Increase in Serum Alkaline Phosphatase Owing to Furosemide

To the Editor:

We reviewed the charts of patients with alcoholic liver disease with ascites who were being treated with intravenous furosemide (Lasix). We noticed that five of nine patients exhibited an increase in serum alkaline phosphatase (ALP; EC 3.1.3.1) about 24–48 h after initiation of furosemide therapy. The mean increase (32% above baseline value) was greater than could be accounted for by analytical and biological variations. There was no significant change in the clinical status (except a brisk diuresis in one patient) or in any of the other laboratory indexes to liver function. All samples were obtained before breakfast, which excludes the increase in serum ALP being attributable to ingestion of a fatty meal (1).

The figure shows the response of serum ALP to various doses of furosemide in one patient. Enzyme fractionation revealed that the increase was due to the hepatic isoenzyme. Three patients with acute renal failure who were given furosemide did not show an increase in ALP.

A possible explanation of this phenomenon in patients with alcoholic liver disease is the reduction in bile flow that occurs with inhibitors of sodium transport such as furosemide (2). Why this happens only in some patients requires further study.

References

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The Bilirubin Content of Serum Does Not Affect \( \gamma \)-Glutamyltransferase Activity

To the Editor:

We determine \( \gamma \)-glutamyltransferase activity (EC 2.3.2.2, GGT) in serum in a bichromatic analyzer (ABA-100) with "A-GEN T GGT" (Abbott Laboratories). This test is based on a procedure described by Szasz (1) in which the substrate \( \gamma \)-glutamyl-4-nitroanilide is split by GGT into glutamate and 4-nitroaniline. The rate of formation of 4-nitroaniline, determined kinetically at 405 nm or 415 nm, is proportional to GGT activity.

For an abnormal serum we found the within-assay precision (CV) to be 1.09% (n = 25, \( \bar{x} \) = 24.8 U/L, SD = 0.76).

Combes et al. (2), on observing low values for GGT activity and high concentrations of bilirubin in patients with acute viral hepatitis, suggested that bilirubin can interfere with GGT activity. When they added crystalline bilirubin, dissolved in 0.1 mol/L KOH, to serum with increased values of GGT and low bilirubin and incubated the mixture for 5 min, they observed that the enzymic activity decreased as the concentration of bilirubin was increased.

Dickson and Beck (3), using both bilirubin from human bile and crystalline bilirubin, found no apparent inhibition. In contrast, they found that GGT activity increased with increasing bilirubin concentration.

Theodorsen and Stromme (4) also concluded that the addition of bilirubin to serum samples in concentrations up to 200 mg/L does not inhibit GGT activity if the nonlinearity of the instrument used was corrected for.

Fraser (5) reported that if serum with 44 U of GGT activity per liter, total bilirubin on the order of 150 mg/L, and conjugated bilirubin on the order of 75 mg/L was diluted 10- and 20-fold with an albumin-based diluent, the apparent GGT activity was 80 and 100 U/L, respectively. However, if samples with high GGT activity were diluted with samples having low GGT activity and conjugated bilirubin >75 mg/L, inhibition by bilirubin does not appear.

Because of the above discrepancies and with a view to giving additional data, we measured the enzymic activity of GGT in serum with different activities of this enzyme and bilirubin (a) in dilutions of human serum with isotonic saline, (b) in mixtures of two different sera, and (c) in mixtures of serum with added bilirubin.

In serum with different GGT activities and different concentrations of bilirubin, we saw no unexpected variation in enzymic activity after various dilutions with isotonic saline.

Thus, mixtures of serum containing from 42 to 1286 U of GGT per liter and from 10 to 420 mg of total bilirubin per liter showed the expected GGT activity, with CVs ranging from -2.2% to +1.0%.

Addition of up to 500 mg of bilirubin (Wiener Lab., Rosario, Argentina) per liter to sera had no effect on the apparent enzymic activity of GGT. (We also used bilirubin from Ortho Diagnostics Inc., Raritan, NJ; Dade, Miami, FL; and Laboratories Knickerbocker, Barcelona, Spain, with similar findings.)

Our results indicate that the procedure we use for enzymic determination is uninfluenced by bilirubin. Therefore, the observations that the apparent activity of GGT in serum is lowered by high concentrations of bilirubin must be ascribed to something else.

Moreover, the increase in the apparent activity of GGT described by Fraser (5) only in serum with low enzymic activity can be simply explained as an artifact of the high dilution (10- and 20-fold) and the standard deviation of the reported values. So the apparent increase in GGT activity in mixtures of sera with solution of increasing concentrations of bilirubin described by Dickson and Beck (3) and also observed by us is simply due to contaminating GGT activity in the preparation of bilirubin used. If corrected for this contaminant activity, no effect of bilirubin on serum GGT is observed. However, this contamination is very small in terms of...
of the high concentration of bilirubin used.

Results were consistent for more than 50 different sera.

We thank the technicians on our service for their collaboration in this work. Detailed data may be obtained from the authors or the Editorial Office of this journal.

References

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**Rapid Detection and Estimation of Hemoglobin by Agarose Gel Electrophoresis**

To the Editor:

It is often necessary to detect hemoglobin F, to diagnose thalassemia. Electrophoresis on agarose gel gives better separations of hemoglobin bands (1). We use a discontinuous buffer system (Tris–EDTA–boric acid, pH 8.1, for gel; barbitral buffer, pH 8.6, for the electrophoretic tank).

Blood was sampled from normal human adults and human umbilical cords into EDTA-containing tubes. Hemolysates were prepared according to Drabkin (2). The concentrations of the hemolysates were adjusted to about 10% and 1%. Gel slides, 2.5 × 7.5 cm or 5 × 7.5 cm, were prepared with 6 g/L agarose (Koch-Light) in Tris buffer. Two to four microliters of the 1% hemolysate was applied to the gel. Electrophoresis was at 5 mA per microscope slide for 45 min. The hemoglobin bands

in the agarose gel were fixed by placing the gel in trichloroacetic acid (100 g/L). The gel was transferred to saline (9 g/L) for about 2 to 3 min, to separate it from the glass slide, and then to filter paper (Whatman No. 1). When it had dried, it was stained with bromphenol blue. The stained hemoglobin bands were cut out, eluted with 0.1 mol/L NaOH, and the color in the eluate was measured at 590 nm. From this, the concentrations of different hemoglobin bands were estimated. The concentration of hemoglobin F was also estimated, for comparison, by the routinely accepted 1-min alkali denaturation method (3), with use of the 10% hemolysate.

This method gives good separations of HbA, HbF, HbS, and HbA2 (Figure 1). Results for HbF by our method were comparable with results by the alkali denaturation method. This simple, rapid method can be easily carried out in the routine laboratory.

References

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**Misuse of Glucose Oxidase Test Strips**

To the Editor:

Glucose oxidase test strips for blood glucose have proven convenient as an aid to immediate decisions in the management of the diabetic patient. In many cases, venous blood is drawn and tested immediately with the test strip, and the rest of the specimen is submitted to the laboratory for a confirmatory glucose determination, and often other analyses as well. Several test-strip preparations are available for use with whole blood and at least one is designed for use with either serum or blood (1). In addition, test strips designed for use with blood have been used, with or without modification, with plasma, serum, or cerebrospinal fluid (2–4). Thus one can use either whole blood or serum for rapid evaluation of glucose before processing for additional analyses.

Recently, we discovered that some attending physicians had adopted the practice of dipping the test strip into the sample for 60 s rather than removing a drop to place on the strip as stated in the manufacturer's instructions. Because of the potential artefacts introduced in the remaining sample by this procedure, we evaluated glucose in control sera at various times after exposure for 60 s to two commonly used glucose oxidase test strips (B–G Chemstrips from BMC Biodynamics, Indianapolis, IN 46250, and Dextrostix from Ames Co., Miles Laboratories, Inc., Elkhart, IN 46515). The procedure resulted in small but significant decreases in glucose values, which became larger with time after exposure, consistent with elution of glucose oxidase (BC 1.1.3.4) from the test strip into the sample. There was a definite, although nonlinear, dose response (Table 1).

In sixteen additional trials carried out by exposing 2.5 mL of serum aliquots to one strip each for 60 s, with glucose measurement at 120 min, the range of decrease found was 90 to 90 mg/L (mean 57, SD 17.5 mg/L).

During these measurements, we noted that treated sera changed color, from yellow to pale green, about 20 min after exposure. Anticipating additional changes, we performed SMA-12 and -6 profiles on samples of both serum and whole blood after exposure to glucose.

**Table 1. Change in Measured Glucose in Control Sera after Exposure to Glucose Oxidase Strips**

<table>
<thead>
<tr>
<th>Time after exposure, min</th>
<th>Measured decrease, mg/L</th>
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<tr>
<td>0–2</td>
<td>30</td>
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<tr>
<td>30</td>
<td>60</td>
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<tr>
<td>120</td>
<td></td>
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<tr>
<td>One strip</td>
<td>0.1</td>
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<td>Three strips</td>
<td>8.4</td>
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* Duplicate aliquots of pooled human serum were exposed by dipping one or three glucose oxidase test strips into the sera for 60 s. Other aliquots were not exposed. All sera subsequently were allowed to stand, covered, at room temperature for the listed times before glucose analysis in an AutoAnalyzer II (Technicon Corp., Tarrytown, NY 10591) by the method of Trinder (3). *One SD = 27.5 mg/L.*