Enzyme activities in human liver biopsies: assay methods and activities of some lysosomal and membrane-bound enzymes in control tissue and serum

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Summary

1. Highly sensitive techniques are described for the assay of plasma membrane (5'-nucleotidase, alkaline phosphatase), microsomal (neutral α-glucosidase, leucyl-2-naphthylamidase) and biliary canalicular (γ-glutamyltransferase) enzymes and for nine acid hydrolases (acid phosphatase, phosphodiesterase, β-glucosidase, α-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase, N-acetyl-β-glucosaminidase, β-glucuronidase) in human liver.

2. Optimum and specific assay systems have been developed which give linear kinetics for all enzymes.

3. The range of enzyme activities in samples of human liver, obtained by closed needle biopsy, and in sera have been determined.

Key words: biliary tract, enzymes, liver, lysosomes, microsomes, needle biopsy.

Introduction

Serum enzyme activities are commonly used as indices of liver disease, although little is known about their concentration in normal liver tissue. In addition, there is little information about the relation between enzyme activities in serum and the liver.

Direct measurement of enzyme activities in the amount of tissue which can be obtained by needle biopsy has not been possible hitherto because of the relatively low sensitivity of previous assay methods.

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Methods

Collection of liver tissue

Samples of normal liver were obtained from patients either by closed biopsy during the routine investigation of pyrexia of unknown origin or by open biopsy at laparotomy for duodenal ulcer. Histologically all these samples were normal. Informed consent of all patients was obtained before biopsy and the studies reported in this paper were approved by the local Ethical Committee.

The tissue from closed biopsies was collected under local anaesthesia with a Menghini needle (length 70 mm and diameter 1.4 mm). This technique was selected so that minimal amounts of fibrous tissue were present in the biopsy. Representeative fragments of the biopsy were placed in 3 ml of ice-cold sucrose solution (0.25 mol/l), containing disodium EDTA (1 mmol/l), pH 7.4, and ethanol (22 mmol/l), SVE medium, and stored at −20°C. The remainder was placed in buffered formalin/saline and processed for routine light-microscopy. Serum specimens were collected and assayed concurrently.

Immediately before enzyme analysis, the liver tissue was disrupted with ten to fifteen strokes...
of a tight-fitting (B) pestle in a small Dounce homogenizer (Kontes Glass Co., Vineland, New Jersey, U.S.A.).

**Enzyme assays**

Optimum conditions were established for each enzyme assay. The variables studied included buffer systems, pH and period of incubation (up to 2 h). The use of cofactors to improve the kinetics and the specificity of the assay was investigated for certain enzymes. A concentration–activity curve was plotted for each enzyme, to show that linear kinetics had been established.

**Membrane enzymes**

**Leucyl-2-naphthylamidase (EC 3.4.11.2).** This enzyme was assayed fluorimetrically according to the method of Panveliwalla & Moss (1966). A diluted sample, 0.1 ml, was incubated for up to 60 min at 37°C with 0.25 ml of leucyl-2-naphthylamide (0.21 mmol/l) (Sigma Chemical Co.) in a variety of different buffers (0.1 mol/l): Hepes/HCl, Tris/HCl, sodium phosphate, sodium cacodylate and Tricine/HCl were used over a pH range 6.0–8.0. In addition the effect of magnesium chloride was tested over the concentration ranges 0–10 mmol/l and 10–100 mmol/l to improve the specificity of the assay. Concentration–activity curves were constructed with various concentrations of diluted liver homogenate to determine whether the kinetics were linear. The reaction was stopped by addition of 2 ml of ice-cold glycine/NaOH buffer (50 mmol/l), pH 10.4, and the 2-napthylamine released was estimated fluorimetrically in a model 203 Perkin-Elmer fluorescence spectrophotometer. The exciting wavelength was 365 nm and emission wavelength setting was 460 nm. Suitable enzyme and substrate blanks were performed with each assay. Calibration was performed with freshly prepared standard solutions of 2-naphthylamine.

**Alkaline phosphatase (EC 3.1.3.1).** This was assayed by a modification of the method of Douglas, Kerley & Isselbacher (1972). The freshly prepared substrate contained adenosine monophosphate (0.12 mmol/l) and 1 μCi of tritiated adenosine 5-monophosphate ([2-3H]adenosine 5-monophosphate, ammonium salt, specific radioactivity 500 μCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) in 50 ml of 60 mmol/l buffer. Nine different buffers were tested, which included ammediol/HCl, Tris/HCl, Tricine/HCl, sodium glycine, piperazine/HCl, diethanolamine/HCl, triethanolamine/HCl, sodium barbital and sodium borate. The effect of adding Triton X-100 (1 g/l) to the buffer solutions was also studied. A portion (0.1 ml) of the sample was mixed with 0.5 ml of substrate and incubated at 37°C for periods up to 90 min. The reaction was stopped and the unhydrolysed adenosine monophosphate was precipitated by the method of Somogyi (1945). Portions (0.25 ml) of zinc sulphate (0.25 mol/l) and 0.25 ml of filtered barium hydroxide (0.25 mol/l) solutions were used. In order to exclude non-specific alkaline phosphatases, and ensure specificity of the assay, the effects of addition of β-glycerophosphate (12 mmol/l) and cofactors such as magnesium chloride (0–100 mmol/l) and manganese chloride (10–100 mmol/l) were investigated. After centrifugation at 1500 g for 30 min, 0.5 ml of the supernatant was counted for radioactivity in 10 ml of a Triton/toluene scintillant (Lazarow & de Duve, 1971) with a Beckman LS 250 scintillation counter.

**5'-Nucleotidase (EC 3.1.3.5).** This was assayed by a modification of the method of Douglas, Kerley & Isselbacher (1972). The freshly prepared substrate contained adenosine monophosphate (0.12 mmol/l) and 1 μCi of tritiated adenosine 5-monophosphate ([2-3H]adenosine 5-monophosphate, ammonium salt, specific radioactivity 500 μCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) in 50 ml of 60 mmol/l buffer. Nine different buffers were tested, which included ammediol/HCl, Tris/HCl, Tricine/HCl, sodium glycine, piperazine/HCl, diethanolamine/HCl, triethanolamine/HCl, sodium barbital and sodium borate. The effect of adding Triton X-100 (1 g/l) to the buffer solutions was also studied. A portion (0.1 ml) of the sample was mixed with 0.5 ml of substrate and incubated at 37°C for periods up to 90 min. The reaction was stopped and the unhydrolysed adenosine monophosphate was precipitated by the method of Somogyi (1945). Portions (0.25 ml) of zinc sulphate (0.25 mol/l) and 0.25 ml of filtered barium hydroxide (0.25 mol/l) solutions were used. In order to exclude non-specific alkaline phosphatases, and ensure specificity of the assay, the effects of addition of β-glycerophosphate (12 mmol/l) and cofactors such as magnesium chloride (0–100 mmol/l) and manganese chloride (10–100 mmol/l) were investigated. After centrifugation at 1500 g for 30 min, 0.5 ml of the supernatant was counted for radioactivity in 10 ml of a Triton/toluene scintillant (Lazarow & de Duve, 1971) with a Beckman LS 250 scintillation counter.

**γ-Glutamyltransferase (EC 2.3.2.2).** This was assayed by a fluorimetric modification of the method of Szasz (1969). A portion (0.1 ml) of
**Enzyme activities in human liver**

**TABLE 1. Optimum assay conditions for membrane enzymes in human liver biopsies**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC no.</th>
<th>Substrate</th>
<th>Optimum buffer</th>
<th>pH</th>
<th>Cofactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucyl-2-naphthylamidase</td>
<td>3.4.11.2</td>
<td>l-Leucyl-2-naphthylamide (0.21 mmol/l)</td>
<td>Piperazine/HCl (0.1 mol/l)</td>
<td>6-75</td>
<td>Magnesium chloride (50 mmol/l), Triton X-100 (1 g/l)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>3.1.3.1</td>
<td>4-Methylumbelliferyl phosphate (0.21 mmol/l)</td>
<td>Diethanolamine/HCl (0.1 mol/l)</td>
<td>9-5</td>
<td>Magnesium chloride, (5 mmol/l), Triton X-100 (1 g/l)</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>3.1.3.5</td>
<td>Adenosine monophosphate (0.12 mmol/l) [(^3)H]Adenosine monophosphate (1 µCi)</td>
<td>Piperazine/HCl (0.06 mol/l)</td>
<td>9-0</td>
<td>2-Glycerophosphate (12 mmol/l), magnesium chloride (24 mmol/l), Triton X-100 (1 g/l)</td>
</tr>
<tr>
<td>γ-Glutamyltransferase</td>
<td>2.3.2.2</td>
<td>γ-Glutamyl-2-naphthylamide (0.21 mmol/l)</td>
<td>Ammediol/HCl (0.05 mol/l)</td>
<td>8-5</td>
<td>Glyceglycine (20 mmol/l), Triton X-100 (1 g/l)</td>
</tr>
<tr>
<td>α-Glucosidase (neutral)</td>
<td>3.2.1.20</td>
<td>4-Methylumbelliferyl α-D-glucopyranoside (0.21 mmol/l)</td>
<td>Sodium cacodylate/ HCl (0.1 mol/l)</td>
<td>7-5</td>
<td>Triton X-100 (1 g/l)</td>
</tr>
</tbody>
</table>

The sample was incubated for various periods of time up to 90 min at 37°C with 0.25 ml of γ-glutamyl-2-naphthylamide (0.21 mmol/l) in ammediol/HCl buffer (0.05 mmol/l), pH 8.5, containing Triton X-100 (1 g/l) and glycylglycine (20 mmol/l). The reaction was stopped with ice-cold glycine/NaOH and the liberated 2-naphthylamine was measured as described for leucyl-2-naphthylamidase. Concentration-activity curves were constructed with 0-0.1 ml of diluted sample.

**Neutral α-glucosidase (EC 3.2.1.20).** This was assayed fluorimetrically as described by Peters, Müller & de Duve (1972). A diluted sample, 0.1 ml, was incubated for up to 90 min at 37°C with 0.25 ml of 4-methylumbelliferyl-α-D-glucopyranoside (0.21 mmol/l) in 0.1 mol/l buffer solution containing Triton X-100 (1 g/l). Six buffers were tested, which included sodium phosphate, Tris/HCl, Heps/HC1, Tricine/HCl, piperazine/HCl and sodium cacodylate, over the range pH 6.0-8.0. The reaction was stopped with buffered glycine as before and the liberated 4-methylumbelliferone assayed as described for alkaline phosphatase.

**Acid hydrolases**

These enzymes were assayed by a modification of the method of Leaback & Walker (1961), with 4-methylumbelliferyl derivatives. A diluted sample, 0.1 ml, was incubated at 37°C for 10-60 min with 0.25 ml of appropriately buffered substrate and cofactors over the range pH 3.5-6.5. The reaction was stopped with buffered glycine and the fluorescence measured exactly as described for neutral α-glucosidase and alkaline phosphatase. Details of substrate, buffers, pH optima and cofactors are shown in Table 3. Addition of cofactors such as sodium chloride and zinc sulphate were used to enhance the activity of certain acid hydrolases. Thus the effects of sodium chloride (0-10 mmol/l) on β-galactosidase activity and zinc sulphate (0-10 mmol/l) on α-mannosidase activity were studied.

**Proteins**

A micro-modification of the technique of Lowry, Rosebrough, Farr & Randall (1951) was employed, 0.2 ml of appropriately diluted sample being used. A standard curve was constructed for each assay with bovine serum albumin (Armour Pharmaceutical Co., Chicago, U.S.A.) in a range 0-10 µg, with 2 cm path-length microcells and with the absorbance measured at 750 nm.

**Presentation of enzyme activities**

All results of tissue enzyme activities were expressed as munits/mg of protein and serum activity as munits/ml of serum. For all enzymes 1 unit of activity corresponded to the hydrolysis of 1 µmol of substrate/min at 37°C. Ratios of tissue to serum enzyme activity were calculated.
Fig. 1. pH optima, time-course and concentration–activity graphs for five membrane-bound enzymes: (a) leucyl-2-naphthylamide; (b) alkaline phosphatase; (c) 5'-nucleotidase; (d) y-glutamyltransferase; (e) α-glucosidase (neutral).
Enzyme activities in human liver

Fig. 2. Effect of increasing concentrations of magnesium chloride on the activity of three membrane-bound enzymes: (a) leucyl-2-naphthylamidase; (b) 5'-nucleotidase; (c) alkaline phosphatase.

as munitis/g of liver tissue divided by munitis/ml of serum. It was assumed that 1 g of liver contained 200 mg of protein (Leighton, Poole, Beaufay, Baudhuin, Coffey, Fowler & de Duve, 1968).

Results

The experiments described in this paper record the activities of several membrane-bound (microsomal) enzymes and of several acid hydrolases (lysosomal enzymes) in closed needle biopsies from human liver. For each enzyme detailed kinetic studies were performed to provide optimum and specific assay conditions. The results of these studies and comments on the assay procedure will be given in this section. Liver biopsies are stored for up to 2 months at -20°C. With the exceptions of acid and alkaline phosphatase, little variation is found in enzyme activities with length of storage time or with repeated freezing and thawing.

Membrane-bound enzymes

Fig. 1 shows the optima from pH 6-0 to 10-5, time-course for 0–90 min and concentration–activity graphs for the five membrane-bound enzymes in liver homogenates. All enzymes show linear kinetics for up to 60 min and linear activity–concentration graphs. Note that for 5'-nucleotidase there is a rapid fall off in activity after 60 min incubation.

Fig. 2 shows the effect of increasing concentrations of magnesium chloride on the activity of leucyl-2-naphthylamidase, 5'-nucleotidase and alkaline phosphatase. All three enzymes are activated by this metal ion, although the optimum concentrations differ for each assay (Table 1).

Optimum assay conditions for the membrane enzymes are summarized in Table 1. Table 2 shows the activities of these enzymes in human liver tissue and serum.

Acid hydrolases

Fig. 3 and Fig. 4 show the pH, time-course and concentration–activity graphs for the eight acid hydrolases in liver homogenates. All show a single distinct pH optimum, with the exception of α-glucosidase. This enzyme shows optima at pH 4-5 and 5-8. The peak at pH 5-8 could well represent neutral α-glucosidase, which is more alkaline—with sodium cacodylate buffer showing a pH optimum of 7-5 (see Fig. 1).

Fig. 5 shows the effect of increasing concentrations of sodium chloride on β-galactosidase activity and zinc sulphate on α-mannosidase activity. The optimum concentrations of these two cofactors are shown in Table 3.

Table 3 shows the optimum assay conditions adopted for the acid hydrolases. Table 4 shows the activities of these lysosomal enzymes in human liver tissue and serum, together with the ratio of activities.

Discussion

Membrane-bound enzymes

Leucyl-2-naphthylamidase showed a distinct pH optimum at 6-5 and the activity was increased approximately fourfold by addition
TABLE 2. Membrane-bound enzymes in control liver and serum
Specific activities are shown as mean values ± SEM with the number of samples given in parentheses.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tissue (munits/mg of protein)</th>
<th>Serum (munits/ml)</th>
<th>10⁻³ Tissue/serum ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucyl-2-naphthylamidase</td>
<td>12.40 ± 0.62 (5)</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>12.08 ± 0.95 (5)</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>5.36 ± 0.76 (5)</td>
<td>0.493</td>
<td></td>
</tr>
<tr>
<td>γ-Glutamyltransferase</td>
<td>5.08 ± 0.81 (5)</td>
<td>0.840</td>
<td></td>
</tr>
<tr>
<td>α-Glucosidase (neutral)</td>
<td>0.60 ± 0.06 (5)</td>
<td>1.967</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Optimum assay conditions for acid hydrolases in human liver biopsies
4-MeUm = 4-methylumbelliferone.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC no.</th>
<th>Substrate (0.21 mmol/l)</th>
<th>Assay conditions</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>3.1.3.2</td>
<td>4-MeUm phosphate</td>
<td>Sodium acetate (0.1 mol/l)</td>
<td>4-4</td>
</tr>
<tr>
<td>Phosphodiesterase</td>
<td>3.1.4.1</td>
<td>4-MeUm pyrophosphate diester</td>
<td>Sodium acetate (0.1 mol/l)</td>
<td>5-5</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>3.2.1.20</td>
<td>4-MeUm β-D-glucopyranoside</td>
<td>Sodium acetate (0.1 mol/l)</td>
<td>5-8</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>3.2.1.21</td>
<td>4-MeUm α-D-glucopyranoside</td>
<td>Sodium acetate (0.1 mol/l)</td>
<td>4-5</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>3.2.1.22</td>
<td>4-MeUm α-D-galactopyranoside</td>
<td>Sodium acetate (0.1 mol/l)</td>
<td>4-5</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>3.2.1.23</td>
<td>4-MeUm β-D-galactopyranoside</td>
<td>Sodium acetate (0.1 mol/l)</td>
<td>4-5</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>3.2.1.24</td>
<td>4-MeUm α-D-mannopyranoside</td>
<td>Sodium acetate (0.1 mol/l),</td>
<td>5-0</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>3.2.1.30</td>
<td>4-MeUm 2-acetamido-2-deoxy-β-D-glucopyranoside</td>
<td>Sodium acetate (0.1 mol/l),</td>
<td>5-7</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>3.2.1.31</td>
<td>4-MeUm β-D-glucuronide trihydrate</td>
<td>Sodium acetate (0.1 mol/l)</td>
<td>4-0</td>
</tr>
</tbody>
</table>

TABLE 4. Acid hydrolase activity in control liver and serum
Specific activities are shown as mean values ± SEM with the number of samples given in parentheses.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tissue (munits/mg of protein)</th>
<th>Serum (munits/ml)</th>
<th>10⁻³ Tissue/serum ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>19.9 ± 0.7 (5)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Phosphodiesterase</td>
<td>0.024 ± 0.007 (5)</td>
<td>3-25</td>
<td></td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>0.008 ± 0.001 (5)</td>
<td>21-00</td>
<td></td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>0.029 ± 0.004 (5)</td>
<td>2-14</td>
<td></td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>0.010 ± 0.001 (5)</td>
<td>1-20</td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>0.073 ± 0.02 (5)</td>
<td>1-15</td>
<td></td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>0.096 ± 0.006 (5)</td>
<td>0-52</td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>0.063 ± 0.06 (5)</td>
<td>0-38</td>
<td></td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>0.613 ± 0.11 (5)</td>
<td>1-58</td>
<td></td>
</tr>
</tbody>
</table>
Enzyme activities in human liver

of magnesium chloride (50 mmol/l) to the incubation medium (Smith & Spackman, 1955; Patterson, Hsiao & Keppel, 1963; Mahadevan & Tappel, 1967).

Alkaline phosphatase was assayed at high sensitivity with 4-methylumbelliferyl phosphate as a substrate. Guilbault, Sadar, Glazer & Haynes (1968) have compared several phosphate derivatives as substrates for alkaline phosphatase assays and found the 4-methylumbelliferyl derivative to be the most satisfactory. The pH optimum of 9.5 is lower than that usually recorded for alkaline phosphatase but is almost certainly due to the low substrate concentration employed in the present work (Motzok & Branion, 1959; Skillen & Harrison, 1973; Morton, 1957). Diethanolamine/HCl, a phosphate-accepting buffer (McComb & Bowers, 1972), was found to be the best of seven buffers tested. Glycine/NaOH buffer (0.1 mol/l) was found to be totally inhibitory for alkaline phosphatase, as was shown by Bodansky (1946) and Bodansky & Schwartz (1968). The enzyme activity was enhanced by addition of magnesium chloride (5 mmol/l) (Morton, 1957; Clark & Porteous, 1965). As with other tissues, alkaline phosphatase was very active and the kinetics were linear during incubation for at least 60 min.

The assay of 5'-nucleotidase presented a special problem because sensitive assays may respond to the various non-specific phosphatases.

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**Fig. 3.** pH optima, time-course and concentration–activity graphs for four acid hydrolases: (a) acid phosphatase; (b) β-glucosidase; (c) α-glucosidase; (d) α-galactosidase.
FIG. 4. pH optima, time-course and concentration–activity graphs for four acid hydrolases; (a) β-galactosidase; (b) α-mannosidase; (c) N-acetyl-β-glucosaminidase; (d) β-glucuronidase.

FIG. 5. Effect of cofactors on the activity of the acid hydrolases (a) β-galactosidase and (b) α-mannosidase.
present in liver tissue. The radiometric assay of Avruch & Wallach (1971) was adopted and various modifications similar to those of Douglas et al. (1972) were employed to enhance sensitivity. Piperazine buffer (0.06 mol/l) gave the highest activity of the nine buffers tested with a pH optimum of 9.0. Addition of magnesium chloride (20 mmol/l) to the incubation medium caused an eightfold increase in activity (Belfield & Goldberg, 1969). Further specificity of the assay towards adenosine monophosphate was obtained by adding 2-glycerophosphate (12 mmol/l) to the medium as a 'substrate divertor' for the alkaline phosphatase (Belfield & Goldberg, 1968). Omission of the 2-glycerophosphate from the incubation medium caused a 16% increase in apparent 5'-nucleotidase activity. Using mouse brain, Suran (1973) found only a 5% increase in activity, indicating that this tissue contains very little non-specific alkaline phosphatase. Nickel chloride (10 mmol/l), a selective inhibitor of 5'-nucleotidase (Ahmed & Reis, 1958; Campbell, 1962), caused a 98% inhibition of human liver alkaline phosphatase with the present assay technique. The specificity of the assay for 5'-nucleotidase might be enhanced by using a glycine buffer which completely inhibits alkaline phosphatase. 5'-Nucleotidase assayed with this buffer, however, gave only 70% of the activity obtained with piperazine buffers. Triton X-100 (1 g/l) was added to the incubation medium as it has been reported (Konopka, Gross-Bellard & Turski, 1972) that partial latency of rat liver 5'-nucleotidase may occur, although this was not found with human liver in the present study.

\[ \gamma \text{-Glutamyl transferase assayed with ammediol/ HCl buffer (0.05 mol/l) showed a distinct optimum at pH 8.5, similar to that of serum (Szasz, 1969).} \]

Neutral \( \alpha \)-glucosidase, partially localized to liver microsomes and which was thought to hydrolyse maltose and maltotriose (Lejeune, Thinès-Sempoux & Hers, 1963; Brown & Brown, 1965; Gamkloou & Scherstén, 1972; Seymour, Neale & Peters, 1974), was assayed with sodium cacodylate/HCl buffer (0.1 mol/l). A broad pH optimum with peak at approximately 7.5 was found and similar values of pH 7.0 have been reported by Lejeune et al. (1963). However, when acetate buffer (0.1 mol/l) was used a double pH optimum was found, with a peak at pH 4.5 corresponding to the lysosomal \( \alpha \)-glucosidase and a peak at 5.8 corresponding to the neutral (alkaline) \( \alpha \)-glucosidase.

The activities of these five membrane-bound enzymes in liver tissue and serum are shown in Table 2. \( \gamma \)-Glutamyl transferase had the highest activity, followed by 5'-nucleotidase and alkaline phosphatase. Similar relative activities were obtained with rat liver homogenates (C. A. Seymour, unpublished work), although the specific enzyme activities were approximately twice those found in human liver.

The tissue/serum ratios for leucyl-2-naphthylamidase and alkaline phosphatase are similar and are significantly lower than the other membrane enzymes. The reason for this variation is uncertain but presumably reflects differences in subcellular localization, tissue release and plasma clearance for these enzymes.

**Acid hydrolases**

The assay of the various acid hydrolases with four methylumbelliferyl derivatives was particularly reliable (Hultberg & Öckerman, 1972). The blank values and the stability of the substrates stored in a desiccator were consistently satisfactory (Guilbault et al., 1968). In view of the extreme sensitivity of the assays it was important to use freshly prepared buffers as even minimal bacterial contamination could give elevated and erratic blank readings. Some of the substrates had only a limited water solubility and for convenience we have prepared 10 mmol/l solutions of the substrates in 2-methoxyethanol. These solutions, which were stable for at least 1 month at 4°C, were diluted with the appropriate buffer immediately before use, to give a final concentration of 0.21 mmol/l. For certain substrates higher concentrations could be used but higher blank values were obtained with a consequently reduced sensitivity. Addition of bovine serum albumin (1 g/l) did not significantly improve the stability of the enzyme and only increased the blank readings.

Preliminary experiments with \( \beta \)-galactosidase had shown a rapid fall-off in activity during the incubation. Rat liver \( \beta \)-galactosidase was markedly affected by ions (Baccino, Zuretti & Pernigotti, 1975) although the effects of added sodium chloride were dependent on pH, with stimulation occurring only at pH greater than 5.0. In human liver, however, the addition of sodium chloride (8 mmol/l) to the incubation
medium appeared to stabilize the enzyme so that linear kinetics were achieved over a 90 min incubation period. Similar findings for N-acetyl-β-glucosaminidase have been reported by Barrett (1972) and by Sellinger, Beaufay, Jacques, Doyen & de Duve (1960).

Low activities of α-mannosidase were found during the preliminary experiments. An approximate twofold activation was produced by adding zinc sulphate for α-mannosidase prepared from a variety of plant and animal sources (Snaith & Levy, 1968). Addition of Zn²⁺ also increases the specificity of the assay for lysosomal α-mannosidase with respect to cytosol α-mannosidase (Marsh & Gourlay, 1971).

Under identical assay conditions the specific activities of acid hydrolases in rat liver were between two and ten times those of human liver (Peters, Neale & Heath, 1975), but their relative activities were similar. Acid phosphatase was the most active, followed by β-glucuronidase and N-acetyl-β-glucosaminidase. The tissue/serum ratios for the different acid hydrolases varied from 0·1 × 10³ to 21·0 × 10³.

With the exception of acid phosphatase and β-glucosidase the tissue/serum ratios for the acid hydrolases were in reasonable agreement. Subcellular fractionation studies on kidney (Price & Dance, 1967) and on human liver (C. A. Seymour & T. J. Peters, unpublished results) have shown that much of β-glucosidase activity is localized to the cytosol fraction rather than to the lysosomes. Acid phosphatase also has major extra lysosomal components (Beaufay, 1972). In addition, other tissues, e.g. prostate and erythrocytes, probably contribute significant amounts to the serum acid phosphatase activity.

In this paper we have described methods of assaying fourteen enzymes in very small samples of human liver (approximately 10 mg) obtained by percutaneous needle biopsy, and we have defined the range of activities for normal tissue. In subsequent papers we shall describe work currently in progress to determine the detailed subcellular localization of each enzyme and the changes which occur in pathological processes affecting the liver.

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References


Enzyme activities in human liver


