TECHNICAL METHODS

Spectrophotometric Estimation of Urinary Taurine

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The amino-acid taurine has been shown to be a normal urinary constituent (Dent, 1948). Employing a quantitative chromatographic technique a diminished taurine excretion in cystinuria (Stein, 1951) and in Wilson's disease (Stein, Bearn, and Moore, 1954) has been detected. An increased taurine excretion, on the other hand, has been used as a sensitive index of minor liver damage in infectious hepatitis and cirrhosis (Dent and Walshe, 1954).

A method of estimation of taurine, without resort to chromatographic techniques, has not hitherto been available. A simple method of estimation of this amino-acid as its dinitrophenyl (D.N.P.) derivative is here presented.

Method

Principle.—Amines, peptides, and all amino-acids except taurine are removed from the urine by treatment with a cation exchange resin. Taurine is converted to its D.N.P. derivative by interaction with fluorodinitrobenzene. A final purification is effected by making use of the unique partition characteristics of D.N.P. taurine, and the concentration estimated by spectrophotometric examination of the solution at the absorption maximum of D.N.P. taurine.

Reagents.—The following reagents were employed:

Fluorodinitrobenzene.—A solution of 2 g. (1.4 ml.) of 1-fluoro 2:4 dinitro benzene is made up to 100 ml. with ethanol. This reagent is a powerful skin irritant.

Cation Exchange Resin.—A sulphonated polystyrene resin in the hydrogen form, free of coloured impurities. The damp A.R. grade of "amberlite" 1R 112 (H) resin in the form of 16 to 50 mesh beads (obtained from British Drug Houses Ltd.) was used throughout the present work.

Ethylene Dichloride.—Some commercial grades are satisfactory without further treatment. Purification, when necessary, may be effected by shaking with concentrated sulphuric acid, followed by washing with water, drying over calcium chloride, and distillation.

Aniline.—Freshly distilled.

Sodium Bicarbonate.—4% w/v.

Technique

To a 3 by $\frac{1}{2}$ in. test-tube containing 0.4 g. of "amberlite" 1R 112 resin 2 ml. of urine is added. The contents are mixed by repeated inversion for at

least one minute and filtered. Of the filtrate 0.5 ml. is pipetted into a centrifuge tube containing sodium bicarbonate (0.7 ml.); fluorodinitrobenzene (0.1 ml.) is added, mixed, and the tube placed in a 60° bath for 30 minutes. A blank (0.5 ml. of water) and a standard taurine solution (0.5 ml. of 0.001 M taurine) are similarly treated.

Then 2N hydrochloric acid (0.7 ml.) and ethylene dichloride (2 ml.) are added and the mixture is shaken and centrifuged briefly. One millilitre of the upper (aqueous) phase is transferred to a centrifuge tube containing 2 ml. water, and 1.2 ml. ethylene dichloride and 1.8 ml. aniline are added, and the mixture is shaken and centrifuged. Most of the upper (aqueous) phase is sucked off and 2 ml. of the organic phase transferred to a centrifuge tube containing 4 ml. sodium bicarbonate, which is shaken and centrifuged. The aqueous phase is read in a "unicam" S.P. 500 spectrophotometer or similar instrument at a wavelength of 360 m μ , using the blank solution for the zero setting.

Standards and Calculations

A millimolar taurine solution is prepared by dissolving 0.125 g. of taurine in N hydrochloric acid (100 ml.) and diluting to one litre with water.

$$\text{mM } \mu\text{l. taurine in urine} = \frac{\text{Extinction of standard at } 360 \text{ m}\mu}{\text{Extinction of test at } 360 \text{ m}\mu}$$

Specificity and Accuracy of Method

The final sodium bicarbonate extract of treated urine was desalted by the addition of "amberlite" 1R 112 (H) resin, filtered, and taken to dryness *in vacuo*. The residue was transferred to a strip of chromatography paper with water and run in butanol-acetic acid-water (40:10:50). A single yellow spot ($R_f = 0.4$) corresponding to that obtained from an authentic specimen of D.N.P.-taurine was always present; a small amount of a brown pigment of low R_f was also present. Recovery of taurine added to urine was 95–105% up to a concentration of 2.0 μM ml., falling to approximately 80% at 3.0 μM /ml. It is advisable, therefore, to dilute concentrated urines to bring the taurine concentration to < 2.0 μM /ml., and to repeat the estimation.

Other solvent systems may be used instead of ethylene dichloride + ethylene dichloride – aniline for the purification of D.N.P. taurine. Ethylene dichloride + ethylene dichloride – benzyl alcohol (20:80) and amyl acetate + amyl acetate – *tert.* amyl alcohol (20:80), for example, were approximately 70% as efficient as the solvent system used, as judged by the extinction at 360 m μ of the final bicarbonate phase.

Equimolar solutions of cysteic acid read approximately 12% of taurine solutions under the conditions adopted. Cysteic acid is not, however, present in significant amounts in untreated urine.

Results

Forty-seven 24-hour urines and 33 early morning specimens have been examined, and the results are presented in Table I.

TABLE I
TAURINE EXCRETION

24-Hour Specimens		Early Morning Specimens	
Type of Case	Taurine (mM)	Type of Case	Taurine (mM l.)
Biliary cirrhosis	2.3	Cirrhosis	3.2
Cirrhosis ..	0.5	Infective hepatitis ..	8.9
Others (45) ..	0.98 ± 0.7	(acute stage) ..	7.2
		Infective hepatitis ..	2.9
		(chronic stage) ..	1.9
		Pancreatic carcinoma (liver involvement)	2.2
		Others (26)	1.0
			1.1 ± 0.6

Discussion

Variations in the concentration of taurine have been noted by several workers during chromatographic investigation of urinary amino-acids (Kay and Entenman, 1954; Souchon and Grunau, 1952; Ishihara, Komori, and Iida, 1951; Nardi, 1954; Beerstecher, Sutton, Berry, Brown, Reed, Rich, Berry, and Williams, 1950). Taurine is present in high concentration in liver (Tallan, 1954), and it is in the liver that taurine is conjugated with cholic acid to form taurocholate. It might be expected, therefore, that pathological involvement of the liver might affect urinary taurine output. Dent and Walshe (1954) detected raised taurine levels in cases of infectious hepatitis and cirrhosis but not in cases of carcinoma of the liver, obstructive jaundice, and liver infiltrations. Other workers (Hsia and Gellis, 1954) have not been able to confirm the rise in hepatitis. The present work indicates that a markedly raised taurine output is a possible but not invariable accompaniment of hepatitis.

Summary

A simple method of taurine estimation as its dinitrophenyl derivative is presented. Urine taurine concentration is sometimes raised in cases of hepatitis.

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Chemical Tests for Ketonuria

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The detection and roughly quantitative assessment of ketonuria is usually accomplished by a combination of Rothera's and Gerhardt's tests. The former reaction is very sensitive, giving colours with both acetone and aceto-acetic acid, whereas the latter is a much less sensitive test for aceto-acetic acid only, a positive reaction therefore being of more significance. Nash, Lister, and Vobes (1954) have remarked on the lack of uniformity in the published methods of carrying out these tests, and stated that they were relatively time-consuming when correctly performed. These workers studied the use of a recently developed tablet preparation containing nitroprusside, and claimed that it compared favourably with the older reactions with respect to speed and simplicity of manipulation. The sensitivity of the tablets appeared to be roughly intermediate between that of the Rothera and that of the Gerhardt when the tests were compared on samples of normal urine to which varying amounts of acetone and aceto-acetic acid had been added.

In the present work the three tests have been applied to urine specimens from diabetic out-patients with the object of obtaining a more accurate comparison of the sensitivities by making quantitative analyses of acetone and aceto-acetic acid in the urines.

Experimental

The urine specimens were either brought by the diabetic patients or were obtained during their attendance at the clinic. With two exceptions only those specimens which gave a positive reaction with Rothera's test were used. Acetone, aceto-acetic acid, and β-hydroxybutyric acid were measured in the specimens by the method of Thin and Robertson (1952).

Rothera's Test (Harrison, 1947).—About 2 g. of a powder, consisting of 100 parts of ammonium sulphate and 1 part of sodium nitroprusside, were placed in a test-tube and 10 ml. of urine added. The mixture was well shaken and then 2 ml. of 880 ammonia solution added. A positive reaction was taken as the development of a permanganate colour in 10 minutes and was graded from + to + + + +.

Gerhardt's Test (Nash et al., 1954).—Ten per cent. (w/v) FeCl₃ solution was added drop by drop to 5 ml. of urine until any precipitate which formed had redissolved. If a reddish colour developed the test