Reinterpretation of Hyperamylasemia in Diabetic Coma

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Serum enzyme activity was sequentially determined in 10 consecutive patients with diabetic ketoacidosis, of whom all had increased \( \beta \)-glucuronidase activity, eight had increased amylase activity, and four had increased acid phosphatase activity. Activity of amylase and that of the two lysosomal enzymes were poorly correlated, irrespective of whether peak activities or activities of all samples were considered. Of 37 cases with acute viral hepatitis, serum \( \beta \)-glucuronidase activity was increased in 33 and amylase activity in four, and the correlation between the two was poor. Study of normal human liver showed that the ratio of its mean enzymatic activity to the upper normal limit for serum was less than 1.0 for amylase, and approximately 80 and 6000 for acid phosphatase and \( \beta \)-glucuronidase, respectively. The hepatocyte cannot be the source of an increased serum amylase activity, and we question whether lysosomes are concerned in its release from other tissues.

Additional Keyphrases: lysosomal enzymes • origin of serum amylase activity • diabetic ketoacidosis • viral hepatitis

Hyperamylasemia is now a recognized feature of diabetic ketoacidosis (1–3). It was present in 57% of 42 consecutive cases, and in 19% of them the value was more than fivefold the upper normal limit (4). We saw no correlation between serum amylase activity and any of the following: blood glucose, blood bicarbonate, serum lipase activity, the activity in serum of hepatobiliary-specific enzymes, or renal function. There was no evidence of macroamylasemia, and no patient had signs or symptoms of acute pancreatitis. We considered transient leakage of the enzyme from the pancreas to be the most probable mechanism for the abnormality, which was in no way related to the prognosis.

It has been suggested that the increased amylase activity in diabetic ketoacidosis is the result of release of the enzyme from the liver after its ultrastructure is damaged by “activated” lysosomal enzymes (5). Support for this hypothesis rested upon the parallel rise and fall of the activities of amylase and lysosomal enzymes in the 14 patients studied (5), and a paper describing the microsomal distribution of hepatic amylase in the rat (6). The negligible amylase content of human liver (7–9) was not considered. We therefore decided to test the validity of this hypothesis on a clinical and experimental basis.

Materials and Methods

Clinical Material

Patients. Blood used in determining enzymatic activity in serum was obtained from 10 consecutive patients with diabetic ketoacidosis on admission and at four further intervals during the next 60 h. We also studied 37 patients with acute viral hepatitis, diagnosed according to published criteria (10), by using the first blood sample obtained during out-patient or in-patient investigation.

Liver tissue. Ten samples of human liver, histologically and macroscopically normal and selected without conscious bias, were obtained within 12 h of death. They were rinsed free of blood in ice-cold water, blotted dry, weighed, finely chopped with scissors, and homogenized for three passes of 10 s each, with use of a Teflon pestle motor-driven at a speed of 6000 rpm, in three volumes of 0.2 mol/liter sucrose, buffered to pH 7.4 with 50 mmol/liter phosphate and containing ethylenediaminetetraacetate and dithiothreitol in final concentrations of 1.0 mmol/liter. This treatment will be referred to as “light homogenization”. Aliquots were then transferred for further homogenization under two sets of conditions:

(a) “Heavy” homogenization was carried out for two additional 1-min periods as above.

(b) “Detergent” homogenization was carried out as for a and for a further two 1-min periods after addition of a surfactant (Triton X-100) to a final concentration of 1 ml/liter.

After these procedures, the aliquot was centrifuged at 100 000 \( \times g \) for 30 min at 4 °C and the supernate used for determination of enzyme activities.

Enzyme Assays

The following assays were carried out on all sera and liver supernates at 37 °C, and the activity is expressed in International Units per liter (U/liter or mU/liter).

Amylase was measured by using a dye covalently linked to dextran (11) and supplied in tablet form as “Phadebas” by Pharmacia (Great Britain) Ltd., London, England. The normal range is 20–300 U/liter.

\( \beta \)-Glucuronidase was assayed by using phenolphthalein monoglucuronide as substrate (12). The normal range, 128–478 mU/liter, is about 0.001 of
Table 1. Peak Serum Enzyme Activities in 10 Consecutive Cases of Diabetic Ketoacidosis

<table>
<thead>
<tr>
<th>Case no.</th>
<th>β-Glucuronidase</th>
<th>Amylase</th>
<th>Acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mU/liter</td>
<td>U/liter</td>
<td>U/liter</td>
</tr>
<tr>
<td>1</td>
<td>1835</td>
<td>295</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>1583</td>
<td>994</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>1562</td>
<td>1500</td>
<td>3.9</td>
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<tr>
<td>4</td>
<td>1551</td>
<td>750</td>
<td>2.4</td>
</tr>
<tr>
<td>5</td>
<td>1234</td>
<td>600</td>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
<td>1114</td>
<td>1200</td>
<td>8.2</td>
</tr>
<tr>
<td>7</td>
<td>983</td>
<td>375</td>
<td>1.9</td>
</tr>
<tr>
<td>8</td>
<td>743</td>
<td>299</td>
<td>5.0</td>
</tr>
<tr>
<td>9</td>
<td>644</td>
<td>3375</td>
<td>5.6</td>
</tr>
<tr>
<td>10</td>
<td>481</td>
<td>4968</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Normal range 128-478  20-300  0-4.1

* Arranged in decreasing order of β-glucuronidase value.

Fig. 1. Sequential changes in serum enzyme activities in two subjects with diabetic ketoacidosis.

the values cited by Belfiore and Napoli (5). (We assume this is a misprint or an arithmetic error on the part of the authors).

Acid phosphatase was assayed by using adenosine 3-monophosphate as substrate (13). The normal range is 0-4.1 U/liter.

Results and Discussion

Diabetic ketoacidosis. Table 1 presents the highest enzyme values for the 10 cases studied. Whereas β-glucuronidase activity was abnormally great at some time during the acute episode in all 10 patients, amylase activity was increased in eight and acid phosphatase in only four. There was no correlation between peak values for β-glucuronidase and amylase in the serum; case 1, with the highest β-glucuronidase value, had a normal amylase value, and case 10, with the lowest β-glucuronidase had the highest amylase value. The time-course of these changes is shown for two typical cases in Figure 1. Figure 2 shows plots of activities of amylase against β-glucuronidase and amylase against acid phosphatase for all specimens drawn from the 10 subjects. Obviously there is little correlation between these various activities.

Acute viral hepatitis. Of the 37 patients studied, β-glucuronidase activity was increased in 33 and amylase in only four. A single case had increased acid phosphatase activity. β-Glucuronidase and amylase activities in the serum of these patients were poorly correlated (Figure 3). In half the cases, aminotransferase activity was >1000 U/liter in the serum examined. If hyperamylasemia could be caused by damage to liver cells, increased serum amylase activity should be an impressive feature of viral hepatitis, which it clearly is not.
**Human liver tissue.** Progressive disruption of lysosomes and endoplasmic reticulum would be expected to follow the progression from "light" through "heavy" to "detergent" homogenization treatment of the tissue, and ought to be reflected in increased solubilization of particulate enzymes. Figure 4 shows that, as expected, increased activity was found in proportion to the severity with which the tissue was treated, although this was less evident for amylase than for the other two enzymes. The amylase activity of human liver was very low, and below the limits of detection in six of the 10 specimens. The 100,000 × g supernate from the detergent-treated homogenate contained all of the amylase present in the homogenate, and about half the activity of the membrane-associated enzymes alkaline phosphatase, γ-glutamyl transpeptidase, and 5′-nucleotidase, although activity of the last enzyme was low in all fractions. Systematic study showed that ethylenediaminetetraacetate, dithiothreitol, sucrose, or phosphate in the concentrations used in the homogenization medium were not inhibitory to human amylase. We also established that incubating the detergent-treated homogenate for intervals up to 6 h at 37 °C before centrifugation did not lead to increased amylase activity of the supernate, as could conceivably occur if activated lysosomal enzymes were needed to strip the amylase from the endoplasmic reticulum.

**Conclusion.** It has been suggested by Nothman and Callow (14) on the basis of extirpation studies in man and dog that the liver may be partially responsible for the normal serum amylase. Fridhandler et al. (9), on the other hand, suggested that the serum is responsible for the normal liver amylase activity. We have been unable to corroborate the indirect evidence advanced by Belfiore and Napoli to substantiate the hypothesis that the liver is the source of the hyperamylasemia in diabetic ketoacidosis (5). Consideration of the gradient between liver and serum for the three enzymes studied (Table 2) renders this highly improbable, although it is likely that the sporadically increased enzymatic activity in serum (aminotransferases, isocitrate dehydrogenase, guanase, glutamate dehydrogenase, adenosine deaminase, and 5′-nucleotidase) that occurs in a proportion of cases during diabetic ketoacidosis may be caused by transient leakage from hepatocytes (15).

**Table 2. Liver-to-Serum Gradient for Three Enzyme Activities**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Liver* (U/kg)</th>
<th>Serum* (U/liter)</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>195†</td>
<td>300</td>
<td>0.65</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>3300</td>
<td>0.48</td>
<td>6875</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>355</td>
<td>4.1</td>
<td>87</td>
</tr>
</tbody>
</table>

* Mean of 10 samples homogenized in Triton X-100, 1 ml/liter. † Upper normal limit.