Laboratory Tests in the Differential Diagnosis of Liver Disease

Report on Two Procedures

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The seromucoid estimation and the Jirgl test were investigated as aids in the differential diagnosis of jaundice or hepatomegaly. Subnormal concentrations of seromucoid strongly suggest hepatocellular disease, while in this series most patients with hepatitis or cirrhosis had normal or raised concentrations. In obstructive jaundice normal or high values may be found. Very high values suggest obstruction due to carcinoma, or the presence of secondary deposits of carcinoma in the liver. However, so many other conditions are associated with a raised seromucoid concentration that this finding must be interpreted with caution. The Jirgl test is not specific for post-hepatic obstruction, being, in the authors’ experience, usually positive in infective hepatitis and in carcinoma with secondary deposits in the liver, and occasionally in cirrhosis. It is suggested that the test responds to intra- as well as to extrahepatic obstruction.

It has been reported by Greenspan et al. that the concentration of blood seromucoid, or at least of its protein component, is frequently low in hepatitis and portal cirrhosis but frequently high in obstructive biliary disease and in hepatomegaly due to metastatic carcinoma; determination of this concentration has been proposed as an aid to the differential diagnosis of jaundice (1-4). Mandel et al. (5) confirmed the presence of subnormal seromucoid concentrations in some patients with viral hepatitis and alcoholic cirrhosis, and found mostly raised and no subnormal concentrations in patients with benign and malig-

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nent biliary obstruction. Since complications likely to raise the seromucoid concentration were present in most of their patients with "primary" hepatic disease, only a minority of those patients showed a subnormal seromucoid concentration; and even after exclusion of the patients with extrahepatic complications the incidence of diagnostic, subnormal seromucoid concentrations was far below that expected from the reports of Greenspan et al. Mandel et al. concluded that although the finding of a subnormal concentration of seromucoid in a jaundiced patient is strongly indicative of parenchymal disease of the liver, this diagnosis is not excluded by a normal or increased concentration; and, furthermore, that the finding of normal or increased concentrations is of very limited value.

**Seromucoid Estimation**

Our own observations confirm the existence of raised seromucoid concentrations in most patients with obstructive jaundice, and especially in patients with obstruction due to carcinoma or with secondary deposits of carcinoma in the liver; they do, however, support the finding of Mandel and his colleagues of a lowered seromucoid concentration in only a minority of patients with hepatocellular disease.

**Material and Methods**

A total of 226 specimens were examined: 90 from 89 healthy people and 136 from 113 patients who were or had recently been jaundiced or who had hepatomegaly at the time of the test. Hepatitis and hemolytic jaundice were diagnosed using clinical and laboratory data. The diagnoses of cirrhosis, obstructive jaundice due to stones or carcinoma, and secondary deposits of carcinoma in the liver were usually made by operation, biopsy or postmortem examination. Postoperative blood specimens were not used for seromucoid estimation, since the seromucoid concentration is influenced by trauma (6).

Thirteen cases are excluded because the cause of the jaundice or hepatomegaly has not yet been determined. Another 23 cases are excluded because of the existence of extrahepatic complications likely to raise the seromucoid concentration (e.g., small-bowel obstruction, bronchopneumonia, rheumatoid arthritis, etc.). Most of those without cirrhosis had raised seromucoid concentrations. Of the 23, 6 were cirrhotic patients with essentially normal seromucoid concentrations.

Seromucoid was separated by the method of Winzler, Devor et al.
(7) and estimated with Folin-Ciocalteu reagent after a preliminary heating with sodium hydroxide in the water bath. This preliminary treatment is based on the work of Andersch and Gibson (8), Greensberg and Mirolubova (9), and Minot and Keller (10) with total serum proteins, and eliminates variations that may occur in the final results when the seromucoid is allowed to stand in sodium hydroxide for varying lengths of time; it also results in the production of somewhat more color.

Eight ml. of 0.75 M perchloric acid was added to 2 ml. of serum and the mixture was shaken. After exactly 15 min. the precipitated proteins were filtered off (Whatman No. 50 9-cm. filter paper). One ml. of the filtrate was mixed with 0.2 ml. of a 5% solution of phosphotungstic acid in 2N hydrochloric acid, and after exactly 15 min. the precipitated seromucoid was centrifuged. The supernatant fluid was decanted and the tube allowed to drain. The precipitate was washed with 2 ml. of 95% ethyl alcohol and recentrifuged, the supernatant fluid being decanted and the tube allowed to drain as before. The precipitate was then dissolved in 2 ml. of 0.5 N sodium hydroxide and the solution heated in a boiling water bath for 5 min. After cooling, about 7 ml. of water was added, followed by 0.3 ml. of Folin-Ciocalteu reagent and finally water to 10 ml., and the contents of the tube were mixed by inversion. The color was compared at 30 min. with that produced by a standard solution of tyrosine (Ilford filter 205, EEL colorimeter). Results were expressed as milligrams of "tyrosine" per 100 ml. of serum. (The method is not specific for this amino acid.) To facilitate comparison with the results of the earlier workers we have also indicated the casein equivalents of the tyrosine values.

Comment

Normals

The results obtained with 89 apparently healthy people (52 men and 37 women; mostly laboratory staff and blood donors) are shown in Fig. 1, and illustrate the sex difference reported by previous investigators, e.g., Greenspan (4). Arbitrarily, we have taken the normal range as 3.0–8.0 mg. tyrosine for men and 2.5–6.5 mg. for women. There appears to be little justification for calculating a "normal" range from the mean and standard deviation, since the distribution of normal values is asymmetrical. The ranges we have chosen to regard as normal include all but one of the men and all the women in the normal series. It is obvious from Fig. 1 that our conclusions would be unaffected by slight adjustments to the upper and lower limits of these ranges.
Jaundiced Patients

These have been divided into the groups shown in Fig. 1. When more than one result was obtained with a given patient, on different dates, both results are shown.

Results obtained from 2 women whose jaundice was believed due to chlorpromazine have been omitted from the hepatitis group. This type of jaundice is thought to be associated with some degree of intrahepatic obstruction, and it is interesting to note that with both these patients results were slightly high (7.8 and 9.4 mg. tyrosine). Of the remaining patients in the hepatitis group, 11 had infective hepatitis.

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Fig. 1. Seromucoid concentrations in normal people and in patients with jaundice and/or hepatomegaly. Filled circles represent men, open, women. Vertical lines indicate normal ranges: unbroken, men; interrupted, women. Where more than one result is shown for a given patient, points are joined.
and 4 others were recovering from it; one had subacute hepatitis (tyrosine, 14.9 mg.); one probably had homologous serum jaundice (tyrosine, 6.5 mg.); and one had chronic hepatitis (tyrosine, 4.3 mg.). None, as far as could be determined, had any extrahepatic complication tending to raise the seromucoid concentration.

**Cirrhosis**

In this group no patients known to have complications likely to raise the seromucoid concentration are included. Six cirrhotic patients with such complications were found to have essentially normal seromucoid concentrations.

**Obstructive Jaundice and Carcinoma**

Obstructive-jaundice patients were divided into: those with stones and/or cholecystitis, and those in whom the obstruction was due to carcinoma of the head of the pancreas or bile ducts. The highest values were obtained in the second of these two groups.

Patients with secondary deposits of carcinoma in the liver comprise the final group represented in Fig. 1.

**Other Conditions**

Two cases did not fit into any of the above groups. In the first, liver biopsy suggested intrahepatic cholangitis, and a seromucoid tyrosine value of 6.8 mg. was obtained. In the second case (Table 3) laparotomy revealed a cirrhotic, multinodular, enlarged, soft liver and biopsy showed the appearances of obstructive jaundice superimposed upon diffuse hepatic fibrosis; no stones were found. The tyrosine value was 4.7 mg.

**Conclusions**

From the results certain conclusions may be drawn as to the value of seromucoid estimations by the method described.

In the absence of the nephrotic syndrome (11), of excessively high serum globulin levels, and possibly of certain endocrine diseases (4) a subnormal seromucoid concentration is strongly suggestive of hepatocellular disease; but this diagnosis is by no means excluded by a normal concentration, which (in agreement with Mandel et al.) we have found in the majority of patients with hepatitis or cirrhosis.

There is a marked tendency toward raised values in obstructive jaundice, though some patients with this condition show values within the normal range. Very high values suggest carcinoma. These findings are in broad agreement with those of Greenspan and his col-
leagues. In interpreting raised values it is most important to bear in mind the possible effect of extrahepatic diseases: the seromucoid fraction appears to be a particularly "labile" constituent—cf. Greenspan and Dreiling (3)—and many conditions may produce striking elevations in its concentration. For this reason subnormal concentrations of seromucoid are more likely to help in diagnosis than are raised concentrations.

The finding of a normal concentration of seromucoid appears to be of value in suggesting the absence of secondary deposits of carcinoma in the liver, but not in differentiating between other causes of jaundice and hepatomegaly.

It is interesting to note that 4 of 5 patients with jaundice of hemolytic origin had raised seromucoid concentrations.

**Jirgl's Test**

In 1957 Jirgl (12) reported a test in which a fraction separated from serum is dissolved in sodium carbonate and treated with Folin-Ciocalteu reagent; a positive result is indicated by opalescence, turbidity, or precipitation. Jirgl found that 44 of 46 cases of obstructive jaundice gave a positive result, whereas in 412 cases of jaundice not due to obstruction (including 147 with infective hepatitis), and in 233 without jaundice, a negative result was obtained. The test is thought to depend on the presence of "abnormal beta-lipoprotein or a very similar substance" (13). Daikos et al. (14) found the test positive in 23 of 25 cases of obstructive jaundice and 4 of 29 cases of nonobstructive jaundice: 24 patients with other conditions yielded only 1 or possibly 2 positive results, the 2 patients concerned having leishmaniasis and being under treatment with antimony preparations. Seven pregnant women and 5 other normal people all gave negative results. Daikos et al. concluded that the test was a reliable one for the differentiation of obstructive from hepatocellular jaundice, but they did not comment on the fact that their findings differed from those of Jirgl in so far as they obtained positive tests in 4 of 14 patients with infective hepatitis.

We find that the test appears to respond to intrahepatic as well as to extrahepatic obstruction.

**Material and Methods**

A total of 56 specimens of serum were examined: from 9 normal, healthy people; from 28 patients who were or had recently been jaun-
diced or who had hepatomegaly at the time of the test; and from 19 patients with miscellaneous nonhepatic conditions. The diagnoses were made as described in the earlier part of this paper.

The test was performed as described by Jirgl, confirmation of doubtful or borderline results being obtained by allowing the test solution to stand for several hours and noting whether flocculation occurred (14).

**Results and Discussion**

The results are summarized in Tables 1-4. In spite of the small number of cases investigated, certain conclusions appear to be legitimate. Clearly, a negative Jirgl test is an indication of the absence of posthepatic obstruction. However, a positive test is evidently very common in infective hepatitis. This finding is in marked contrast to Jirgl's negative results in a series of 147 patients with this condition, and suggests that the test responds to intra- as well as to extrahepatic obstruction.

Of the patients with infective hepatitis in our small series, the 4 who gave positive Jirgl tests had been jaundiced, respectively, for 4 days, about 5 days, about 10 days and 1 month at the time blood was taken; the patient who gave a negative result had been jaundiced for about 5

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**Table 1. Results of Jirgl Test in Posthepatic Obstructive Jaundice**

<table>
<thead>
<tr>
<th>Cause of obstructive jaundice</th>
<th>No. of cases</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stones</td>
<td>6</td>
<td>5*</td>
<td>1</td>
</tr>
<tr>
<td>Carcinoma of head of pancreas</td>
<td>1</td>
<td>1†</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma of bile duct</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9</strong></td>
<td><strong>8</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>

*One faint positive; 2 very faint.
†Exceedingly faint positive.

**Table 2. Results of Jirgl Test in Hepatocellular Jaundice**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic cirrhosis</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Infective hepatitis</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Hepatitis presumed due to chlorpromazine</td>
<td>1</td>
<td>1*</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>12</strong></td>
<td><strong>7</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>

*Exceedingly faint positive.
Table 3. Results of Jirgl Test in Other Hepatic Conditions and in Patients with Both Obstruction and Hepatocellular Disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse hepatic fibrosis and intra-hepatic obstruction</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma with secondary deposits in liver</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Cirrhosis, and stones in common bile duct</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>7</strong></td>
<td><strong>5</strong></td>
<td><strong>2</strong></td>
</tr>
</tbody>
</table>

Table 4. Results of Jirgl Test in Normal Individuals and in Patients with Miscellaneous Nonhepatic Conditions

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>19</td>
<td>1*</td>
<td>18†</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>28</strong></td>
<td><strong>1</strong></td>
<td><strong>27</strong></td>
</tr>
</tbody>
</table>

*Very faint positive (ulcerative colitis).
†Two were borderline results (solutions not “crystal clear”).

It is possible that negative results might regularly be obtained at an earlier stage, but we have no information on this point.

There appeared to be no correlation between the result of the Jirgl test and the seromucoid concentration.

References