Multiple Forms of \( \gamma \)-Glutamyltransferase: A Clinical Study

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We have measured the amounts of different molecular forms of \( \gamma \)-glutamyltransferase (EC 2.3.2.2), leucine aminopeptidase (EC 3.4.11.2), and alkaline phosphatase (EC 3.1.3.1) in serum of patients with different types of liver disease. A high-molecular-mass (greater than 1 000 000 Da) form of \( \gamma \)-glutamyltransferase and of each of the other enzymes is present in greatest amounts in patients with jaundice from extrahepatic obstruction. A \( \gamma \)-glutamyltransferase form of intermediate molecular mass (250 000 to 500 000 Da) is present in the serum from most patients with liver disease and can be separated by electrophoresis into several bands. We found that one of these bands predominated in patients with extrahepatic obstructive jaundice, whereas the others predominated in patients with other liver diseases. Electrophoresis of serum \( \gamma \)-glutamyltransferase may be of clinical value in distinguishing extrahepatic from intrahepatic causes of jaundice.

Additional Keyphrases: jaundice · liver disease · isoenzymes · electrophoresis, polyacrylamide gel · chromatography, gel · cancer · leucine aminopeptidase · alkaline phosphatase · relation to bile salt concentrations

It has long been known that serum \( \gamma \)-glutamyltransferase (\( \gamma \)GT; EC 2.3.2.2) activity is increased in most patients with liver disease (1–4), with the highest activities believed to occur in serum of patients with extrahepatic biliary obstruction (5). However, \( \gamma \)GT activity in serum discriminates poorly among different forms of liver disease.

Previous studies indicate that \( \gamma \)GT is present in serum in various forms, some representing aggregates of \( \gamma \)GT with serum lipids, others as fragments of liver cell membrane (6, 7). The nature of, and the relationships among, these various forms are complex, but we have been able to distinguish, on the basis of relative molecular mass (\( M_r \)) and electrophoretic behavior, three major categories of \( \gamma \)GT in the serum of patients with liver disease (8). The first is a high-\( M_r \) fraction (high-\( M_r \) \( \gamma \)GT), greater than 1 000 000 Da, which we have previously designated as Peak 1, which is eluted in the void volume on gel chromatography on Sephacryl S300 and remains at the origin on polyacrylamide gel electrophoresis. This fraction is present in normal serum and tends to predominate in serum from patients with obstructive liver disease. The second, an intermediate-\( M_r \), \( \gamma \)GT fraction (\( M_r \) 250 000 to 500 000 Da) that has an electrophoretic mobility between 8 and 55% that of albumin, may be of two types, most easily distinguished by electrophoresis: One is heterogeneous, which we have called Bands IIA (\( \gamma \)GT), with mobilities between 8 and 40% that of albumin; the other, Band IIB (\( \gamma \)GT), is discrete, with a mobility of 45 to 55% that of albumin. Previous studies (8) suggest that Bands IIA are usually the main intermediate-\( M_r \) \( \gamma \)GT fractions present in liver disease, but Band IIB predominates in patients with jaundice due to extrahepatic obstruction. The third, a low-\( M_r \) \( \gamma \)GT fraction (\( M_r \) about 120 000), is a hydrophilic form of the enzyme, in contrast to the two other forms, which are hydrophobic.

Our previous studies have also suggested that the relative amounts of the various \( \gamma \)GT peaks differ for sera from groups of patients with different types of liver disease. Here we have tried to define these differences more clearly and, in particular, to determine whether jaundice from extrahepatic obstruction, possibly remediable by surgery, can be distinguished from "medical" causes of jaundice by measurement of \( \gamma \)GT.

Microsomal aminopeptidase (leucine aminopeptidase, LAP; EC 3.4.11.2) and alkaline phosphatase (ALP; EC 3.1.3.1) are two other enzymes on the hepatocyte plasma membrane that appear in serum in liver disease in several high-\( M_r \) forms (8–10). We have also studied the high-\( M_r \) forms of these enzymes, to compare the mechanism of their release and their diagnostic efficiency with those of \( \gamma \)GT.

Materials and Methods

Samples

We selected sera from 100 patients with liver disease, from specimens submitted for \( \gamma \)GT measurement. Selection was based on the diagnosis, confirmed by liver biopsy, liver scan, or laparotomy, and clinical and laboratory findings and not on the result for \( \gamma \)GT. Sera were also sampled from 10 apparently healthy individuals with no clinical or biochemical evidence of liver damage.

Procedures

General. Measurement of bile salt concentrations, localization of \( \gamma \)GT activity after electrophoresis, and measurement of enzyme activities were all performed as described previously (11–16). Reference intervals were as follows: \( \gamma \)GT 6–31 U/L for females, 8–49 U/L for males; LAP 27–70 U/L; and ALP 30–140 U/L.

Measurement of high-\( M_r \) enzymes by gel chromatography. Apply 1 to 2 mL of serum to a 950 × 26 mm column of Sephacryl S300 (Pharmacia, Uppsala, Sweden) equilibrated with Tris HCl buffer (20 mmol/L, pH 8.0) containing 50 mmol of sodium chloride per liter. Collect 3.5-mL fractions with an upward flow rate of 28 mL/h. In our hands the high-\( M_r \) enzymes are all eluted in the void volume (\( M_r \) > 1 000 000) and are easily resolved from the fractions of lower \( M_r \) (8).

Combine the fractions containing the high-\( M_r \) peaks and calculate the enzyme activity of the high-\( M_r \) fraction as follows:

\[
\text{High-} M_r \text{ enzyme, } \% = \frac{A}{(A + B)} \times 100
\]

where \( A \) = the sum of the activities of the fractions in the
Measurement of Band IIB. Separate intermediate-$M_r$ $\gamma$GT into its subfractions, Bands IIA and Band IIB, by electrophoresis on 40 to 300 g/L polyacrylamide gradient gels (Phar-macia) (8). Stain the gels for $\gamma$GT activity and quantify Bands IIA and Band IIB by absorption densitometric scanning at 525 nm (we used an Auto Scanner Flur-Vis; Helena Laboratories, Beaumont, TX). Estimate Band IIB as a percentage of the total intermediate-$M_r$ $\gamma$GT from the formula:

$$\text{Band IIB}(\gamma\text{GT}) = \frac{C}{(C + D)} \times 100$$

where C is the area of Band IIB and D is the area of Bands IIA.

Statistical analysis. We used nonparametric analysis (Kruskal–Wallis one-way analysis of variance) (17) to compare results of tests in different disease categories, and parametric methods for correlation analysis.

Results

Analytical quality. The enzyme activity that we could account for analytically was variable; for $\gamma$GT, the mean analytical recovery was 101.7% (range 81–146%); for LAP 104% (81–155%); for ALP, 105.3% (82–143%). Electrophoresis of a concentrated pool corresponding to the high-$M_r$ enzyme fraction confirmed that no other fractions were present that could explain the recoveries that exceeded 100%. These unexpected high recoveries perhaps may be attributed to the presence of inhibitors in serum, which were removed or diluted during gel chromatography. The precision (CV) of the method, as assessed by gel chromatography of a single serum sample on 11 different days, with measurement of the high-$M_r$ fraction each day, was 3.7% for high-$M_r$ $\gamma$GT (mean activity concentration, 58 U/L), 10.2% for high-$M_r$ LAP (mean, 18 U/L), and 7.9% for high-$M_r$ ALP (mean, 22 U/L).

Between-batch precision for determining intermediate-$M_r$ $\gamma$GT was estimated by electrophoresing 10 sera on two different days. The CV was 11.2% for Band IIB at a mean proportion of total intermediate-$M_r$ enzyme of 55% (range 0–100%). The mean $\gamma$GT activity in these sera was 309 U/L (range 28–625 U/L).

High-$M_r$ $\gamma$GT. Figures 1 and 2 show the distributions of total $\gamma$GT, high-$M_r$ $\gamma$GT (U/L), and high-$M_r$ $\gamma$GT (%) in the different groups of patients. All groups show considerable overlap. The results for patients with metastatic carcinoma in liver were similar, whether or not there was jaundice, so we combined these two groups. However, high-$M_r$ $\gamma$GT, expressed as a percentage (Figure 2, bottom), was:

- greatest in patients with extrahepatic obstruction ($p < 0.00001$).
- greater in patients with liver metastases than in other groups, if patients with extrahepatic obstruction were excluded from the analysis ($p < 0.00001$).
- greater in patients with extrahepatic obstruction than in those with liver metastases ($p < 0.00001$).
- greater in patients with extrahepatic obstruction than in jaundiced patients in other disease categories ($p < 0.00001$).

High-$M_r$ $\gamma$GT (expressed as U/L) appears to be normal or only slightly increased in patients receiving anticonvulsants...
Fig. 3. Distribution of high-Mr LAP (top) and of high-Mr ALP (bottom), expressed as the percentage of the total activity in the different patient groups.
Abbreviations as in Fig. 1.

or those with hemochromatosis, or after liver transplantation.

High-Mr LAP and high-Mr ALP. The results for high-Mr LAP (Figure 3, top) were very similar to those for high-Mr γGT in all aspects in patients with extrahepatic obstruction or liver secondaries. The results for high-Mr ALP (Figure 3, bottom), although similar, showed fewer differences between the different disease categories. For example, whereas high-Mr ALP was greatest in extrahepatic obstruction (p <0.001), a direct comparison between the patients with extrahepatic obstruction and those with liver metastases or those with jaundice from other causes showed no significant difference.

Relationship between high-Mr γGT and other high-Mr enzymes. There was a close relationship between high-Mr γGT (%) and high-Mr LAP (%), r = 0.84 (Figure 4). The correlation between high-Mr ALP (%) and high-Mr γGT (%), r = 0.66, or high-Mr ALP (%), r = 0.67, was less good.

Relationship between high-Mr enzymes and bile salt concentrations. We investigated the relationship between the high-Mr enzymes and bile salt concentrations, the latter being widely regarded as good indices of cholestasis. Bile salt concentrations were also significantly higher in extrahepatic obstruction than in other forms of liver disease (p <0.000001). There was a low but significant association between serum bile salt concentrations and high-Mr γGT (%) and also high-Mr LAP (%) but not high-Mr ALP (%). For total conjugated cholate, the correlation coefficient with high-Mr γGT (%) was 0.47, and with high-Mr LAP (%) it was 0.38 (p <0.001). For total conjugated chenodeoxycholate the respective correlation coefficients were 0.32 (p <0.01) and 0.24 (p <0.05).

Quantification of Band IIIB. Band IIB was determined in 64 of the patients with liver disease, including 16 of the 25 patients with jaundice due to extrahepatic obstruction. Typical electrophoretic patterns are shown in Figure 5 and the results plotted in relation to high-Mr γGT (%) in Figure 6. In the occasional cases where Bands IIA and IIB were present together in the serum and they were not completely separated—e.g., patients 5 and 8 (Figure 5)—the percentages were calculated by extrapolation of the individual peaks. In 11 of the 16 patients (69%) with obstructive jaundice, all of the intermediate-Mr γGT was present as Band IIB, as seen by visual inspection of the gels and confirmed by densitometric scanning. In none of the patients from any of the other groups was all the intermediate-Mr γGT present as Band IIB. Although the numbers are quite small, the data in Figure 6 suggest that the electrophoretic subdivision of Band II discriminates the patients with extrahepatic obstruction from the rest better than does the percentage of high-Mr γGT. In an attempt to determine whether the combination of the two results further improved discrimination, we divided Figure 6 into four quadrants, using arbitrary cutoff values derived from the value of the mean +2 SD for each variable in the patients without obstructive jaundice (40% for high-Mr γGT, 57% for Band IIB). A value in the top right quadrant (i.e., both variables above the cutoff limit) was defined as a positive test for obstructive jaundice. In Table 1, the results obtained are compared to those derived from those using only one of the
variables (with the same cutoff limits), together with equivalent results obtained by using high-Mr ALP (cutoff value 32%) and high-Mr ALP (cutoff value 26%). They suggest that little improvement in discrimination is obtained by measuring high-Mr γGT and Band IIB together as compared with that obtained from electrophoretic fractionation alone. The results also show that, whereas all three high-Mr enzymes had similar values for specificity in the detection of extrahepatic biliary obstruction, high-Mr ALP was much less sensitive than either high-Mr γGT or high-Mr LAP.

Discussion

Our results confirm many previous reports that serum γGT activity is increased in most types of liver disease but that it discriminates poorly between different causes. The present study has been directed towards determining whether more complex analysis of serum γGT patterns in liver disease increases its discriminatory capacity.

We have shown that high-Mr γGT, particularly when the results are expressed as a percentage, is (a) higher in obstructive than non-obstructive lesions and (b) higher in extrahepatic than in intrahepatic obstruction. Our results suggest that measurement of high-Mr γGT, particularly when results are expressed as a percentage, can help to distinguish extrahepatic from (a) intrahepatic obstruction and from (b) almost every other form of liver disease, whether or not the patient is jaundiced. Moreover, a high value (greater than 50%) appears to be almost diagnostic of obstructive jaundice (Figure 2, bottom).

We know of only one other clinical study of high-Mr γGT (18). Significantly higher values were found in 38 patients with malignant infiltration of liver than in 17 patients with alcoholic liver disease. These findings have been confirmed in the present report, although there was considerable overlap between these groups (Figure 2, bottom). In a recent study of total hydrophobic γGT, Selvaraj et al. (19) were unable to distinguish between different types of liver disease. However, their method was less specific than ours and also measured intermediate-Mr γGT which, with high-Mr γGT, constitutes total hydrophobic γGT.

High-Mr LAP behaved similarly to high-Mr γGT and was also present in greater amounts in obstructive than non-obstructive lesions.

In contrast to high-Mr γGT and high-Mr LAP, several clinical studies (20-23) involve measurement of high-Mr ALP. In general, these investigators have found, as we have, higher values in obstructive than in non-obstructive lesions, although there is some disagreement as to whether the highest values in extrahepatic biliary obstruction (Figure 3, bottom), there was no significant difference between these and the values seen in patients with metastases in the liver.

Measurement of high-Mr ALP has been advocated as having particular value in the detection of liver metastases (24, 25). Our study confirms that higher levels are present in patients with secondary deposits than in healthy individuals, or patients with liver disease other than extrahepatic biliary obstruction.

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Our results do not suggest that measurement of high-Mr fractions of ALP, or of γGT or LAP, is likely to be of practical value in the diagnosis and detection of liver metastases, because the overlap with other categories of liver disease is so great. We have not, however, investigated the value of high-Mr enzyme measurements in patients with minimal abnormalities of liver function—a group in whom detection of liver metastases might be particularly important.

Our results do suggest that measurement of Band IIB may distinguish extrahepatic biliary obstruction from other types of liver disease. We were unable, however, to equate this band with any of the bands obtained on non-sieving media by other authors because of the considerable difficulties in inter-relating the results of different groups of workers. The proportion of intermediate-Mr γGT present as Band IIB reached 100% only in extrahepatic biliary obstruction, and in these cases it could be visualized without the need for scanning (Figure 5). We attempted to increase the discrimination between patients who did and did not have extrahepatic obstructive jaundice by combining the measurement of Band IIB with that of high-Mr γGT, but the attempt was unsuccessful (Table 1). No greater discrimination was obtained than by measuring Band IIB alone.

The percentages of each of the three high-Mr enzymes present in serum were seen to be correlated with one

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<tr>
<th>Table 1. Diagnostic Value of High-Mr γGT (%) and Band IIB (%), Singly or Together, High-Mr LAP (%), and High-Mr ALP (%), in the Detection of Extrahepatic Biliary Obstruction</th>
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<tbody>
<tr>
<td><strong>High-Mr γGT</strong></td>
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<tr>
<td>Sensitivity *</td>
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<td>Predictive value of positive test a</td>
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Proportion of patients * with extrahepatic obstruction giving a positive test, * without extrahepatic obstruction giving a negative test, a with positive test who had extrahepatic obstruction, and d with negative test who did not have extrahepatic obstruction.

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another. Furthermore, the highest concentrations of the high-Mᵢ forms were found in patients with extrahepatic obstruction, who also showed the highest concentrations of total conjugated bile salts in serum. The most likely explanation of these findings is that a hydrophobic but low-Mᵢ form of γGT (or LAP or ALP) is eluted from the cell membrane, to which it is attached by the hydrophobic domain of the molecule, by high local intracellular concentrations of bile salts (26). On passing into the circulation, where the concentrations of bile salts are lower than in the liver cell, the γGT aggregates either with itself or with other lipid-containing particles (6). We have previously shown that, in bile, high concentrations of bile salts are needed to maintain γGT in a hydrophobic low-Mᵢ form (27). The relatively poor correlation between high-Mᵢ γGT and bile salt concentrations in the serum may be due to the fact that intracellular bile acid concentrations and those in serum do not always parallel one another. This explanation for the presence of high-Mᵢ γGT in serum is also consistent with findings for high-Mᵢ ALP and LAP. High-Mᵢ γGT correlates well with γGT and is known to be readily accessible on the membrane surface, whereas high-Mᵢ ALP, which correlates poorly with γGT, is much less accessible to the dissociating action of, for example, papain than is LAP or γGT (28). It is therefore quite likely that ALP is much less accessible to the detergent action of bile salts than are γGT and LAP.

Intermediate-Mᵢ γGT has been shown to consist of a complex between low-Mᵢ hydrophobic γGT and high-density lipoprotein (8, 29). However, the factors involved in the preferential formation of the Band IIIB in patients with extrahepatic obstructive jaundice remain to be determined.

In this report we have shown that measurement of high-Mᵢ γGT, or of the intermediate-Mᵢ form, which we have termed Band IIIB, may aid diagnosis in liver disease. High-Mᵢ γGT may distinguish extrahepatic obstructive causes of jaundice from other causes, but the method that we have used is time-consuming and tedious; it is not suitable for use in busy diagnostic departments. Alternative methods of measuring this fraction, such as electrophoresis on non-sieving media, may be less time consuming, but we have not studied them quantitatively. In contrast, estimation of Band IIIB is relatively simple and is probably a better discriminator. Extrahepatic and other causes of jaundice can often be discriminated by simple inspection of the gels. We consider that electrophoresis of γGT in polyacrylamide gel (or possibly other gels as well) can aid the important clinical differentiation between extrahepatic and intrahepatic causes of jaundice, and that it is sufficiently practical for use in a service laboratory.

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References