Thin-Layer Chromatography of Urinary Imidazoles

R. Humbel

A simple technique is described for the identification of urinary imidazoles by thin-layer chromatography on cellulose. It is especially suitable for the diagnosis of histidinemia, and it can give information about other anomalies in histidine metabolism.

During a study on the metabolism of histidine in a child affected with histidinemia (Beauvais, P., and Humbel, R., to be published), we became interested in the chromatography of imidazoles. In this disease the urine contains numerous imidazoles: histidine and its metabolites, imidazole-pyruvic, imidazole-lactic and imidazole-acetic acids and N-acetylhistidine. I developed a chromatographic method on thin-layer cellulose which permits rapid resolution of these urinary imidazoles.

Method

The chromatography is done on ready-made cellulose sheets 20 cm long (Merck, Darmstadt, Germany). The solvent is an ethanol-water mixture (9:1). After development, the sheets are dried in air and sprayed with a solution of ninhydrin in ethanol-water (1:1). They are then heated for 10 minutes at 100°C and developed for 15 minutes. The characteristic spots are shown in Fig. 1.
Germany). Ten µl of untreated urine is applied to 2 cm of the starting line, which is 2 cm above the lower edge of the sheet.

The chromatogram is developed in acetone–acetic acid–water, 70:10:20 (v/v), until the solvent reaches the upper edge of the sheet.

The plate is then dried in a stream of warm air, and developed once again in the same solvent and in the same direction. This double chromatography practically eliminates the “salt-effect” that results from the application of undesalted urine.

The sheet is dried in warm air and sprayed with freshly prepared Pauly’s reagent: 10 vol sulfanilic acid, (0.9 g/100 ml 0.2N HCl) plus 10 vol of a freshly prepared solution of sodium nitrite (5 g/100 ml); and, after 5 min, plus 20 vol of a solution of sodium carbonate (10 g/100 ml).

Imidazoles appear as red or red-tinged orange spots on a pale yellow background.

Results

Figure 1 shows the chromatogram obtained with seven pure reference substances obtained through the courtesy of Dr. S. K. Wadman, Department of Pediatrics, University of Utrecht, The Netherlands. The imidazoles are well separated.

The chromatogram of Fig. 2 illustrates the results obtained with urines. The reference substances were added to normal urine to cover the salt effect in the left-hand pattern. Normal urine alone is seen in the middle pattern; histidine, imidazole–lactic acid, and imidazole–acetic acid (faint) are visible. The urine applied on the right was obtained from the child affected with histidinemia. It contains high concentrations of histidine, imidazole–pyruvic acid, imidazole–lactic acid, imidazole–acetic acid, and N-acetylhistidine; no urocanic acid and only traces of imidazole–propionic acid are present.

Fig. 2. Chromatography of urinary imidazoles. From left to right, the patterns are: reference substances added to a normal urine, 10 µl of a normal urine, and 10 µl of urine from a histidinemic child.