Analytical Subcellular Fractionation of Needle-Biopsy Specimens from Human Liver

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1. Fragments (2–20 mg wet wt.) of closed needle-biopsy specimens from human liver were disrupted in iso-osmotic sucrose and subjected to low-speed centrifugation. The supernatant was layered on a linear sucrose-density gradient in the Beaufay small-volume automatic zonal rotor. The following organelles, with equilibrium densities (g/ml) and principal marker enzyme shown in parentheses, were resolved: plasma membrane (1.12–1.14; 5'-nucleotidase); lysosomes (1.15–1.20; *N*-acetyl- β -glucosaminidase); mitochondria (1.20; malate dehydrogenase); endoplasmic reticulum (1.17–1.21; neutral α -glucosidase); peroxisomes (1.22–1.24; catalase). 2. The distribution of particulate alkaline phosphatase and, to a lesser degree, leucine 2-naphthylamidase, followed that of 5'-nucleotidase. γ -Glutamyltransferase was associated with membranes of significantly higher equilibrium density than was 5'-nucleotidase. 3. The distribution of 12 acid hydrolases was determined in the density-gradient fractions. β -Glucosidase had a predominantly cytosolic localization, but the other enzymes showed a broad distribution of activity throughout the gradient. Evidence was presented for two populations of lysosomes with equilibrium densities of 1.15 and 1.20 g/ml, but containing differing amounts of each enzyme. Further evidence of lysosomal heterogeneity was demonstrated by studying the distribution of isoenzymes of hexosaminidase and of acid phosphatase. 4. The resolving power of the centrifugation procedure can be further enhanced with membrane perturbants. Digitonin (0.12 mM) selectively disrupted lysosomes, markedly increased the equilibrium density of plasma-membrane components and lowered the density of the endoplasmic reticulum, but did not affect the mitochondria or peroxisomes. Pyrophosphate (15 mM) selectively lowered the equilibrium density of the endoplasmic reticulum.

Although the various subcellular organelles of liver tissue from various experimental animals, particularly the rat, have been extensively studied, there have been few investigations on human liver. By using electron-microscopic procedures, considerable information has been obtained about the alterations in certain subcellular organelles in hepatic diseases. However, because of the very limited amount of tissue obtained by needle biopsy, it has not hitherto been possible to apply detailed subcellular-fractionation procedures to human tissue. Combining a single-step centrifugation procedure (Peters *et al.*, 1972) in the small-volume automatic zonal rotor (Beaufay, 1966) with highly sensitive marker enzymes acting on radiolabelled and fluorogenic substrates (Seymour & Peters, 1977) it is now possible to examine human liver biopsy specimens in this manner. The present paper reports baseline data on control tissue in which the enzyme activities

and centrifugation properties of the principal hepatocyte organelles are described.

Materials and Methods

Homogenization and subcellular fractionation

Portions (5–20 mg) of liver biopsy specimens were placed in 3 ml of ice-cold 0.25 M-sucrose containing 1 mM-EDTA (disodium salt), pH 7.2, and 20 mM-ethanol (SVE medium). The specimens were obtained with a Menghini needle, a technique that ensures that only a minimal amount of fibrous tissue is present in the biopsy. Representative portions were processed for routine histological examination. All specimens were histologically normal and the various clinical tests revealed no abnormality of liver function. The tissue was disrupted with 15 strokes of a loose-fitting (type A) pestle in a small Dounce homogenizer (Kontes Glass Co., Vineland, NJ, U.S.A.) and the homogenate centrifuged at 600 *g* for 10 min in a swing-out rotor (r_{av} , 16.0 cm) in a MSE 4L

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centrifuge. The pellet was resuspended in a further 2 ml of SVE medium with three strokes of the Dounce homogenizer, re-centrifuged as before and the post-nuclear supernatants were combined.

A portion of the liver extract (3–4 ml) was layered on a 28 ml sucrose density gradient extending linearly with respect to volume from a density of 1.05 g/ml to one of 1.28 g/ml and resting on a cushion of 6 ml of sucrose (density 1.32 g/ml) in the Beaufay small-volume (type E50) automatic zonal rotor as described previously (Peters *et al.*, 1972). All density-gradient solutions contained 1 mM-EDTA (disodium salt), pH 7.2, and 20 mM-ethanol. After centrifugation at 35000 rev./min for 35 min [W (integrated angular velocity) = $\int_0^t \omega^2 \cdot dt = 3.3 \times 10^{10} \text{ rad}^2/\text{s}$] the rotor was slowed and some 16 fractions were collected into tared tubes. After reweighing and mixing, the density of the fractions was determined indirectly by refractometry. Results are expressed as frequency-density histograms and were computed as described by Leighton *et al.* (1968). A crude lysosomal fraction was prepared as described by Peters *et al.* (1975).

Treatment with membrane perturbants

Biopsies were homogenized in either 0.25 M-sucrose containing 0.12 mM-digtonin [Sigma (London) Chemical Co., Kingston, Surrey, U.K.] or 15 mM-sodium pyrophosphate, pH 8.2. Both solutions contained 1 mM-EDTA (disodium salt), pH 7.2, and 20 mM-ethanol. Postnuclear-supernatant fractions were prepared and subjected to analytical subcellular fractionation as described above.

Analytical methods

Enzyme assays were performed as described previously (Peters, 1976; Seymour & Peters, 1977). Acid phosphatase was also assayed in the presence of 2 mM-KF. Ribonuclease was assayed by the method of de Duve *et al.* (1955). Cathepsin D was assayed with ^{14}C -labelled haemoglobin as described by Roth & Losty (1971). D-Amino acid oxidase was assayed fluorimetrically by the method of Guilbault *et al.* (1968), with D-proline as substrate. The isoenzymes of *N*-acetyl- β -glucosaminidase were separated and measured by the technique of Ellis *et al.* (1974). Latent and sedimentable acid hydrolases were assayed as described by Peters *et al.* (1975). Protein content of the homogenate was measured by the method of Lowry *et al.* (1951) and of the gradient fractions by a fluorescent method described by Peters *et al.* (1976), with bovine serum albumin as standard.

The studies described in this paper have been approved by the local ethical committee.

Results

Enzyme activities

Table 1 shows the specific activities (munits/mg of protein) of liver homogenates and the activity in the post-nuclear supernatant, expressed as a percentage of the activity in the whole homogenate. At least three-quarters of the activity in the whole homogenate is recovered in the post-nuclear supernatant fraction. Table 2 shows the latent and sedimentable enzyme activity in the tissue extracts for four acid hydrolases.

Subcellular-fractionation experiments

Fig. 1 shows the distribution of the marker enzymes for the principal organelles after density-gradient centrifugation of liver extracts. Malate dehydrogenase shows a bimodal distribution with a distinct mitochondrial peak of activity containing approx. half of the total activity at a modal density of 1.20 g/ml. Identical distributions for particulate succinate dehydrogenase, glutamate dehydrogenase and aspartate aminotransferase (results not shown) were obtained. Approx. two-thirds of the *N*-acetyl- β -glucosaminidase, a typical lysosomal marker enzyme, sediments into the gradient and shows a very broad distribution with a modal density of 1.19 g/ml. Lactate dehydrogenase, a putative cytosol marker, shows a major soluble component, but significant amounts of activity are recovered deeper in the gradient. 5'-Nucleotidase, a marker for plasma-membrane fragments, shows a sharp peak in the density region 1.13–1.15 g/ml.

Catalase shows a bimodal distribution with a major soluble component and a slightly smaller particulate component in the density region 1.22–1.24 g/ml. This is the densest of the organelles and, in a single experiment, the distribution of D-amino acid oxidase was similar to that of particulate catalase. No uricase was detected in human liver homogenates.

Neutral α -glucosidase shows a small soluble component and a major sedimentable peak with a modal density of 1.20 g/ml, which is skewed towards lighter densities. Protein shows a major soluble component with a broad distribution throughout the gradient. A peak of protein at density 1.20 g/ml corresponds to the highest activity of the mitochondrial, lysosomal and endoplasmic reticulum marker enzymes.

Fig. 2 compares the distribution of 5'-nucleotidase with that of three other putative plasma-membrane marker enzymes. Alkaline phosphatase shows an almost identical distribution with that of 5'-nucleotidase. Leucine 2-naphthylamidase shows a similar distribution with a peak at a modal density of 1.13 g/ml. However, there is more soluble activity

Table 1. *Enzyme specific activities and percentage activity in postnuclear supernatant from liver-biopsy homogenates*
Enzyme activities ($\pm$ S.E.) are expressed as units (μ mol/min) with number of specimens assayed shown between parentheses.

Enzyme	EC no.	$10^{-3} \times$ Specific activity (munits/mg of protein)	Percentage activity in postnuclear supernatant
Alkaline phosphatase	3.1.3.1	1.36 ± 0.16 (39)	78 ± 4 (7)
5'-Nucleotidase	3.1.3.5	13.2 ± 2.3 (40)	72 ± 3 (6)
Leucine 2-naphthylamidase	3.4.11.2	1.27 ± 0.16 (40)	75 ± 6 (5)
γ -Glutamyltransferase	2.3.2.2	5.08 ± 0.70 (38)	73 ± 4 (7)
Neutral α -glucosidase	3.2.1.20	0.601 ± 0.061 (36)	88 ± 4 (8)
Catalase	1.11.1.6	228 ± 16 (8)	88 ± 2 (10)
Malate dehydrogenase	1.1.1.37	3060 ± 280 (16)	87 ± 2 (11)
Acid phosphatase	3.1.3.2	12.9 ± 0.81 (41)	82 ± 4 (7)
β -Glucuronidase	3.2.1.31	4.92 ± 0.40 (37)	82 ± 4 (5)
<i>N</i> -Acetyl- β -glucosaminidase	3.2.1.30	2.03 ± 0.28 (36)	86 ± 2 (8)
Cathepsin C	3.4.14.1	1.83 ± 0.31 (5)	78 ± 3 (4)
β -Glucosidase	3.2.1.21	0.831 ± 0.17 (38)	88 ± 4 (5)
β -Galactosidase	3.2.1.23	0.420 ± 0.12 (36)	87 ± 3 (4)
Acid diesterase	3.1.4.1	0.395 ± 0.081 (35)	86 ± 3 (5)
Acid α -glucosidase	3.2.1.20	0.312 ± 0.021 (37)	81 ± 4 (5)
α -Mannosidase	3.2.1.24	0.245 ± 0.102 (40)	81 ± 3 (4)
α -Galactosidase	3.2.1.33	0.062 ± 0.011 (35)	73 ± 4 (4)
Lactate dehydrogenase	1.1.1.27	345 ± 31 (5)	87 ± 4 (5)
Protein (mg)	—	2.45 ± 0.58 (10)	86 ± 5 (10)

Table 2. *Latent and sedimentable acid hydrolase activities in postnuclear supernatant from liver biopsy homogenates*
Results are means $\pm$ S.E.M. with numbers of experiments between parentheses.

	Latent activity (%)	Sedimentable activity (%)
<i>N</i> -Acetyl- β -glucosaminidase	64.7 ± 1.7 (23)	56.1 ± 2.8 (9)
Acid phosphatase	55.4 ± 3.9 (5)	50.6 ± 2.4 (9)
β -Glucuronidase	—	71.3 ± 2.5 (4)
β -Galactosidase	—	51.4 ± 4.4 (4)

and the activity is found throughout the gradient. γ -Glutamyl transpeptidase shows a distinct peak in the density region 1.14–1.16 g/ml which is denser than that for the 5'-nucleotidase. In each individual experiment the separation of the particulate components is clearly seen, with the γ -glutamyl transpeptidase sedimenting significantly further into the gradients.

Figs. 3 and 4 show the distribution of 12 acid hydrolases in the density gradient fractions. β -Glucosidase shows a predominantly soluble localization, but the other enzymes show significant particulate components. Comparison of the various distributions suggests a bimodal distribution with peaks at densities of 1.15 and 1.20 g/ml. This is most clearly seen for cathepsin C and ribonuclease. Some enzymes are predominantly recovered in the lower-density peak; these include acid phosphodiesterase, cathepsin D, acid α -glucosidase, acid phosphatase and β -glucuronidase. *N*-Acetyl- β -glucosaminidase, β -galactosidase and α -galactosidase have major peaks in the 1.20 g/ml density region of the gradient.

Fig. 5 shows the result of a single experiment in

which the differential localization of the multiple forms of acid phosphatase and *N*-acetyl- β -glucosaminidase were investigated. The fluoride-resistant acid phosphatase shows a lower median density than the fluoride-inhibitable activity. Note that the latter activity shows a distinct peak at a density of 1.19 g/ml. Hexosaminidases A and B show different distributions, with the latter enzyme showing a distinct component at density region of 1.14 g/ml. The distribution of total *N*-acetyl- β -glucosaminidase is rather atypical, with most of the activity in the density region 1.14 g/ml. These experiments, however, do emphasize the lysosomal heterogeneity.

Fig. 6 shows the elution pattern of *N*-acetyl- β -glucosaminidase isoenzymes from a crude lysosomal fraction and from a cytosol fraction isolated from human liver biopsy homogenates subjected to ion-exchange chromatography. The lysosomal fraction shows a larger proportion of B component, whereas the cytosol fraction shows a much larger proportion of component A. There is also a more distinct intermediate component. Quantitative analysis by using planimetry indicates that the lysosomal fraction con-

Fig. 1. Isopycnic centrifugation of 6000g-min supernatant from human liver biopsy homogenate showing frequency-density histograms for principal organelle marker enzymes

Frequency (mean $\pm$ s.d.) is defined as the fraction of total recovered activity in the gradient fraction divided by the density span covered. The hatched area represents, over an arbitrary abscissa interval, the enzyme remaining in the sample layer. The percentages ($\pm$ s.d.) of activity recovered, with numbers of specimens analysed in parentheses, are: malate dehydrogenase, 91 ± 15 (7); catalase, 102 ± 14 (10); *N*-acetyl- β -glucosaminidase, 78 ± 7 (4); neutral α -glucosidase, 111 ± 5 (3); lactate dehydrogenase, 101 ± 11 (3); protein, 88 ± 12 (5); 5'-nucleotidase, 108 ± 8 (4); D-amino acid oxidase, 87 (1).

tains $52 \pm 8\%$ (S.E., $n = 4$) and cytosol contains $85 \pm 6\%$ (S.E., $n = 3$) of component A.

Fig. 7 shows how the resolving power of the analytical fractionation procedure can be enhanced by the addition of membrane perturbants to the homogenizing medium. Digitonin causes a striking increase in the equilibrium density of the plasma-

membrane enzymes 5'-nucleotidase and γ -glutamyl transpeptidase. Neutral α -glucosidase shows a decrease in density and the *N*-acetyl- β -glucosaminidase is recovered almost entirely in the soluble fractions, indicating selective, but complete disruption of the lysosomes. Preliminary experiments indicate a partial resistance of the low-density

Fig. 2. Isopycnic centrifugation of 6000g-min supernatant from human liver biopsy homogenate showing frequency-density histograms of plasma-membrane enzymes

Details are as in Fig. 1. The percentage recoveries and numbers of specimens are: 5'-nucleotidase, 108 ± 8 (4); alkaline phosphatase, 88 ± 15 (6); γ -glutamyl transpeptidase, 104 ± 8 (8); leucine 2-naphthylamidase, 104 ± 15 (5).

lysosomes to digitonin disruption. Mitochondria and peroxisomes are unaffected by digitonin treatment. Addition of pyrophosphate to the incubation medium has a highly specific effect on the distribution of particulate α -glucosidase, which shows a marked decrease in density. Other organelles are relatively unaffected.

Discussion

The experiments in this paper describe a single-step centrifugation procedure which permits the resolution of the principal organelles from human liver biopsy specimens. Routinely approx. 2mg of protein, but as little as 0.2mg, corresponding to 1mg of tissue, can be satisfactorily processed in this manner. Thus new approaches are possible to the study of physiological and pathological problems when only small quantities of tissue can be obtained. The present study describes the properties of the

major organelles in normal liver from adult subjects and forms the basis against which similar studies with pathological tissue can be compared. The properties of the various organelles will be considered under their appropriate subheadings and will be compared with those in rat liver, the usual reference tissue in this type of study.

Cytosol

Lactate dehydrogenase is the marker enzyme for this compartment and, although mainly recovered with the soluble fractions, significant amounts are found throughout the gradient. This probably represents enzyme adsorbed to several subcellular organelles. In rat liver (de Duve *et al.*, 1962) up to 50% of the activity may be recovered with particulate components. There is at least a partial localization to the rougher endoplasmic reticulum, and this activity can be readily desorbed with 50mM-KCl (Tilleray & Peters, 1976).

Fig. 3. Isopycnic centrifugation of 6000g-min supernatant from human liver biopsy homogenate showing frequency-density histograms for six acid hydrolases

Details are as in Fig. 1. The percentage recoveries and numbers of specimens are: *N*-acetyl- β -glucosaminidase, 78 ± 7 (4); β -glucuronidase, 97 ± 6 (4); acid phosphatase, 83 ± 14 (8); α -galactosidase, 92 ± 5 (3); β -galactosidase, 107 ± 11 (3); β -glucosidase, 89 ± 14 (4).

Plasma membrane

5'-Nucleotidase, especially when assayed under the highly specific conditions used in the present study, is a satisfactory marker for this organelle (Solyom & Trams, 1972). The equilibrium density

of 1.13 g/ml is significantly less than that of rat liver, 1.15 g/ml (Peters & Shio, 1976; Tilleray & Peters, 1976) when tissue extracts are centrifuged in a similar manner. Alkaline phosphatase has an identical distribution with that of 5'-nucleotidase, but leucine 2-naphthylamidase, although having

Fig. 4. Isopycnic centrifugation of 6000g-min supernatant from human liver biopsy homogenate showing frequency-density histograms for a further six acid hydrolases

Details are as in Fig. 1. The percentage recoveries and numbers of specimens are: α -mannosidase, 105 ± 5 (3); acid α -glucosidase, 81 ± 8 (4); acid diesterase, 85 ± 5 (3); cathepsin D, 81 ± 7 (3); cathepsin C, 95 ± 3 (3); ribonuclease, 104 ± 6 (3).

a significant plasma-membrane component, has additional activity deeper in the gradient as well as a greater proportion of cytosolic activity. Experiments on rat liver have suggested a partial localization to microsomal fraction and lysosomes (Mahadevan & Tappel, 1967), although recent studies on the microsomal fraction indicate a localization to the plasma-membrane components rather than to the endoplasmic reticulum (Tilleray & Peters, 1976).

γ -Glutamyltransferase appears to have a distinctive subcellular localization. It is associated with membranes having a distinctly higher equilibrium density than the typical plasma-membrane marker. Both membranes complex with digitonin and yield fragments with almost identical equilibrium densities. Studies on rat liver indicate that γ -glutamyl transpeptidase is localized in particularly high concentrations to the biliary-canalicular cells (Wootton *et al.*, 1977). Examination of the rat microsomal fraction by analytical density-gradient centrifugation (Tilleray

& Peters, 1976) indicates that γ -glutamyltransferase has an identical localization with 5'-nucleotidase and other plasma-membrane marker enzymes.

Thus as well as exhibiting cellular heterogeneity the liver shows subcellular heterogeneity. Preparative fractionation procedures (Wisher & Evans, 1975) have isolated three distinct fractions from different regions of the plasma membrane. It is possible that in human liver the differential distribution of 5'-nucleotidase and γ -glutamyltransferase reflects different subcomponents of the plasma membrane.

Lysosomes

In the present study marked lysosomal heterogeneity has been clearly demonstrated; latent and sedimentable acid hydrolases clearly establishes the biochemical criteria for lysosomes in human liver. Some heterogeneity has been demonstrated, particularly when ribonuclease and cathepsin D are compared, in rat liver (de Duve *et al.*, 1955), but

Fig. 5. Isopycnic centrifugation of 6000g-min supernatant from human liver biopsy homogenate showing frequency-density histograms for acid hydrolase isoenzymes

Details are as in Fig. 1. The percentage recoveries are: total acid phosphatase, 87; fluoride-resistant acid phosphatase, 79; fluoride-sensitive acid phosphatase, 83; *N*-acetyl- β -glucosaminidase, 93; *N*-acetyl- β -glucosaminidase isoenzyme A, 84; *N*-acetyl- β -glucosaminidase isoenzyme B, 79.

this is far more striking in human liver. This heterogeneity may reflect the different cell types, e.g. Kupffer and parenchymal cells, in the liver. The lower density of these organelles presumably reflects lipid accumulation, possibly as lipofuscin (Essner &

Novikoff, 1960; Goldfischer *et al.*, 1966; Porta & Hartroft, 1969).

The selective inhibition of lysosomal acid phosphatase by fluoride is similar to that found with rat liver (Shibko & Tappel, 1963). The demonstration of

Fig. 6. Chromatograms of *N*-acetyl- β -glucosaminidase isoenzymes of lysosomal fraction (a) and of cytosol fraction (b). The peaks correspond, in order of elution, from right to left, to hexosaminidase B, intermediate component and hexosaminidase A. For further details see Ellis *et al.* (1974).

differential concentration of hexosaminidase A to the soluble fractions agrees with the studies of Robinson & Stirling (1968) on human spleen. The analysis of the density-gradient fractions suggests a differential localization of these isoenzymes to separate populations of lysosomes.

It is possible that for certain hydrolases significant extralysosomal localizations will have complicated the distribution patterns. Acid phosphatase, particularly when assayed with chromogenic substrates (Beaufay, 1972), and β -glucuronidase (Fishman *et al.*, 1969) have significant microsomal localizations. Similarly, α -mannosidase has a cytosolic component (Marsh & Gourlay, 1971) as well as a lysosomal localization.

β -Glucosidase is almost exclusively localized to cytosol. Although earlier studies (Lloyd, 1969) suggested that this enzyme, in rat liver, was localized to the outer aspect of lysosomes, more recent experiments (Burton & Lloyd, 1976) indicate that this result was an artifact due to inhibition of β -glucosidase by Triton X-100. In human liver, whether assayed in the presence or absence of Triton X-100, this enzyme was largely recovered in the soluble fraction. Studies with rat kidney (Price & Dance, 1967) and rabbit aortic cells (Peters *et al.*, 1972) have reached similar conclusions.

The use of 0.12 mM-digitonin in the selective disruption of lysosomes is valuable in increasing the resolving power of the fractionation procedure. Clear separation of mitochondrial, endoplasmic-reticulum and lysosomal components can be achieved.

Endoplasmic reticulum

Neutral α -glucosidase (Lejeune *et al.*, 1963; Peters & de Duve, 1974; Peters, 1976; Tilleray & Peters, 1976; Bloomfield *et al.*, 1977) has been used as the marker enzyme for this organelle. Because of the extremely limited amounts of tissue available, more classical marker enzymes (glucose 6-phosphatase, NADPH-cytochrome *c* reductase) could not be satisfactorily assayed in the human liver density-gradient fractions.

The neutral α -glucosidase appears to be distributed throughout the endoplasmic reticulum. The marked decrease in median density after homogenization in pyrophosphate is due to the stripping of ribosomes from the rough endoplasmic reticulum with subsequent density decrease (Beaufay *et al.*, 1974; Tilleray & Peters, 1976). The decrease in density after digitonin treatment is surprising, but similar results were obtained with rat liver microsomal fraction (Tilleray & Peters, 1976; Mitropoulos *et al.*, 1978).

Fig. 7. Isopycnic centrifugation of 6000g-min supernatant from human liver biopsies homogenized in iso-osmotic sucrose containing either 0.12 mM-digitonin (a) or 15 mM-sodium pyrophosphate (b) (continuous lines)

Averaged control data from Fig. 1 are shown by broken lines. Details are as in Fig. 1. The percentage recoveries (digitonin-treated; pyrophosphate-treated) are: 5'-nucleotidase (93; 92); γ -glutamyl transpeptidase (105; 85); neutral α -glucosidase (109; 109); N-acetyl- β -glucosaminidase (100; 70).

and presumably reflects the detergent action of the digitonin, as some loss of RNA and protein from the microsomal fraction occurs after digitonin treatment.

Mitochondria

Malate dehydrogenase was used as the marker enzyme for this organelle, which has a slightly higher

equilibrium density (1.20) in human as compared with rat (1.19) liver (Baudhuin *et al.*, 1964; Peters & Shio, 1976). Other marker enzymes for mitochondrial subcomponents had identical distributions.

If in human, as in rat, liver (Baudhuin *et al.*, 1964; Schnaitman *et al.*, 1967) monoamine oxidase is a selective marker for the outer mitochondrial membrane, the coincidental distribution of succinate dehydrogenase (inner membrane) and monoamine oxidase in the density-gradient fractions indicates that the mitochondria are isolated intact. Similarly, the symmetry of the particulate malate dehydrogenase indicates that negligible hydrostatic damage to these organelles has been sustained (Wattiaux, 1974). Assays of density-gradient fractions for glutamate dehydrogenase, which, unlike malate dehydrogenase, is localized exclusively to the mitochondrial matrix reveals less than 10% of total recovered activity in the soluble fractions. This is further evidence of the maintenance of mitochondrial integrity during the fractionation procedure.

Peroxisomes

The demonstration of particulate catalase in association with D-amino acid oxidase firmly establishes the biochemical criteria (de Duve & Baudhuin, 1966) for peroxisomes in human liver. The equilibrium density of 1.23 g/ml is slightly less than that reported for rat liver, 1.25 (Leighton *et al.*, 1968; Peters & Shio, 1976), but the trace amounts of soluble D-amino acid oxidase indicate that excellent integrity of this rather fragile organelle has been maintained. The technique of analytical subcellular fractionation procedures has been successfully applied by our group to a variety of congenital and acquired diseases of human tissues (Peters, 1977) and the present study extends this approach to liver. Reports of the investigation of hepatic disease by these techniques (Segal *et al.*, 1976; Seymour *et al.*, 1977; Peters & Seymour, 1978; Jenkins & Peters, 1978) have already indicated the potential value of this approach to the investigation of hepatic pathology.

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