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Note

Thin-layer chromatography of kynurenin metabolites

The identification of metabolites of kynurenin is of interest in several diseases, and particularly in the study of inborn errors in tryptophan metabolism.

Thin-layer chromatography (TLC) on ECTEOLA-cellulose for the separation of some kynurenin metabolites was first proposed by McMANUS AND JACKSON[1]. We have modified this method slightly so as to obtain excellent separation of the principal metabolites of kynurenin.

Materials and methods

Thin-layer plates of ECTEOLA-cellulose (20 × 10 cm) on a flexible support (supplied by Macherey, Nagel and Co., Düren, G.F.R.) were used. Purification of the thin layer was necessary and was achieved by running in distilled water up to the top of the plate. The layer was then dried in cold air.

Standard solutions of 3-hydroxyanthranilic acid, kynurenin and 3-hydroxykynurenin were prepared in 0.1 *N* HCl at a concentration of 1 mg/ml. Anthranilic acid, kynurenic acid, xanthurenic acid, the 8-methyl ester of xanthurenic acid and *o*-aminohippuric acid were prepared as above in 0.2 *N* ammonia solution.

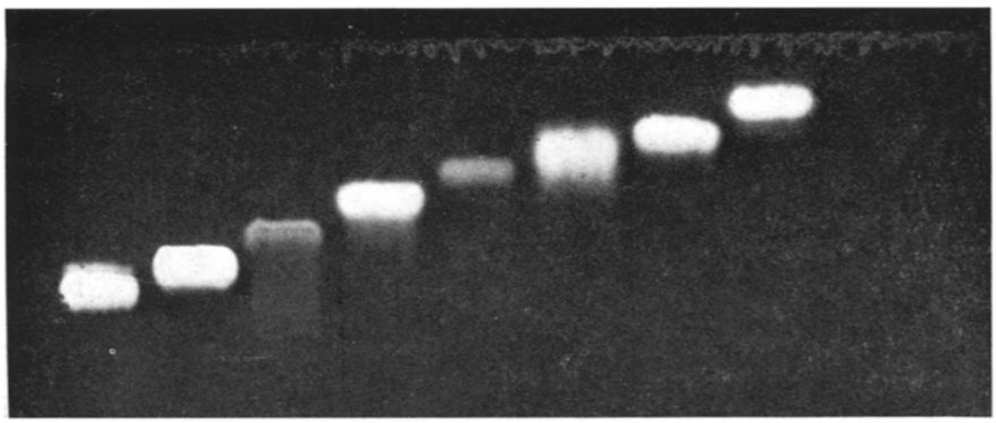

Fig. 1. TLC of kynurenin metabolites.

No.	*Compounds tested*	*Fluorescence at 254nm*
1	Xanthurenic acid	light blue
2	8-Methyl ester of xanthurenic acid	light blue
3	Kynurenic acid	green
4	3-Hydroxyanthranilic acid	green–blue
5	3-Hydroxykynurenin	yellow–green
6	Anthranilic acid	light blue
7	Kynurenin	green–blue
8	*o*-Aminohippuric acid	light blue

The solvent mixture that gave the best results in chromatography consisted of 10 g sodium chloride and 1 ml of glacial acetic acid per 100 ml of water.

Volumes of 2 μl of each standard solution were applied over 1.5 cm at a distance of 2 cm from the lower edge of the plate. Chromatography was carried out in the ascending direction until the solvent reached the upper edge of the plate, which required about 15–20 min. The chromatogram was air-dried in a vertical position and examined under UV light at 254 nm.

Results

Fig. 1 shows the chromatogram obtained. The method has also been used successfully in the analysis of urine specimens obtained from patients after an oral loading test with L-tryptophan (0.3 g/kg). The kynurenin metabolites are clearly visible under these conditions with only 5 μl of urine.

The above method gave an excellent separation of the principal metabolites of kynurenin within 15–20 min. It can be used for the rapid identification of these compounds in urine under pathological conditions after a tryptophan loading test.

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1 R. MCMANUS AND M. JACKSON, *Anal. Biochem.*, 23 (1968) 163.

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