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Ultracentrifugation Does Not Affect Results of Analyses for Digoxin, Cortisol, and Calcium in Lipemic Sera

To the Editor:
Free fatty acids form micelles and entrap steroids (1, 2), and triglycerides can complex with calcium to form insoluble carboxylate salts (3). Thus we were interested in what effect the removal of lipids would have on analytical results for digoxin, cortisol, and calcium in lipemic sera. Specifically, would the removal of chylomicrons from serum by ultracentrifugation lead to spuriously low results?

To answer this question, we checked the distribution of these constituents in lipemic sera before and after ultracentrifugation. Aliquots of pooled specimens of human serum containing triglyceride concentrations of 1.6 g/L (Pool A) and 0.13 g/L (Pool B) were supplemented with tritiated digoxin (Kallestad Laboratories, Inc., Austin, TX 78701; 0.28 mCi/L; 20.5 μg of digoxin per liter) and hydroxycortisone (Sigma Chemical Co., St. Louis, MO 63178; 5.4 mg of cortisol per liter) to give pharmacological and physiological concentrations. We removed calcium from each pool with Dowex “Macroporous” resin, then supplemented each pool with Standard Reference Material (National Bureau of Standards, Washington, DC 20234) calcium to a final concentration of 100 mg/mL and centrifuged at 10 T 000 × g (Airfuge, ACR-90 chylomicron rotor; Beckman Instruments Inc., Palo Alto, CA 94304) for 10 min. After centrifuga-

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Hypophosphatasia in Renal-Transplant Patients

To the Editor:
Eight of 37 patients who had undergone renal transplantation within the last three years were found to have low serum alkaline phosphate (ALP; EC 3.1.3.1) activity. The mean and SD for ALP in the eight patients was 40 (SD 3) U/L (the reference interval for our laboratory is 45–125 U/L). In six of the above patients, the difference was consistently present during periods ranging from three months to a year. In the re-

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Glucose Phosphate Isomerase and Glutathione Reductase in Benign and Malignant Extrahepatic Cholestasis

To the Editor:
Over the last two decades, variations in activity of glucose phosphate isomerase (GPI, EC 5.3.1.9) and glutathione reductase (GR, EC 1.6.4.2) in serum have been studied extensively, not only in different neoplasias (1–3) but in some types of hepatopathies and cholestasis as well (4–6), so far without any definitive diagnostic value having been established for them in cholestasis.

On the other hand, the usefulness of such enzymic activity data in patients with cholestasis has been only partly evaluated, without any comparisons with the other enzymes of recognized importance in this type of pathology having been established [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and gamma glutamyltransferase (GGT)].

To evaluate the behavior of GR and GPI in different types of cholestasis, both benign and malignant, as well as their comparative value with regard to the other enzymes mentioned, we determined the activity of GR (7), GPI (8), AP, AST, ALT (9), and GGT (10) in serum of 47 patients with serum bilirubin >20 mg/L. In 31 of these cases, laparatomy revealed a benign obstruction of the biliary tract caused by cholelithiasis, and in 16 patients the obstruction was due to either neoplasia of the head of the pancreas or of the extrahepatic bile ducts, with no hepatic
metastasis, as confirmed by either laparotomy or necropsy. Our control group consisted of 53 ostensibly healthy volunteer blood donors.

When the patients with benign biliary obstruction were compared with the control group, the mean values for GR activity were significantly higher (p <0.01), but we saw no important differences in GPI. However, in those patients with neoplastic obstruction of the biliary tract with hepatic metastasis, the mean values for GR and GPI were higher than those of the control group (p <0.001 and p <0.02, respectively). The difference between this group and those with benign biliary obstruction was significant only for GR (p <0.02). Table 1 gives the mean values found for all these analyses in the three groups.

While in 13 (81.2%) of the 16 patients with obstructive neoplastic icterus GR values were high, this was the case in only seven (22.5%) of the 31 patients with benign biliary obstruction (p <0.001). In the cases with benign obstructive icterus the GR was above the normal range in 16.1%, and in 43.7% of those with neoplastic obstruction (p <0.05). Simultaneously measured GR and GPI values were normal in 22 (71.0%) of 31 patients with benign obstruction and in one case with neoplastic obstruction (p <0.001). Activities of the remaining enzymes studied were increased significantly in both types of icterus (Figure 1).

From our results we conclude that determination of GR is useful in the differential diagnosis of benign and malignant obstructive icterus.

References

J. Tor
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Colorimetry vs Column Chromatography in Hb A1 Assay

To the Editor:

Hemoglobin A1 is a glycosylated form of hemoglobin A, the concentration of which is an integrated index to a person's blood sugar concentration during the previous several weeks. Thus, measurement of Hb A1 concentration may be used in assessing diabetic control. A column-chromatographic method (1) is widely used because it is simple and can be performed in any laboratory. However, separations of glycosylated hemoglobins are affected by pH (2), osmolality (3), and temperature (2). Moreover, whole blood or hemolysate cannot be stored for more than one week. The Hb A1 fraction, usually 80% of Hb A1, may increase by 179% when hemolysate is stored for 10 days at -20 °C or even by 314% when stored at room temperature, in contrast to Hb A1c, which remains constant at these temperatures for two weeks (4). Finally, Hb F is known to interfere (5).

We compared the column-chromatographic method (1) with a modified colorimetric method (6) that is done as follows:

To 2 mL of hemolysate, containing 60 mg of Hb, add 1 mL of 1 mol/L oxalic acid and digest the mixture at 100 °C for 90 min (into each digestion tube insert a rubber stopper, punctured with a 38 × 0.8 mm hypodermic needle; this minimizes evaporation). After adding 1 mL of a 400 g/L solution of trichloroacetic

Table 1. Analytical Results for Serum (X ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Benign Obstruction</th>
<th>Neoplastic Obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 53)</td>
<td>(n = 31)</td>
<td>(n = 18)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin, mg/L</td>
<td>5 ± 0.4</td>
<td>74 ± 11</td>
<td>142 ± 19</td>
</tr>
<tr>
<td>Enzyme activities, U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPI</td>
<td>56.2 ± 2.2</td>
<td>69.3 ± 6.9</td>
<td>86.8 ± 12.1</td>
</tr>
<tr>
<td>GR</td>
<td>25.8 ± 0.5</td>
<td>29.6 ± 1.2</td>
<td>41.9 ± 4.3</td>
</tr>
<tr>
<td>AST</td>
<td>9.9 ± 0.4</td>
<td>49.9 ± 7.9</td>
<td>69.9 ± 12.1</td>
</tr>
<tr>
<td>ALT</td>
<td>12.6 ± 0.9</td>
<td>81.5 ± 11.1</td>
<td>121.0 ± 40.9</td>
</tr>
<tr>
<td>AP</td>
<td>111.7 ± 4.6</td>
<td>578.7 ± 65.2</td>
<td>896.5 ± 101</td>
</tr>
<tr>
<td>GGT</td>
<td>14.4 ± 1.4</td>
<td>236.2 ± 30.9</td>
<td>341.0 ± 93.5</td>
</tr>
</tbody>
</table>

Fig. 1. Enzymic activity in patients with benign and malignant obstructive jaundice.