AN ASSESSMENT OF NEW TESTS FOR DETECTING BILIRUBIN IN URINE

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Recently a simple tablet test for detecting bilirubin in urine has been introduced, and since this test is claimed to have considerable advantages over existing ones used by clinicians we have compared it with others commonly used to detect bilirubinuria. A comparison has been made of the tablet test ("ictotest") with the Harrison-Fouchet and iodine ring tests—selected because they are so commonly used—as well as with a modified Fouchet test which will be referred to as the mat Fouchet test. The last is very simple and quick, and unlike the standard Harrison-Fouchet test is suitable for routine use in the consulting room. The sensitivity of the tests has been compared and the effect of urobilinogen, drugs, and other interfering substances noted.

In the "ictotest" bilirubin is concentrated by adsorption on a cellulose asbestos mat and then allowed to couple with a diazo compound, in tablet form, to give a coloured complex. It has been tried in America by Klatskin and Bungards (1953), Sobotka, Luisada-Opper, and Reiner (1953), Giordano and Winsted (1953), and by Free and Free (1953), and in this country by Tallack and Sherlock (1954). All these authors have commented favourably on its simplicity, sensitivity, and specificity. As we also consider that the "ictotest" and the mat Fouchet test are superior to the crude iodine test still used by many, we record our findings in the hope that we may encourage their general adoption.

Material

Eighty consecutive urine specimens, sent to the laboratory in connexion with other liver function studies, were tested, as well as 10 specially selected because they were normal, contained salicylates, or had a very high concentration of bilirubin.

Methods

The urines were submitted to some or all of the following tests:—The "ictotest," mat Fouchet, Harrison-Fouchet, iodine ring, Schlesinger test for urobilin, Obermayer test for indican, ferric chloride test for salicylate, and the heat test for protein. All these tests, with the exception of the first two, were carried out as described by Harrison (1947), and because of the known instability of bilirubin in urine (Klatskin and Bungards, 1953) all were performed as soon as possible on the day the specimens were received. Positive results were assessed on a rough quantitative basis, being graded as strong, moderate, or weak; doubtful positives were recorded as such. Ease of assessment and especial difficulties were also noted.

The "ictotest" and the mat Fouchet test were carried out as follows:

"Ictotest."—The tablets and mats manufactured by the Ames Company were used. Each tablet contains 0.2 mg. p-nitrobenzene diazionium p-toluene sulphonate, 100 mg. sulphasalicylic acid, 10–20 mg. sodium bicarbonate, and 15–25 mg. boric acid. The mats measure about 20 × 20 × 4 mm. and consist of a cellulose asbestos mixture. Five drops of urine were allowed to soak into a test mat and a tablet was then placed on the moistened area. One drop of water was placed on top of the tablet and allowed to remain for a few seconds before a second drop was added, carrying the fluid on to the test mat below. The result was read within 30 seconds; the development of a purple colour on the mat was recorded as positive and all other colours, and any change after 30 seconds, were recorded as negative. The speed of development and intensity of the purple were used to assess the concentration of bilirubin.

Mat Fouchet Test.—Five drops of urine were allowed to soak into an "ictotest" mat and one drop of Fouchet reagent was added in the centre. The result was recorded as positive if a bluish colour developed within a few seconds and as negative if purple or pink colours appeared or there was just blanching of the yellow background. Positive results were graded according to the depth of colour produced.

The manufacturers state that either surface of the "ictotest" mat may be used. We found that using the original upper and lower surfaces of mats after splitting them in the horizontal plane in no way reduced the sensitivity of the test. Having performed many tests in parallel the use of half-mats was adopted for the remainder of the trial.

Attempts were made to find an alternative to the "ictotest" mat. Asbestos filter pads, filter paper of various grades, cellulose wadding, and the Franklin
barium chloride-impregnated plaster of paris tablet (Franklin, 1949) were tried, but all except the last were found inferior to the "ictotest" mat. The Franklin tablet was found slightly less efficient in adsorbing bilirubin on to the surface, but the smooth, pure white background was found advantageous when reading the result.

**RESULTS**

**Sensitivity of Tests.**—Parallel tests on the 80 urines from patients with suspected liver impairment gave the results shown in the table. This shows that the Harrison-Fouchet and mat Fouchet tests give similar results, that the "ictotest" is considerably less sensitive than these, and that the iodine test is very unsatisfactory. The number of doubtful positives, which reflects the difficulty of assessing the result of a test, shows that the "ictotest" and the mat Fouchet test are often difficult to read, but even the Harrison-Fouchet test is not free from this objection.

<table>
<thead>
<tr>
<th>Result of Test</th>
<th>Test</th>
<th>Ictotest</th>
<th>Iodine Ring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Harrison-Fouchet</td>
<td>Mat Fouchet</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>15 urines</td>
<td>14 urines</td>
<td>28 urines</td>
</tr>
<tr>
<td>Doubtful positive</td>
<td>12</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Weak</td>
<td>31</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Strong</td>
<td>15</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Total number of definite positives</td>
<td>53</td>
<td>50</td>
<td>33</td>
</tr>
</tbody>
</table>

To make a better assessment of the relative sensitivity of the four tests, serial dilutions were made with distilled water of six urines from markedly jaundiced patients. The results of the tests on these specimens showed that in each case the Harrison-Fouchet method detected bilirubin in the greatest dilution; in two cases down to 1 in 128. The Harrison-Fouchet test detected bilirubin at twice the dilution giving a positive mat Fouchet test and three times that giving a positive "ictotest." In all cases the iodine test was found to be markedly less sensitive than the other tests.

**Effect of Interfering Substances.**—Previous experience had shown that the presence of urobilin, indican, and salicylates in urine occasionally caused difficulty in interpreting the Harrison-Fouchet test, so the effect of these substances on the three more sensitive tests was investigated. Our results (see below) showed that each may be associated with difficulty when only small amounts of bilirubin are present. The effect of proteinuria on the "ictotest" was also noted.

Urobilin.—Of the 80 urines tested, 18 had a slight excess, nine a moderate, and nine a marked excess of urobilin. Analysis of the results showed that the relative sensitivities of the bilirubin tests were the same regardless of the concentration of urobilin. Therefore, as far as difficulties caused by urobilin are concerned the tests appear of equal status. In some cases where excess urobilin was present a brownish purple spot developed in the Harrison-Fouchet test and a red colour in the "ictotest."

Indican.—Of 54 urines tested for indican, 14 had abnormally large quantities. In these 14 urines the bilirubin tests showed agreement in all except four. In each of the latter an intense red colour was produced in the "ictotest," and in three a purplish brown spot in the Harrison-Fouchet test.

Salicylate.—Of 23 urines tested for the presence of salicylate, five gave positive ferric chloride tests which were shown conclusively to be due to salicylate. The tests for bilirubin on these five urines gave more strongly positive results with the mat Fouchet test than with the Harrison-Fouchet test. That this was attributable to salicylate was shown by testing two specimens of urine from non-icteric patients receiving large doses of salicylate —calcium salicylate and sodium p-aminosalicylate (P.A.S.). The results showed that, not only does salicylate cause strong false positives with the mat Fouchet test, but may sometimes cause difficulty in interpreting the "ictotest," for a doubtful positive was recorded in the case of the patient receiving P.A.S. In the mat Fouchet test salicylates may produce a colour indistinguishable from that due to bilirubin, but sometimes the spot has a pinkish hue or a red halo surrounds a bluish centre; either of the latter findings should arouse suspicion of a false positive. In the "ictotest" P.A.S. therapy may account for a permanganate colour developing within 30 seconds; earlier development of an immediate orange-yellow (P.A.S.) or red colour (calcium salicylate) should arouse suspicion of a false positive. In the Harrison-Fouchet test salicylates cause a greyish-red spot quite unlike the bluish-green of a positive test for bilirubin; the appearance of a pink colour when a drop of Fouchet reagent is allowed to fall on the filter paper where there is no precipitate will serve to confirm that salicylates are present.

Protein.—Klatskin and Bungards (1953) stated that protein lowered the sensitivity of the "ictotest," and caused the production of a pink colour. Tallack and Sherlock (1954) did not confirm this and analysis of our results does not show a higher
incidence of proteinuria in urines giving a red colour with this test.

Ease of Assessment of Tests. — In most cases there was nothing to choose between the tests as regards ease of reading. On 30 occasions when a definite preference was noted, the Harrison-Fouchet test was preferred in 25, the mat Fouchet test in three, and the "ictotest" in two.

Discussion

Our results as regards the comparative sensitivity of the tests agree with those of other workers in pointing to the relative insensitivity of the iodine test. Our findings differ from those of Tallack and Sherlock (1954), however, in placing the "ictotest" as less sensitive than the Harrison-Fouchet and even the mat Fouchet test. In this connexion it is unfortunate that we were unable to compare the tests on a larger number of urines.

Considering the specificity of the tests the "ictotest" would appear to be very satisfactory, for on only three occasions in 180 tests were apparent false positives recorded, and even then the results were graded as doubtful positives in two. This very low incidence of false positives with the "ictotest" is in accord with the findings of previous workers. Turning to the occurrence of false negatives, however, the masking of positive tests by other intense colours occurs particularly with the "ictotest," though even the Harrison-Fouchet test is not free of this difficulty. Such was seen in some urines which contained excess urobin and indican, although the nature of the substance responsible remains uncertain. False positives due to salicylates can occur with the mat Fouchet test and this represents an important drawback to this method.

When sensitivity, specificity, and ease of assessment are all taken into account, there would seem to be no case, in the laboratory, for replacing the Harrison-Fouchet test by the newer tests. In the choice of test for routine use by the clinician, however, where its technical simplicity has led to the general adoption of the iodine test, the advantages of the new tests would seem to justify a change. There is little to choose between the "ictotest" and the mat Fouchet test for this purpose. Both require little equipment; both use reasonably stable reagents, although that for the mat Fouchet is liquid; both can be performed in a few seconds, and the higher sensitivity of the mat Fouchet test is offset by its greater liability to yield false positives.

Summary

A tablet test for bilirubinuria has been compared with the Harrison-Fouchet and iodine tests, and also with a simple modification of the Fouchet test.

The Harrison-Fouchet test is considered to be the best, but the new tests have been shown to be greatly superior to the iodine test.

For routine use outside the laboratory it is recommended that the iodine test be replaced by one or other of the new tests.

We wish to thank the Ames Company, Ltd., for providing the tablets and test mats used in the trial, and Professor M. Maizels for helpful criticism.

References


Addendum

Since this paper was submitted for publication we have had the opportunity of performing over 200 tests with three other batches of "ictotest" reagents, using both whole and half-mats. Results on 26 routine specimens, and also on serial dilutions of urine containing much bilirubin, have confirmed our previous findings as to the relative sensitivities of the tests.
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