is only suitable when the urinary amounts of metabolites are above the normal levels of "spontaneous" excretion.

Experimental

Materials. Kynurenine, 3-hydroxykynurenine, kynurenic acid, xanthurenic acid, xanthurenic acid 8-methyl ether, and *o*-aminohippuric acid were prepared in this laboratory. N-α-Acetylkynurenine and N-α-acetyl-3-hydroxykynurenine, chromatographically pure, were isolated from human urine of subjects loaded with L-tryptophan. 3-Hydroxyanthranilic acid was a commercial product. Urine of human beings who had been subjected to a loading test of 100 mg/kg of L-tryptophan was also analysed, the content of the above metabolites being markedly elevated in this case. The polyamide powder was the standardized product of Woelm.

Solvent systems. Of the many solvents tried, formic acid 99 %–methanol–water (2.5 : 37.5 : 60 v/v), gave the best results.

Detection of the spots. For detecting the above tryptophan derivatives, a Philips HPW-125 lamp at 3655 Å, was used.

Method. Smooth glass strips (4 × 20 or 4 × 30 cm) were coated with a 0.1 mm thick layer of standardized polyamide (0.3 g suspended in a mixture of methanol–chloroform, 3 : 2), by means of a home-made applicator. The plates were then dried in an oven at 40° for 10 min.

Spots of the standard solutions of the above compounds in 50 % acetone were applied from a capillary pipette on a point 2.5 cm from the edge of the plate. Amounts of each metabolite ranging between 0.05–3 μg gave good resolutions.

Urine of subjects loaded with L-tryptophan was directly spotted in volumes of 0.001–0.05 ml, depending upon the content of metabolites, and a mixture of pure substances was run in parallel on the same chromatostrips.

Development was carried out by the ascending method in closed cylindrical chambers (6 × 40 cm) in which saturation was not necessary. The time required for the development of short and long chromatostrips (the solvent front moved 15 and 26 cm) was 60 and 150 min, respectively.

The developed chromatograms were then removed from the tanks, dried in an oven at 60° and observed under U.V. light.

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