TECHNICAL METHODS

Results

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

<table>
<thead>
<tr>
<th>Type of Case</th>
<th>Early Morning Specimens</th>
<th>Type of Case</th>
<th>Early Morning Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-Hour Specimens (mM)</td>
<td>Early Morning Specimens (mM 1.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary cirrhosis</td>
<td>2.3-0.5</td>
<td>Cirrhosis</td>
<td>3.2-2.9</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>0.98±0.5</td>
<td>Infective hepatitis (acute stage)</td>
<td>2.9-7.2</td>
</tr>
<tr>
<td>Others (45)</td>
<td></td>
<td>Infective hepatitis (chronic stage)</td>
<td>1.9-2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pancreatic carcinoma</td>
<td>1.0-0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(liver involvement)</td>
<td>1.1±0.6</td>
</tr>
</tbody>
</table>

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.

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

References


TECHNICAL METHODS

was repeated after boiling the urine for 15 minutes in a beaker and cooling before the addition of the FeCl₃ solution. The reaction was again graded by a combination of + symbols.

Table Test (Acetest Reagent Tablet).—The tablet contained sodium nitroprusside, glycine, disodium phosphate, and lactose. The technique and grading used was that recommended by the manufacturers. A tablet was placed on a clean white surface and one drop of urine allowed to fall gently on it. After 30 seconds the colour developed was compared with a colour scale provided with the tablets, the reaction being graded as negative, trace, moderate, and strongly positive.

Results

In the 41 urines examined acetone was found in concentrations ranging from 0.5 to 37 mg./100 ml., aceto-acetic acid from 1.7 to 220 mg./100 ml., and ß-hydroxybutyric acid from 5 to 490 mg./100 ml. None of the tests gave a colour with ß-hydroxybutyric acid, and the contribution of the acetone towards the colour development of the Rothera and tablet tests was probably negligible, because Nash et al. (1954) reported that Rothera's test gave only a trace reaction with 25 mg./100 ml. of acetone in normal urine. The relative sensitivities of the three tests, with respect to the aceto-acetic acid content of the urines, are given in Table I.

Table I

<table>
<thead>
<tr>
<th>Grading of Test</th>
<th>Rothera</th>
<th>Gerhardt</th>
<th>Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0-3 *</td>
<td>0-50</td>
<td>0-14</td>
</tr>
<tr>
<td>+</td>
<td>&gt;8-20</td>
<td>&gt;140</td>
<td>15-34 (trace)</td>
</tr>
<tr>
<td>++</td>
<td>&gt;20</td>
<td>&gt;70 (moderate)</td>
<td>&gt;70 (strong)</td>
</tr>
</tbody>
</table>

* Figures represent urinary aceto-acetic acid concentrations in mg. 100 ml.

It is seen that the Rothera's test is easily the most sensitive reaction, giving a definite positive result with concentrations of aceto-acetic acid from 3 mg./100 ml. upwards. The tablet test became positive at a concentration of approximately 15 mg./100 ml. of aceto-acetic acid, moderate positive reactions were obtained above 35 mg./100 ml. concentration, and strong positive reactions above 70 mg./100 ml. concentrations. Unequivocal positive Gerhardt reactions were only obtained at aceto-acetic acid concentrations above 50 mg./100 ml.

Discussion

Although the detection and semi-quantitative evaluation of ketonuria cannot give more than an approximate guide to the extent of ketosis in any patient, the urinary tests for ketonuria are very valuable aids to the clinician. The most important criteria for such tests are that the colour change should be easily recognized and capable of some degree of grading, that the manipulations should be simple, and that the test should be sensitive enough to detect ketone-bodies in concentrations of clinical significance.

In the present work the colour change in the Rothera test was the most easily recognized, and the tablet test, essentially a Rothera reaction, also gave an easily detectable colour, whereas the Gerhardt test gave some equivocal reactions. The tablet test was most suitable for a semi-quantitative evaluation of the colour because an artificial colour scale was available for comparison, whereas the assessment of the intensity of colour in the other two tests was a matter of personal judgment and experience. None of the tests needed very skilled or complicated manipulations, but the tablet test had the merit of dispensing with reagent solutions and test-tubes.

The most useful range of sensitivity cannot easily be decided because, first, there is no exact relationship between the amount of ketonuria and the patient's clinical state, and, secondly, none of the tests detect ß-hydroxybutyric acid which was found to form a large but variable proportion of the urinary ketone bodies.

The Rothera test, which is sensitive to as little as 3 mg. per 100 ml. of aceto-acetic acid, may give positive results with urines from normal individuals who have fasted overnight. Nevertheless, a positive reaction, accompanied by glycosuria, may indicate a tendency to ketosis in a diabetic patient which will not be detected by the other tests. However, with urinary concentrations of aceto-acetic acid above 25 mg. per 100 ml., it gives very strong colours which cannot be easily graded, and the use of a second, less sensitive test is necessary in order to assess these degrees of ketonuria. The Gerhardt test should be adequate for this purpose when used as a diagnostic aid in cases of diabetic coma, because clinical signs of ketosis do not develop when the urine contains less than about 50 mg. per 100 ml. of aceto-acetic acid (Nash et al., 1954). In many cases during the early development of diabetic coma, the ketonuria may be insufficient to cause positive Gerhardt's reactions, yet Rothera's test may be too strongly positive for accurate assessment. The tablet test covers this range of ketonuria and should be of value, particularly as it possesses other advantages such as the ease of grading the colour change. Whether the tablet test should supplement or replace either or both of the older tests is, however, a matter for experienced physicians in the diabetic field to decide.

Summary

The sensitivities of three tests for ketonuria have been compared on urine samples from diabetic patients and the suitability of the tests for clinical use have been discussed.

References
