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Note

Simple method of measurement of orotic acid and orotidine in urine

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Orotic acid and orotidine are intermediary metabolites in the biosynthesis of pyrimidine nucleosides and nucleotides and their bases. They occur in increased concentration in urine in a number of clinical circumstances which require their accurate determination for diagnostic and therapeutic purposes.

There are a number of methods for the analysis of orotate and orotidine each of which has its own disadvantages. The assay based on bromination of orotate followed by color development with *p*-dimethylaminobenzaldehyde is not specific in that it measures both orotate and orotidine and may have other interfering compounds [1,2]. The normal range of urine orotic acid by this method is reported to be 0.4–1.2 μmol/mmol of creatinine.

Enzymatic [3] and isotope dilution [4] techniques have been reported but both are complex compared to the high-performance liquid chromatographic (HPLC) method presented here. Previous HPLC techniques have been promising but required considerable pre-column sample manipulation and/or multiple columns [5–7] or incomplete resolution in human urine [8,9].

Presented here is a method for measuring orotate and orotidine by isocratic HPLC with UV detection in urine requiring no sample preparation apart from filtration.

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EXPERIMENTAL

The HPLC equipment included a Waters Assoc. (Milford, MA, U.S.A.) Model M6000A pump, a WISP 710A injector, a systems programmer and a Model 450 variable-wavelength detector, 0.1 a.u.f.s.

A strong anion-exchange column (Whatman Partisil 10 SAX, 250 mm × 4.6 mm I.D., particle size 10 μm, Parsipanny, NJ, U.S.A.) was used throughout.

The mobile phase, pumped at a flow-rate of 1.2 ml/min, consisted of 0.8 *M* formic acid in 35% methanol adjusted to pH 2.8. The absorbances were recorded at 275 nm. An internal standard consisting of 2-nitrobenzoic acid was added to urine samples before filtration.

Procedure

To urine containing no detectable orotate or orotidine was added orotate and orotidine (Sigma, Chicago, IL, U.S.A.) to result in a final concentration of each of 20, 30, 40, 60, 80 and 100 μM. To 4 ml of each sample were added 2 nmol of 2-nitrobenzoic acid (10 μl of a 200 μM solution) following which the

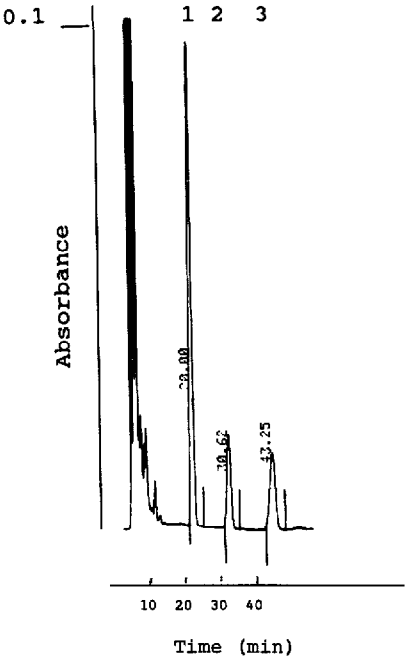

Fig. 1. Chromatogram of 50 μl of normal urine containing 0.5 μM 2-nitrobenzoic acid (peak 1), 0.08 μM orotic acid (peak 2) and 0.07 m*M* orotidine (peak 3). All are well separated from endogenous UV-absorbing compounds which appear early in the elution profile. Chromatographic conditions were: column, anion exchange; mobile phase, 0.8 *M* formic acid in 35% methanol (pH 2.8); flow-rate, 1.2 ml/min.

samples were filtered (Gelman Acrodisk, 0.2 μm). A 50-μl aliquot of the filtered urine was injected.

RESULTS

Fig. 1 shows the chromatogram of human urine to which orotate, orotidine and 2-nitrobenzoic acid had been added. Complete separation of endogenous UV-absorbing compounds, internal standard, orotate and orotidine is apparent.

Table I shows the recovery of varying amounts of orotate and orotidine added to human urine. Recoveries of orotate varied from 103 to 110% with a mean (±S.D.) recovery of 107±2.7%. Recoveries of orotidine varied from 95 to 113% with a mean (±S.D.) recovery of 109±6.9%.

Fasting urine orotic acid and orotidine levels were measured on 27 normal adult women; 23 of these subjects had levels below the detection limits of this method. The four other subjects had the following respective levels of orotic and orotidine: orotate, 0.47, 0.59, 0.81 and 6.37 μmol/mmol of creatinine; orotidine, 0.36, 2.56, 3.79 and 4.85 μmol/mmol of creatinine.

Following a single 300-mg dose of allopurinol to 26 normal women and collection of four 6-h urine samples the range of peak urine orotate and orotidine levels were, respectively, 2.02–15.4 and 2.02–7.23 μmol/mmol of creatinine.

TABLE I

RECOVERIES OF OROTIC ACID AND OROTIDINE ADDED TO NORMAL URINE

Compound	Amount injected (nmol)	Amount recovered (nmol)	Recovery (%)
Orotic acid	5	5.45	109
	4	4.40	110
	3	3.25	108
	2	2.10	105
	1.5	1.55	103
	1.0	1.05	105
Mean ± S.D.			107±2.7
Orotidine	5	5.40	108
	4	4.45	111
	3	3.35	112
	2	2.25	113
	1.5	1.70	113
	1.0	0.95	95
Mean ± S.D.			109±6.9

DISCUSSION

There are a number of pathophysiological states in which it is desireable to measure urinary excretion of orotate and orotidine [10]: orotate phosphoribosyltransferase deficiency, administration of 6-azauridine or allopurinol, inborn errors of ureagenesis (apart from carbamyl phosphate synthetase deficiency), lysinuric protein intolerance, the hyperammonemia, hyperornithenemia, homocitrullinuria syndrome, purine nucleoside phosphorylase deficiency.

The method described avoids many of the complexities and difficulties of previous techniques. In particular, it separates orotate from orotidine, an important advantage over the most commonly used method which measures both compounds by spectrophotometry in the visible range. This may have special importance to the recently described allopurinol test that identifies carriers of a mutant ornithine transcarbamylase gene [11]. These subjects respond to a single dose of allopurinol with a much greater degree of orotic aciduria and orotidinuria than normal subjects. Attempts to measure orotate and orotidine levels in plasma ultrafiltrates were unsuccessful presumably because normal plasma levels are below the sensitivity of this method.

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