Analytical subcellular fractionation studies on rat liver and on isolated jejunal enterocytes with special reference to the separation of lysosomes, peroxisomes and mitochondria

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Summary

1. Enterocytes were isolated from rat jejunum and characterized morphologically.

2. Attempts to separate the enterocyte subcellular organelles, characterized by their marker enzymes, with isopycnic centrifugation were unsuccessful but good separation of peroxisomes, lysosomes and mitochondria was achieved by sedimentation through a shallow sucrose density gradient with a superimposed inverse gradient of low-molecular-weight dextran.

3. The properties and enzyme activities of the principal subcellular organelles in rat liver cells and enterocytes were compared.

Key words: brush borders, cytosol, enterocytes, intestine, liver, lysosomes, microsomes, mitochondria, peroxisomes, subcellular fractionation.

Introduction

There have been several previous attempts to perform subcellular fractionation of cells of the small intestine but most of these studies have used homogenates of whole mucosa as the starting material. These scrapings contain villus core, crypt and other mucosal cells as well as enterocytes. This is reasonably satisfactory if the aim of the investigation is the preparation of brush-border fractions from enterocytes. If, however, one wishes to study the other subcellular organelles of the enterocyte, for example lysosomes, mucosal scrapings do not provide a satisfactory starting material as these organelles are found in other cells of the intestine.

The aim of the present study was to characterize the peroxisomes, lysosomes and mitochondria of the enterocytes and thus it was necessary to employ a technique for enterocyte isolation. With the procedure, the presence of lysosomes and peroxisomes, as defined biochemically, was conclusively demonstrated for the first time in rat enterocytes. In this paper, some of their properties are described and comparisons are made with rat liver.

Materials and methods

Enterocytes

Preparation. Male rats (Sprague-Dawley strain) weighing 150–250 g were killed by a blow on the head and a 25 cm segment of jejunum was taken and washed with 20 ml of the appropriate isolation fluid. Several techniques for preparing enterocytes were tried, including those of Clark & Porteous (1965), Perris (1966), Kimmich (1970), Evans, Wrigglesworth, Burdett & Pover (1971) and Padron, Gallagher & Kent (1973). The different preparations were assessed by phase-contrast and electron microscopy and by estimation of latent N-acetyl-β-glucosaminidase as a measure of lysosomal integrity in homogenized cell suspensions. Best results, in terms of purity, yield and lysosomal integrity, were obtained with the technique of Reiser & Christiansen (1971). This was slightly modified in that the cell dissociation was performed at 25°C rather than 33°C and the cells were washed by suspension and
centrifugation in sucrose (0.3 mol/l) containing disodium EDTA (1 mmol/l) and ethanol (22 mmol/l) (SVE medium).

Morphological examination of cells. Samples of the cell suspension were fixed for 2 h in 3% (v/v) glutaraldehyde in sodium cacodylate buffer (0.1 mol/l; pH 7.4), centrifuged at 10 000 g for 15 min and post-fixed in osmium tetroxide (10 g/l). The cell pellets were stained in block with uranyl acetate (5 g/l), dehydrated and embedded in Epon. For light-microscopy, thick sections (1 μm) were cut with a glass knife and stained with Azure II–Methylene Blue according to Richardson, Jarett & Finke (1960). For electron microscopy the sections were cut with a diamond knife and examined in a Philips EM 300 microscope. Demonstration of acid phosphatase with the electron microscope was performed by the technique of Barka & Anderson (1962).

Homogenates

Preparation of liver homogenates. Portions (1 g) of rat liver were homogenized in 15 ml of sucrose (0.25 mol/l; pH 7.4) containing disodium EDTA.

<table>
<thead>
<tr>
<th>Table 1. Enzyme assay conditions</th>
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</thead>
<tbody>
<tr>
<td>Enzyme</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
</tr>
<tr>
<td>Monoamine oxidase</td>
</tr>
<tr>
<td>D-Amino acid oxidase</td>
</tr>
<tr>
<td>Catalase</td>
</tr>
<tr>
<td>NADPH–cytochrome c reductase</td>
</tr>
<tr>
<td>Galactosyl transferase (EC 2.3.1.18)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
</tr>
<tr>
<td>Acid hydrolases</td>
</tr>
<tr>
<td>Cathepsin D</td>
</tr>
<tr>
<td>Leucyl-β-naphthalamidase</td>
</tr>
</tbody>
</table>

Enzyme Commission numbers of other enzymes are listed in Table 2.

<sup>(1)</sup> Buffer containing 0.01% Triton X-100.

<sup>(2)</sup> Buffer containing 0.1% Triton X-100.
Fractionation of enterocytes

(1 mmol/l) and ethanol (22 mmol/l) with fifteen passes of a loose-fitting (type A) pestle in a medium-sized Dounce homogenizer (Kontes Glass Co., Vineland, N.J., U.S.A.). The homogenate was centrifuged at 600 g for 10 min for separation of the nuclei, undisrupted cells and debris. The supernatant was subjected to analytical subcellular fractionation.

Preparation of enterocyte homogenate. The pellet of washed cells was suspended in 15 ml of SVE medium and disrupted with ten strokes of a loose-fitting pestle in a Dounce homogenizer. The homogenate was centrifuged at 800 g for 10 min for separation of the nuclear and brush-border fraction and the supernatant was subjected to subcellular fractionation.

Analytical subcellular fractionation

Isopycnic centrifugation of tissue extracts. The analytical subcellular fractionations were carried out in a Beaufay automatic zonal rotor type E40. The use of the rotor and associated equipment has been described previously (Beaufay, 1966; Leighton, Poole, Beaufay, Baudhuin, Coffey, Fowler & de Duve, 1968).

A portion (5 ml) of post-nuclear supernatant from either liver or enterocyte homogenates was layered on to a 24 ml sucrose gradient, extending linearly with respect to volume, from density 1.05 to 1.28 and resting on a 6 ml cushion of density 1.32. All solutions contained disodium EDTA (1 mmol/l; pH 7.4) and ethanol (22 mmol/l). The rotor was run at 35 000 rev./min for 35 min \[W \text{ (integrated angular velocity)} = \frac{\omega^2 df}{dt} = 3.3 \times 10^{10} \text{ rad}^2 \text{s}^{-1} \] at 0°C, and some fifteen fractions were collected into tared tubes. After weighing, the density of each fraction was determined with an Abbé refractometer.

Rate-zonal centrifugation of tissue extracts. Several different systems were tried but the best results were obtained with the following technique. Post-nuclear supernatant (2.0 ml) was layered on to a 40 ml sucrose gradient extending linearly, with respect to volume, from density 1.04 to 1.22. The lighter solution in the gradient-making device contained 5% (w/w) of Dextran 10 (Pharmacia, Uppsala, Sweden) so that an inverse gradient of dextran was superimposed on the sucrose gradient. The gradient rested on a 4 ml cushion of sucrose of density 1.30. All solutions contained disodium EDTA (1 mmol/l; pH 7.4) and ethanol (22 mmol/l). The total integrated angular velocity (W) from injection of the cell extract to collection of the last fraction was 1.2–1.4 \times 10^{9} \text{ rad}^2 \text{s}^{-1}.

Marker enzyme assays

Table 1 shows the enzyme assay techniques used. Protein content of the homogenates was assayed with the technique of Lowry, Rosebrough, Farr & Randall (1951). Protein in the subcellular fractions was assayed by the fluorimetric technique of Hiraoka & Glick (1963). Bovine serum albumin (Armour Pharmaceuticals) was used as a standard. Latent enzyme activity was determined by assaying in isotonic sucrose with (total activity), or without (free activity), 0.1% Triton X-100. Latency is the difference between total and free activity expressed as a percentage of total activity.

Results

Morphological studies

Fig. 1(a) shows a low-power view of a stained Epon section of enterocytes isolated by the modification of the method of Reiser & Christiansen (1971). Most of the cells are present as sheets with a continuous brush border. No crypt or Paneth cells were present in the preparations but occasional goblet cells are seen in sheets of enterocytes. Fig. 1(b) shows an electron micrograph of a section of an isolated enterocyte that had been stained for acid phosphatase, demonstrating that lysosomal integrity has been preserved in the isolated cells.

Enzyme activities of enterocytes and liver

Table 2 shows the specific activities (munits/mg of protein) of various marker enzymes in isolated jejunal enterocytes and in liver. The first three enzymes, \(\alpha\)-glucosidase, alkaline phosphatase and leucyl-\(\beta\)-naphthylamidase, are considerably more active in the enterocyte than in the liver although the ratio of the activities in the two tissues differs for each enzyme. The pH optima for alkaline phosphatase and leucyl-\(\beta\)-naphthylamidase are similar in liver and intestine but the pH profiles for \(\alpha\)-glucosidase differ in the two tissues. In intestine there is a single clearly defined pH optimum at 6.0, but in liver the situation is more complex, with a major peak at pH 7.5–8.0 and a much smaller peak at 4.5.
### Table 2. Enzyme activities of rat jejunal enterocytes and liver

Mean values ± se are shown for specific activities and the number of observations is given in parentheses.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC no.</th>
<th>pH optimum</th>
<th>Jejunal enterocytes (munits/mg of protein)</th>
<th>pH optimum</th>
<th>Liver (munits/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase</td>
<td>3.2.1.20</td>
<td>6.0(1)</td>
<td>19.2 ± 1.4 (6)</td>
<td>4.0(1,6)</td>
<td>0.088 ± 0.017 (4)</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>3.2.1.20</td>
<td>—</td>
<td>—</td>
<td>8.2(2,6)</td>
<td>1.69 ± 0.42 (5)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>3.1.3.1</td>
<td>8.8(2)</td>
<td>67.1 ± 11.0 (6)</td>
<td>8.8(2)</td>
<td>0.56 ± 0.24 (6)</td>
</tr>
<tr>
<td>Leucyl-β-naphthylamidase</td>
<td>3.4.11.2</td>
<td>7.3(3)</td>
<td>51.6 ± 1.10 (6)</td>
<td>7.3(3)</td>
<td>6.19 ± 1.10 (6)</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>3.1.3.5</td>
<td>9.0(6)</td>
<td>13.9 ± 1.1 (3)</td>
<td>9.0(6)</td>
<td>11.7 ± 0.8 (3)</td>
</tr>
<tr>
<td>Catalase</td>
<td>1.11.1.6</td>
<td>7.4(6)</td>
<td>6.61 ± 1.67 (6)</td>
<td>7.4(6)</td>
<td>724 ± 120 (4)</td>
</tr>
<tr>
<td>d-Amino acid oxidase</td>
<td>1.4.3.3</td>
<td>8.5(6)</td>
<td>0.196 ± 0.051 (6)</td>
<td>8.5(6)</td>
<td>2.34 ± 0.12 (4)</td>
</tr>
<tr>
<td>Monoamine oxidase</td>
<td>1.4.3.4</td>
<td>7.4(6)</td>
<td>0.630 ± 0.058 (6)</td>
<td>7.4(6)</td>
<td>1.84 ± 0.41 (6)</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>1.9.3.1</td>
<td>7.8(6)</td>
<td>130 ± 10 (3)</td>
<td>7.8(6)</td>
<td>85 ± 15 (3)</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>1.1.1.37</td>
<td>7.4(6)</td>
<td>478 ± 580 (6)</td>
<td>7.4(6)</td>
<td>8220 ± 310 (6)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>3.1.3.2</td>
<td>4.0(1)</td>
<td>19.0 ± 5.4 (6)</td>
<td>5.0(1)</td>
<td>46.3 ± 7.2 (4)</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>3.2.1.30</td>
<td>5.7(1)</td>
<td>3.41 ± 0.44 (6)</td>
<td>5.5(1)</td>
<td>3.13 ± 0.12 (6)</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>3.2.1.31</td>
<td>4.5(2)</td>
<td>1.60 ± 0.25 (6)</td>
<td>4.5(2)</td>
<td>11.7 ± 1.9 (6)</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>3.2.1.23</td>
<td>4.0(1)</td>
<td>2.00 ± 0.29 (6)</td>
<td>4.0(1,4)</td>
<td>1.75 ± 0.28 (6)</td>
</tr>
<tr>
<td>N-Acetyl-β-galactosaminidase</td>
<td>3.2.1.53</td>
<td>4.7(1)</td>
<td>1.06 ± 0.18 (6)</td>
<td>5.0(1)</td>
<td>0.64 ± 0.09 (6)</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>3.2.1.23</td>
<td>3.6(1)</td>
<td>0.62 ± 0.10 (6)</td>
<td>4.0(1)</td>
<td>0.38 ± 0.13 (6)</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>3.2.1.24</td>
<td>4.4(1,5)</td>
<td>0.50 ± 0.10 (6)</td>
<td>5.0(1,5)</td>
<td>0.68 ± 0.17 (6)</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>3.4.23.5</td>
<td>3.6(6)</td>
<td>13.3 ± 2.1 (3)</td>
<td>3.6(6)</td>
<td>30.8 ± 4.2 (3)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1.1.1.27</td>
<td>7.4(6)</td>
<td>1489 ± 160 (3)</td>
<td>7.4(6)</td>
<td>5790 ± 470 (3)</td>
</tr>
</tbody>
</table>

(1) Acetate (0.1 mol/l).
(2) Borate (0.1 mol/l).
(3) Phosphate (0.1 mol/l).
(4) Tris-HCl (0.1 mol/l).
(5) ZnSO₄ (0.5 mmol/l) present in buffered substrate.
(6) pH of assay.

### Table 3. Relative activities of rat jejunal enterocytes, rat liver homogenate and purified rabbit kidney D-amino acid oxidase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rat jejunal enterocytes</th>
<th>Rat liver homogenate</th>
<th>Rabbit kidney D-amino acid oxidase(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Proline</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>d-Alanine</td>
<td>42</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>d-Methionine</td>
<td>40</td>
<td>48</td>
<td>54</td>
</tr>
<tr>
<td>d-Tyrosine</td>
<td>33</td>
<td>30</td>
<td>128</td>
</tr>
<tr>
<td>d-Valine</td>
<td>30</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>d-Tryptophan</td>
<td>25</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>d-Phenylalanine</td>
<td>23</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>d-Lysine</td>
<td>15</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>d-Leucine</td>
<td>14</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>d-Aspartic acid</td>
<td>5</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>d-Threonine</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>d-Serine</td>
<td>10</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>d-Histidine</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Glycine</td>
<td>5</td>
<td>7</td>
<td>—</td>
</tr>
</tbody>
</table>

(1) Calculated from the data of Bender & Krebs (1950).

### Table 4. Latent enzymic activity in post-nuclear supernatant fraction from homogenates of liver and enterocytes

Mean values ± se are shown with the number of specimens in parentheses.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>85.8 ± 3.1 (8)</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>77.4 ± 2.4 (5)</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>68.2 ± 5.1 (3)</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>72.1 ± 1.5 (3)</td>
</tr>
<tr>
<td>Catalase</td>
<td>78.3 ± 2.1 (5)</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>88.5 ± 2.4 (4)</td>
</tr>
</tbody>
</table>
FIG. 1. (a) Epon sections (1 μm) of enterocyte preparations. Cells are isolated as sheets with continuous brush borders (bb). Goblet cells (Gb) are visible between the enterocytes (x 1200). (b) Portion of an isolated sheet of enterocytes tested for acid phosphatase. The cell maintains its integrity and is joined to a neighbouring cell by several junctional complexes. Electron-dense reaction product is localized in several large lysosomes. Scattered reaction product associated with the microvilli probably reflects surface alkaline phosphatase activity; mv, microvilli; tw, terminal web; r, rootlet or microvilli extending into the terminal web; N, nucleus; m, mitochondrion; er, endoplasmic reticulum; Ig, lysosome; zo, zonula occludens or tight junction; d, desmosomes or macula adherens (x 15000).

(Facing p. 358)
Assays on liver homogenates were performed at pH 8.5 and 4.0, as recommended by Lejeune, Thinès-Sempoux & Hers (1963). 5'-Nucleotidase has a similar level of activity in both liver and enterocyte.

Catalase and D-amino acid oxidase activities are considerably higher in liver than in intestine. This is especially so for catalase, which has a specific activity in liver over 100 times that in enterocytes. Cytochrome c oxidase has a higher specific activity in the enterocyte than in liver but the two dehydrogenases and monoamine oxidase are 2–3 times more active in liver than in the enterocyte.

The nine acid hydrolases have different relative activities in the two tissues. The acid phosphatase activities cannot be directly compared owing to the differences in the pH chosen for the assay. The pH optimum of the enterocyte acid phosphatase appears to be in the region of 5.0, but at this pH alkaline...
phosphatase still has significant activity and therefore assays of acid phosphatase were made at pH 4.0. β-Glucuronidase and cathepsin D were significantly more active in liver than in intestine but the other acid hydrolases, in particular N-acetyl-β-glucosaminidase, had similar activities in both tissues.

Table 3 shows the relative D-amino acid oxidase activity against different amino acids, of homogenates of liver and isolated enterocytes as compared with a purified preparation of rabbit kidney D-amino acid oxidase. The liver and enterocytes have similar specificities with D-proline being the most active substrate. Apart from a higher activity against D-tyrosine, purified rabbit kidney D-amino acid oxidase has similar relative activities to those of the tissue preparations.

As shown in Table 4, several acid hydrolases, as well as catalase, displayed a considerable degree of latency in the post-nuclear supernatant fraction prepared from the enterocytes. The latent N-acetyl-β-glucosaminidase in the enterocytes was similar to that in liver.

Analytical subcellular fractionation

Isopycnic centrifugation experiments. Fig. 2 shows the distribution of a number of enzymes from an enterocyte post-nuclear supernatant fraction after isopycnic centrifugation in a sucrose gradient. All enzymes assayed are, totally or in major part, associated with particles that have moved significantly into the main body of the gradient under the influence of the centrifugal force. Some differences exist between the distribution patterns of the individual enzymes, suggesting the presence of several distinct particles, but the modal densities of all enzymes are almost identical at 1.16–1.17. 5′-Nucleotidase (not shown) had a significantly lower
Fractionation of enterocytes

Fig. 4. Results of rate-zonal centrifugation of post-nuclear supernatant fraction from jejunal enterocyte homogenate. Experimental conditions are described in the Materials and methods section. For details see the legend to Fig. 2. The percentage recovered activity for each enzyme was: N-acetyl-β-glucosaminidase, 80%; α-glucosidase, 89%; catalase, 103%; cytochrome oxidase, 102%; D-amino acid oxidase, 85%; monoamine oxidase, 81.

Rate-zonal centrifugation experiments. Fig. 4 and Fig. 5 show the results that are obtained after centrifugation through the shallow sucrose-dextran gradient. They are representative results from a total of six separate experiments. Much of the cytochrome oxidase activity reaches its equilibrium density (compared with Fig. 2). Most of the monoamine oxidase shows a similar distribution to cytochrome oxidase. All other enzymes are still in the process of sedimenting when the gradient fractions are collected. Closest to its equilibrium position is N-acetyl-β-glucosaminidase, which is accompanied by α-mannosidase and β-galactosidase (not shown). Next is found catalase, which moves more slowly than the preceding hydrolases and also forms a narrower band. It is accompanied by D-amino acid oxidase, which shows a distribution almost identical with that of catalase. Sedimenting even more slowly modal density of 1.13 but no useful resolution of the other organelles could be obtained. Numerous attempts were made to improve the resolution by the use of different solutes for the preparation of the gradient. These included sorbitol, combination of sucrose or mannitol with Ficoll (Pharmacia) or with Stractan (Stein Hall Co. Inc., New York, U.S.A.) as well as sucrose in KCl (0.02 mol/l or 0.20 mol/l). No significant improvement in resolution of these organelles was obtained.

Fig. 3 shows the distribution of a number of marker enzymes when a post-nuclear supernatant fraction from rat liver homogenate is subjected to isopycnic centrifugation. Here there is distinct resolution of the enzymes, in particular those associated with lysosomes (N-acetyl-β-glucosaminidase), mitochondria (cytochrome oxidase) and peroxisomes (catalase).
FIG. 5. Results of rate-zonal centrifugation of post-nuclear supernatant fraction from jejunal enterocyte homogenate. Experimental conditions are described in the Materials and methods section. For details see the legend to Fig. 2. The percentage recovered activity for each enzyme was: catalase, 80; α-glucosidase, 86; N-acetyl-β-glucosaminidase, 82; galactosyl transferase, 79; cytochrome oxidase, 109; NADPH-cytochrome c reductase, 91; protein, 107; β-glucuronidase, 83.

Discussion

In this paper a method for the isolation of jejunal enterocytes relatively uncontaminated by other components has been described and the properties of the subcellular organelles from enterocytes have been compared with those of the organelles of liver.

Mitochondria

Enterocytes are abundantly provided with mitochondria, as can be readily observed morphologically
and is confirmed by their high cytochrome c oxidase activity. According to their centrifugal behaviour, the enterocyte mitochondria appear to form a relatively homogeneous population of particles with an equilibrium density of 1.16–1.17, slightly lower than that of liver mitochondria (1.19). In liver monoamine oxidase activity is largely associated with the outer membrane of the mitochondria (Schnaitman, Erwin & Greenawalt, 1967). The remainder occurs as a component of the microsomal fraction, also possibly related to the outer mitochondrial membrane (Amar-Costesc, Wibo, Thines-Sempoux, Beaufay & Berthet, 1974). The same may well be true for the enterocyte, where the distribution of monoamine oxidase activity remains in the sample layer. This is probably associated with slowly sedimenting particles of the microsome fraction, but may also belong to soluble enzyme activity.

**Lysosomes**

Three acid glycosidases commonly associated with lysosomes, N-acetyl-β-glucosaminidase, α-mannosidase, and β-galactosidase, were found to be largely sedimentable and latent in homogenates of enterocytes, and, upon rate-zonal centrifugation, to sediment as a distinct group of particles. Thus the main biochemical criteria for lysosomes are satisfied. Previous workers have indicated that these and other acid hydrolases are localized to lysosomes within intestinal mucosa (Clark & Porteous, 1965; Hsu & Tappel, 1965; Hübscher, West & Brindley, 1965; Danovitch & Laster, 1969; Peters, 1970; Koldovský, Palmieri & Jamawan, 1972; Raychaudhuri & Desai, 1972; Seetharam & Radhakrishnan, 1972; Lebenthal, Tsuibo & Kretchmer, 1974). These studies, however, have used homogenates of either whole intestine or of mucosal scrapings and thus the cellular origin of the lysosome is uncertain. Histochemical studies have also provided data consistent with the demonstration of lysosomes within enterocytes (Pugh & Walker, 1961; Behnke, 1963; Jervis, 1963; Riecken, Stewart, Booth & Pearse, 1966; Hugon & Borgers, 1968) and fractionation studies with isolated guinea-pig enterocytes (Wrigglesworth & Pover, 1966) have identified lysosomes within these cells. β-Glucuronidase, although assumed by most workers to have a lysosomal localization (Hsu & Tappel, 1964; Riecken et al., 1966; Peters, 1970; Connock & Pover, 1970; Desai, 1971; Koldovský et al., 1972), appears to have a different distribution in the sucrose gradients from the other lysosomal enzymes. Although approximately one-quarter of the activity appears to be associated with the lysosomes the majority belongs to a slow-moving component of microsomal nature. There may also be a soluble component. The dual lysosomal and microsomal localization of β-glucuronidase in the enterocyte is similar to that observed in liver (Fishman, Goldman & Delellis, 1967).

The acid hydrolases have similar enzyme activities in the enterocytes and in liver, except for β-glucuronidase, which is considerably higher in the latter tissue. This suggests a relative abundance of lysosomes in the enterocytes but their equilibrium density of 1.16–1.17 is much lower than that of liver (1.20–1.23) and it is therefore likely that their morphological appearance differs from the typical pericanalicular dense bodies of liver lysosomes. Electron micrographs show many vesicular structures containing material of intermediate density, which may be the morphological counterparts of lysosomes in the enterocytes. The rate-zonal centrifugation experiments indicate that the lysosomes show considerable size heterogeneity and this is supported by histochemical studies, which show a marked variation in size and shape of the acid phosphatase-containing structures.

**Peroxisomes**

The demonstration of latent and sedimentable catalase, together with the observation that this enzyme and d-amino acid oxidase display characteristic and almost identical distribution patterns upon centrifugal fractionation, provides strong evidence for the presence of peroxisomes in rat enterocytes. A recent paper by Connock, Kirk & Sturdee (1974) has reported the presence of peroxisomes in isolated guinea-pig enterocytes. The enterocyte peroxisomes do, however, differ in several respects from those of liver (or kidney). First, the specific activities of the peroxisomal enzymes are considerably lower in the enterocytes, about one-hundredth the activity of liver for catalase and one-tenth that of liver for d-amino acid oxidase. Secondly, attempts to demonstrate other peroxisomal enzymes including urate oxidase or L-α-hydroxy acid oxidase in enterocyte preparations were unsuccessful. Thirdly, the centrifugal behaviour of enterocytes peroxisomes differs
greatly from those of liver, indicating considerable differences in their physical properties. The enterocyte peroxisomes have an equilibrium density of only 1.16-1.17, compared with 1.24 for liver peroxisomes, and their slow sedimentation rate suggests that they may be distinctly smaller than the liver particles, confirming the histochemical observations of Novikoff & Novikoff (1972). In an extensive study of intestinal mucosa using an alkaline diaminobenzidine staining technique, believed to reveal catalase activity, these authors have demonstrated the widespread occurrence in mucosal cells of small diaminobenzidine-positive particles, closely associated with the endoplasmic reticulum (Novikoff, Novikoff, Quintana & Davis, 1973). They have proposed the term 'microperoxisomes' for these particles, which are also found in numerous other tissues (Novikoff, Novikoff, Davis & Quintana, 1973) and are distinctly different morphologically from the characteristic 'microbodies' of liver and kidney.

**Brush-border fragments and microsomes**

Brush-border marker enzymes are largely (approximately 75%) sedimented by the first low-speed centrifugation used to remove the nuclei and undisrupted cells (Hübscher et al., 1965; Porteous & Clark, 1965; Peters, 1970). However, a certain amount of disruption of the microvilli occurs and these small vesicles usually sediment in the microsomal fraction when an extract of intestinal mucosa, particularly after vigorous homogenization, is subjected to differential centrifugation (Hers, Berthet, Berthet & de Duve, 1951; Louvard, Maroux, Baratti, Desnuelle & Mutafshiev, 1973). Microsomes is an operational term for a highly heterogeneous population of slowly moving particles (Claude, 1950). In rat liver the following distinct membrane components have been identified: smooth and rough endoplasmic reticulum, plasma membrane, Golgi and outer mitochondrial membrane fragments (Amar-Costesc et al., 1974). Marker enzymes for some of these components have been assayed in the enterocyte fractions but no useful resolution of these elements (except plasma membrane) was obtained in the present study. Application of the analytical approach of Amar-Costesc et al. (1974) to the subfractionation of enterocyte microsomes may be expected to achieve similar separations to those obtained with liver microsomes.

The techniques and assay methods described in this paper are sufficiently sensitive for direct application to human closed intestinal biopsy specimens. Subsequent papers will report the application of the analytical subcellular fractionation technique to both normal and pathological human jejunal mucosa (Peters, Heath, Jones & Peacham, 1975).

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**References**


