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Note

A simple screening method for urinary purines

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Although urinary screening methods are widely used in clinical chemistry as aids in the detection of abnormalities of amino acid and carbohydrate metabolism, relatively little attention has been paid to the use of such methods in the detection of disorders of purine metabolism[1]. Most of the methods available are cumbersome and can take several days to complete[2–6]. This paper describes a procedure that makes it possible in 1 day of purify, concentrate and separate the purines found in human urine.

MATERIALS AND METHODS

Urine (200 ml, containing approximately 200 mg of creatinine) was acidified by adding a few drops of concentrated hydrochloric acid. It was then applied to the top of a previously washed Dowex 250 × 15 mm 50W-X8 (H^+) column (20–50 mesh) and washed through with distilled water (200 ml). The adsorbed material, including the purines, was then removed from the column by washing with 5 *M* ammonia solution (200 ml). The eluate obtained was applied to the top of a 130 × 15 mm Dowex 2-X8-100 (OH^-) column (50–100 mesh), was then washed through with distilled water (200 ml) and the adsorbed material removed with 1 *M* hydrochloric acid (200 ml). The final acidic eluate, which contained the urinary purines, except for uric acid, was evaporated to dryness on a rotary evaporator. The dried material was dissolved in 6 *M* potassium hydroxide solution (1 ml), the solution neutralized by adding 6 *M* perchloric acid and the precipitated potassium perchlorate removed by centrifugation[7]. The final clear supernatant solution was spotted (1–10 μl) on thin layers of polyethyleneimine (PEI) cellulose pre-coated on plastic sheets (200 × 200 mm; Schleicher & Schüll, Dassel, G.F.R.) containing a fluorescent dye (maximum wavelength of absorption 254 nm). The plates were developed in the first direction with distilled water[8], dried and then in the second direction with 1.25 *M* sodium chloride solution[9]. Ultraviolet-absorbing spots were located by irradiating the plates with a short-wavelength (254-nm) lamp.

RESULTS AND DISCUSSION

The initial chromatographic steps on the Dowex 50W-X8 and Dowex 2-X8-100

resins are necessary in order to remove interfering substances from the urine. Tests showed that the following compounds were removed on washing the Dowex 50 column with water: uric acid, uracil, orotidine, orotic acid (partially), ψ-uridine and protein (albumin). The second column was shown to remove creatinine and urea. All of these interfering compounds are found in normal urine[4]. Purines were recovered quantitatively in the final acidic eluate from the second column.

The two-dimensional thin-layer chromatography was rapid and was completed in about 3 h. Purine extracts were prepared from both normal urine and pregnancy urine samples sent to the laboratory for routine oestrogen screening. No differences were observed between the two groups on chromatography and up to 19 spots could be located under ultraviolet light (Fig. 1). On the basis of their chromatographic mobilities, seven of these spots could be identified as xanthine, hypoxanthine, adenine, guanine, 1-methylhypoxanthine, 7-methylguanine and N^2-methylguanine. The most intense spots were those due to xanthine, hypoxanthine and 1-methylhypoxanthine. Other samples of purine bases were not available for comparison.

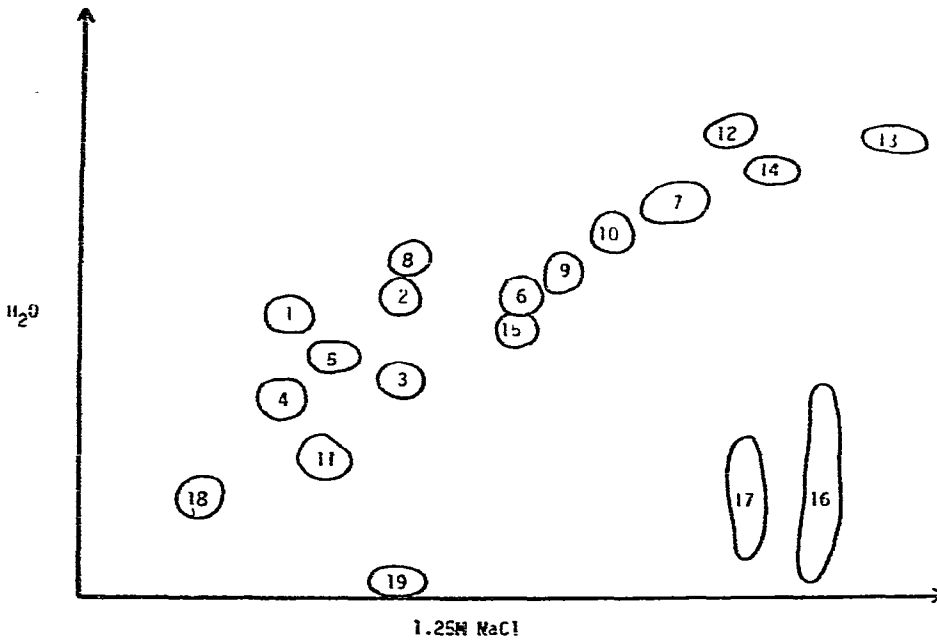

Fig. 1. Two-dimensional chromatogram of urinary purines. Spots: 1, adenine; 2, 7-methylguanine; 3, xanthine; 4, guanine; 5, N^2-methylguanine; 6, hypoxanthine; 7, 1-methylhypoxanthine; 8–19, unidentified. Spots 10, 15 and 17 were highly fluorescent.

The results obtained are comparable with those of other workers. Up to 22 spots have been noted in the paper chromatography of urinary purine preparations[5], although only about 15 purine bases have actually been identified in normal urine[3–5]. The present method should be useful in detecting such well documented inborn errors of purine metabolism as xanthinuria[10], in looking at the changes in purine urinary excretion patterns that occur in several types of cancer[11] and also in screening for

other as yet undiscovered metabolic disorders, particularly those relating to the pathogenesis of primary gout.

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