Changes in hepatic enzymes and organelles in alcoholic liver disease

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Summary

1. Liver biopsy specimens obtained from patients with alcoholic liver disease of varying severity were assayed for lysosomal and microsomal enzyme activities, the results being compared with values previously obtained in control subjects.

2. Analytical subcellular fractionation by sucrose-density-gradient centrifugation was performed on extracts of the biopsies and the properties of the lysosomes, plasma membrane, biliary canaliculi and endoplasmic reticulum membranes were determined. Increased activities of plasma membrane marker enzymes, particularly y-glutamyl transpeptidase believed to be localized to the biliary canicular membrane, were demonstrated. These findings were most marked in alcoholic cirrhosis. The centrifugation studies revealed no abnormalities in the properties of these membranes.

3. Although the total activities of the endoplasmic reticulum marker enzyme neutral α-glucosidase were unaltered in alcoholic liver disease, centrifugation studies showed a decrease in the density distribution of the membrane-bound enzyme in cirrhosis indicating an increase in the proportion of smooth endoplasmic reticulum membranes.

4. Apart from a small decrease in activity of certain acid hydrolases in fatty liver and in cirrhosis the activities of the lysosomal enzymes were unaffected by alcoholic liver disease.

5. Measurements of lysosomal integrity and density-gradient-centrifugation studies revealed no significant abnormalities in the various patient groups apart from increased stability and reduced equilibrium density of certain lysosomes in fatty liver. It is concluded that lysosomal disruption is not implicated in the pathogenesis of alcoholic liver disease.

Key words: alcoholic liver disease, enzymes, ethanol, lysosomes, microsomes, mitochondria, subcellular fractionation, plasma membrane.

Introduction

The toxic effects of ethanol on the liver are well known (see Lieber, 1973). However, the mechanism by which ethanol damages the liver or what factors determine the susceptibility of any individual to liver damage is unknown. There is no direct correlation between the amount of ethanol consumed and the severity of liver damage though the incidence of cirrhosis has been noted to increase with the number of drinking years (Brunt, 1974; Lelbach, 1975).

The measurement of serum 5′-nucleotidase, alkaline phosphatase, leucyl-2-naphthylamidase and particularly of y-glutamyl transpeptidase is standard practice in the assessment of patients with suspected liver disease but the serum activities of these enzymes unfortunately do not discriminate between the different forms of alcoholic liver disease.

We now report the activities of certain plasma membrane and lysosomal enzyme activities in the liver tissue itself, and attempt to correlate the tissue and circulating enzyme activities, with analytical
subcellular fractionation procedures in combination with marker enzyme microassays to study the effects of ethanol upon intracellular membranes and organelles.

Patients and methods

Fifty-six patients with suspected alcoholic liver disease were studied. Each had a history of excessive alcohol intake, of more than 100 g per day, for at least 2 years before study and had abnormal serum liver function tests. Liver biopsies for diagnostic purposes were carried out within the first 24 h of admission to hospital. Control data on 37 patients have been reported previously (Seymour & Peters, 1977; Peters & Seymour, 1978a).

The patients were classified into four groups according to the hepatic histology. One group (12 patients) had histologically normal livers. This group was termed 'alcoholics with normal histology', and the other three groups were: fatty liver (21 patients); alcoholic hepatitis (seven patients, defined by the presence of alcoholic hyaline and inflammatory cells); cirrhosis (16 patients) (Scheuer, 1970). The liver tissue was obtained with a Menghini needle and was washed with ice-cold sodium chloride solution (0.15 mol/l). A portion was taken in formalin for routine histology.

The remaining portion of the biopsy tissue (2–10 mg wet weight) was homogenized in ice-cold isotonic sucrose solution (0.25 mol/l) containing ethanol (20 mmol/l) and disodium EDTA (1 mmol/l), pH 7.2 (Leighton, Poole, Beaufay, Baudhuin, Coffey, Fowler & de Duve, 1968). Analytical subcellular fractionation procedures were carried out as described by Seymour, Neale & Peters (1974) and Peters (1976). Enzyme analyses of all the liver biopsy homogenates and of the density gradient fractions were as described by Seymour & Peters (1977). Properties of the lysosomes were studied by measurement of latent and sedimentable β-N-acetylglucosaminidase and acid phosphatase (Peters, Heath, Wansbrough-Jones & Doe, 1975). Protein in the whole homogenates was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) and in the density gradient fractions by the method of Peters, Batt, Heath & Tilleray (1976) with bovine serum albumin as standard. DNA was assayed by the technique of LePecq & Paoletti (1966).

The studies in this paper have been approved by the local Ethical Committee. Significances of differences were assessed by unpaired t-test.

| Table 1. Hepatic acid hydrolyases in alcoholic liver disease |
| Values are mean±S.E. of protein ± mg with the number of specimens in parentheses. Probability values (P) indicate significance of difference between values in the patient groups and control subjects. Not significant. | |
| **Category** | **β-N-Acetylglucosaminidase (mg/mg of protein)** | **Acid phosphatase (mg/mg of protein)** | **β-Glucuronidase (EC 3.2.1.32)** | **α-Galactosidase (EC 3.2.1.23)** | **Protein (mg/mg of DNA)** |
| Control (37) | 2.03±0.28 | 4.94±0.43 | 10.90±0.91 | 4.2±0.10 | 0.95±0.05 |
| Alcoholic patients with normal histology (12) | 2.44±0.37 | 11.31±1.8 | 10.90±0.91 | 0.66±0.01 | 0.28±0.07 |
| Fatty liver (21) | 2.89±0.31 | 10.29±1.5 | 10.92±0.91 | 0.4±0.01 | 0.19±0.05 |
| Alcoholic hepatitis (7) | 3.07±0.94 | 10.59±1.10 | 10.92±0.91 | 0.4±0.01 | 0.2±0.05 |
| Cirrhosis (16) | 2.66±0.27 | 9.07±0.75 | 10.92±0.91 | 0.42±0.19 | 0.04±0.01 |

DNA was assayed by the technique of LePecq & Paoletti (1966).
Results

Acid hydrolase activities were not significantly different in control patients and alcoholic patients with normal histology (Table 1). Similarly, no significant difference is noted between alcoholic patients with normal histology and the other three groups (Table 1). In the patients with fatty livers there was a reduction in enzyme activity (β-glucuronidase, α-galactosidase and β-galactosidase) as compared with control subjects. The cirrhotic patients showed a reduction in β-glucuronidase and acid diesterase. The protein/DNA ratio was significantly elevated in the fatty liver group, presumably reflecting a decreased cellularity of the tissue or possibly an accumulation of protein by the hepatocyte (Barona, Leo, Borowsky & Lieber, 1977).

Table 2 shows the activities of five membrane-bound enzymes in the various patient groups. 5'-Nucleotidase alone is significantly elevated in the alcoholic normal group. All four plasma membrane enzymes were raised in the biopsies from patients with cirrhosis, but there were no alterations of these enzymes in the fatty liver and alcoholic hepatitis groups as compared with normal subjects. Neutral α-glucosidase, which has an intracellular localization to the endoplasmic reticulum, is similar in all groups.

Table 3 shows tissue/serum enzyme activities expressed as ratios (Seymour & Peters 1977). For β-N-acetylglucosaminidase and acid phosphatase the tissue/serum ratio was increased but there was no correlation with the severity of liver damage as assessed histologically. Alkaline phosphatase and 5'-nucleotidase show no significant differences between the various patient groups. γ-Glutamyl transpeptidase shows a striking decrease in tissue/serum activity ratio in the alcoholic patients (Table 3) but there is no significant relationship with the severity of liver damage.

Latent and sedimentable acid hydrolase activities in the tissue extracts showed no significant differences between the various groups (Table 4) except that the sedimentable and latent β-N-acetylglucosaminidase is increased in the patients with fatty liver.

Fig. 1 shows the results of subcellular fractionation studies on liver biopsy homogenates from alcoholic patients with normal histology compared with control. There were no differences in the density distribution of 5'-nucleotidase, leucyl-2-

<table>
<thead>
<tr>
<th>Category</th>
<th>5'-Nucleotidase (EC 3.1.3.5)</th>
<th>Alkaline phosphatase (EC 3.1.3.1)</th>
<th>Leucyl-2-naphthylamidase (EC 3.4.11.1)</th>
<th>γ-Glutamyl transpeptidase (EC 2.3.2.2)</th>
<th>Neutral α-glucosidase (EC 3.2.1.20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (37)</td>
<td>13.7 ± 2.4</td>
<td>1.35 ± 0.17</td>
<td>1.28 ± 0.17</td>
<td>5.08 ± 0.81</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td>Alcoholic patients with normal histology (12)</td>
<td>24.8 ± 4.3</td>
<td>1.82 ± 0.31</td>
<td>2.41 ± 0.58</td>
<td>6.99 ± 1.8</td>
<td>0.90 ± 0.20</td>
</tr>
<tr>
<td>Fatty liver (21)</td>
<td>16.3 ± 2.9</td>
<td>1.85 ± 0.45</td>
<td>1.35 ± 0.28</td>
<td>4.48 ± 1.2</td>
<td>0.58 ± 0.16</td>
</tr>
<tr>
<td>Alcoholic hepatitis (7)</td>
<td>21.2 ± 5.4</td>
<td>5.38 ± 2.1</td>
<td>1.65 ± 0.36</td>
<td>6.91 ± 2.2</td>
<td>0.76 ± 0.14</td>
</tr>
<tr>
<td>Cirrhosis (16)</td>
<td>22.3 ± 3.6</td>
<td>4.44 ± 1.0</td>
<td>2.17 ± 0.31</td>
<td>15.15 ± 2.1</td>
<td>0.63 ± 0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>10^-3 x Ratio</th>
<th>β-N-Acetyl-glucosaminidase</th>
<th>Acid phosphatase</th>
<th>5'-Nucleotidase</th>
<th>Alkaline phosphatase</th>
<th>γ-Glutamyl transpeptidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.383</td>
<td>0.110</td>
<td>0.493</td>
<td>0.022</td>
<td>0.840</td>
<td></td>
</tr>
<tr>
<td>Alcoholic patients with normal histology</td>
<td>0.775</td>
<td>0.685</td>
<td>0.470</td>
<td>0.025</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>Fatty liver</td>
<td>0.865</td>
<td>0.446</td>
<td>0.515</td>
<td>0.027</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td>Alcoholic hepatitis</td>
<td>0.768</td>
<td>0.553</td>
<td>0.352</td>
<td>0.052</td>
<td>0.194</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>0.548</td>
<td>0.399</td>
<td>0.378</td>
<td>0.056</td>
<td>0.091</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4. Lysosomal integrity in alcoholic liver disease

Latent and sedimentable enzyme activity is shown as a percentage ± SEM, with the number of specimens in parentheses. Statistics: see Table 1.

<table>
<thead>
<tr>
<th>Category</th>
<th>β-N-Acetylglucosaminidase</th>
<th>Acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latent (%)</td>
<td>Sedimentable (%)</td>
</tr>
<tr>
<td>Control</td>
<td>64.7 ± 1.7 (23)</td>
<td>56.1 ± 2.8 (9)</td>
</tr>
<tr>
<td>Alcoholic patients with normal</td>
<td>63.4 ± 1.7 (12)</td>
<td>47.7 ± 3.6 (5)</td>
</tr>
<tr>
<td>histology</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fatty liver</td>
<td>71.8 ± 1.7 (13)</td>
<td>69.4 ± 1.0 (8)</td>
</tr>
<tr>
<td>P &lt; 0.01</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Alcoholic hepatitis</td>
<td>58.8 ± 4.4 (10)</td>
<td>52.7 ± 4.5 (7)</td>
</tr>
<tr>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>57.5 ± 2.6 (11)</td>
<td>57.1 ± 5.3 (7)</td>
</tr>
<tr>
<td>n.s.</td>
<td>n.s.</td>
<td>46.0 ± 3.0 (6)</td>
</tr>
</tbody>
</table>

naphthylamidase, γ-glutamyl transpeptidase and neutral α-glucosidase. The lysosomal enzyme β-N-acetylglucosaminidase shows a shift towards a lighter median density; β-glucuronidase and acid phosphatase show no significant changes. Fig. 2 shows similar profiles for patients with a fatty liver.

![Fig. 1. Isopycnic centrifugation of 8000 g-min supernatant from liver biopsy homogenates from alcoholic patients with normal hepatic histology. Graphs show frequency–density histograms for marker enzymes for patients with alcoholic liver disease (—) and control subjects(—). Frequency (mean ± SD) is defined as fraction of total recovered activity present in the subcellular fraction divided by the density span covered. The cross-hatched area represents, over an arbitrary abscissa interval, the enzyme remaining in the sample layer. The percentages (±SD) of recovered activity (with number of experiments in parentheses): 5'-nucleotidase, 88 ± 7 (3); γ-glutamyl transpeptidase, 73 ± 10 (3); leucyl-2-naphthylamidase, 97 ± 4 (3); neutral α-glucosidase, 103 ± 7 (3); β-glucuronidase, 73 ± 8 (3); β-N-acetylglucosaminidase, 76 ± 4 (3); acid phosphatase, 92 ± 8 (3); protein, 100 ± 5 (2).](image1)

![Fig. 2. Isopycnic centrifugation of 8000 g-min supernatant from liver biopsy homogenates from patients with fatty liver. Details are as given in Fig. 1. Percentage recoveries and number of experiments: 5'-nucleotidase, 84 ± 7 (3); γ-glutamyl transpeptidase, 82 ± 9 (3); leucyl-2-naphthylamidase, 101 ± 14 (3); neutral α-glucosidase, 106 ± 13 (4); β-glucuronidase, 91 ± 14 (5); β-N-acetylglucosaminidase, 75 ± 7 (5); acid phosphatase, 83 ± 5 (3); protein, 68 ± 8 (3).](image2)
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10
5
0
-5
-10
Acid phosphatase

1

Leucyl-2-naphthylamidase
Neutral α-glucosidase

1

y-Glutamyl transpeptidase

1

β-glucuronidase
β-N-Acetylglucosaminidase

1

ρ-Glucuronidase
ρ-N-Acetylglucosaminidase

1

Alkaline phosphatase

1

5'-Nucleotidase

FIG. 3. Isopycnic centrifugation of 8000 g-min supernatant from liver biopsy homogenates from patients with alcoholic hepatitis. Details are as given in Fig. 1. Percentage recoveries (and number of experiments): 5'-nucleotidase, 106 ± 5 (3); y-glutamyl transpeptidase, 76 ± 11 (3); leucyl-2-naphthylamidase, 93 ± 8 (3); neutral α-glucosidase, 105 ± 15 (3); β-glucuronidase, 76 ± 12 (3); β-N-acetylglucosaminidase, 75 ± 9 (3); acid phosphatase, 72 ± 5 (3); protein, 70 ± 7 (3).

Apart from minor changes in the distribution of the lysosomal changes there are no significant alterations in this patient group. Fig. 3 shows the results from patients with alcoholic hepatitis compared with those of control subjects. There is a decrease in the proportion of sedimentable β-N-acetylglucosaminidase with more activity in the soluble fraction; other marker enzymes are relatively unaffected. The patients with cirrhosis showed a decrease in the median density of the endoplasmic reticulum marker enzyme neutral α-glucosidase (Fig. 4). This change is also just discernible in the results from the patients with alcoholic hepatitis. The two lysosome enzymes, β-N-acetylglucosaminidase and acid phosphatase, again show a reduced particulate activity.

Discussion
These studies used enzymic analysis coupled with analytical subcellular fractionation procedures to explore the pathogenesis of alcoholic liver disease. The properties of plasma membrane, biliary canalicular membranes, endoplasmic reticulum and lysosomes were compared in biopsies from patients with alcoholic liver disease of varying severity.

Plasma membrane. There were no significant changes in the centrifugation properties of the plasma membrane fragments in any of the patient groups. There were, however, small increases in the total activity of certain enzymes localized to this organelle, particularly in the patients with cirrhosis. The ratio of tissue to serum activities remained relatively constant, indicating that there was a parallel increase in activity in both sites. Previous studies also indicated a surprisingly significant positive correlation coefficient between tissue and serum enzymes in alcoholic liver disease (Peters & Seymour, 1978b). The significance of these tissue/enzyme changes is not clear but probably reflects both liver cell hyperplasia (W. J. Jenkins & T. J. Peters, unpublished work) and the increased

FIG. 4. Isopycnic centrifugation of 8000 g-min supernatant from liver biopsy homogenates from patients with alcoholic cirrhosis. Details are as given in Fig. 1. Percentage recoveries: 5'-nucleotidase, 81 ± 7 (3); ρ-glutamyl transpeptidase, 82 ± 10 (3); leucyl-2-naphthylamidase, 88 ± 10 (3); neutral α-glucosidase, 108 ± 6 (3); β-glucuronidase, 93 ± 7 (3); β-N-acetyl-β-glucosaminidase, 73 ± 9 (3); acid phosphatase, 83 ± 7 (3); protein, 91 ± 7 (3).
hepatic protein of the ethanol-fed rats (Barona et al., 1977).

Biliary canalicular membranes, as reflected by the changes in \( y \)-glutamyl transpeptidase (Peters, Seymour & Neale, 1974), show no significant change in centrifugation properties. The tissue activities of this enzyme were only significantly increased in the cirrhotic group but there was a marked fall in the tissue/serum ratio reflecting the very high circulating \( y \)-glutamyl transpeptidase activity known to occur in alcoholic liver disease (Zein & Discombe, 1970; Rosalki & Rau, 1972).

Endoplasmic reticulum. The marker for the intracellular membranes of the endoplasmic reticulum shows no significant change in either tissue or serum activities in any of the forms of alcoholic liver disease. In contrast, the centrifugation studies indicate a decrease in equilibrium density of these membranes, particularly in the cirrhotic group. It is clear that the degree of ribosomal granulation is the major factor in determining the distribution of this enzyme in the sucrose gradients (Beaufay, Amar-Costesec, Thines-Sempoux, Wibo, Robbi & Berthet, 1974; Tilleray & Peters, 1976). This result, therefore, indicates an increased proportion of degranulated smooth endoplasmic reticulum membranes relative to the rough endoplasmic reticulum. Morphological studies have indicated a predominance of smooth endoplasmic reticulum membranes in alcoholic liver disease (Lieber & Rubin, 1968). The enhanced drug oxidation in alcoholic liver disease, which is a function of the smooth endoplasmic reticulum membranes and particularly the rough endoplasmic reticulum components but an increase in enzymes associated with the smooth membranes.

It is not clear, however, whether our findings are directly attributable to a toxic effect of ethanol or merely reflect the cirrhotic process. Similar studies on patients with cirrhosis secondary to both primary haemochromatosis or to transfusional siderosis do not show this density shift in membrane-bound \( \alpha \)-glucosidase (C. A. Seymour & T. J. Peters, unpublished work). However, these changes were not detectable unless gross liver damage has been induced by the ethanol.

Lysosomes. Apart from two enzymes (\( \beta \)-glucuronidase and acid diesterase) there were no changes in the activity of lysosomal enzymes in alcoholic liver disease. Similarly, measurements of lysosomal integrity did not show any significant alteration, apart from a decreased fragility in patients with fatty infiltration. Previous measurements of lysosomal integrity in viral hepatitis and cirrhosis have shown evidence of enhanced fragility (Pagliaro, Giglio, Lemol, Catania & Citarrella, 1964), so that it is unlikely that the lysosomal changes reflect a direct toxic effect of ethanol, or that lysosomal disruption is implicated in the pathogenesis of liver damage. Studies in experimental alcoholic liver disease in the rat (W. J. Jenkins & T. J. Peters, unpublished work) and studies with isolated hepatocytes in vitro (Lundquist, 1975) have also provided no evidence for lysosomal disruption due to ethanol.

The subcellular fractionation studies show a decrease in median density of certain of the lysosomal marker enzymes. Elsewhere we have produced evidence for the presence of two distinct populations of lysosomes in human liver biopsies (Peters & Seymour, 1978b). Centrifugation experiments distinguished lysosomes with equilibrium densities of 1.15 from these with densities of 1.20.

In all the groups of patients studied there appears to be a decrease in the proportion of the denser population as characterized by the distribution of \( \beta \)-N-acetylglucosaminidase. This is most marked in the cirrhotic patients. The less-dense population, characterized by acid phosphatase and particularly \( \beta \)-glucuronidase, are relatively unaffected. This decrease in density of the sedimentable \( \beta \)-N-acetylglucosaminidase is likely to be due to lipid accumulation within these lysosomes (Peters & de Duve, 1974). Similar results have been obtained in patients with fatty liver from causes other than ethanol (W. J. Jenkins & T. J. Peters, unpublished work). The significance of these lysosomal changes to disease processes and in particular to any interference with normal lysosomal function remains to be determined.

These studies demonstrate abnormalities in the three organelles investigated in alcoholic liver disease. It is unlikely that they are primarily implicated in the pathogenesis of the hepatic damage, but they undoubtedly contribute to the disorder of liver function.

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References


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