Digitonin perfusion of rat liver

A new approach in the study of intra-acinar and intracellular compartmentation in the liver

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(Received 14 September 1984/Accepted 18 October 1984)

Perfusing a rat liver with digitonin in the concentration range 2–20 mg/ml results in complete decolorization of the organ within 45–250 s. Decolorization progresses with time in the direction of flow, and it is therefore possible, by collecting the eluate, to obtain material from specific intracellular compartments of hepatocytes in different zones in the microcirculatory unit of the liver. The results demonstrate that cytoplasmic marker enzymes from periportal or perivenous hepatocytes can be collected with as little contamination from the other compartment as is obtained in microdissection studies. Furthermore, a fraction enriched in mitochondrial marker enzymes can be achieved with only 10–20% contamination by cytoplasmic material.

The digitonin fractionation of isolated liver cells was originally developed as a means of studying the intracellular compartmentation of metabolites (Zuurendonk & Tager, 1974). Applying a modification of this procedure (Janski & Cornell, 1980) to primary cultures of rat hepatocytes, we noted that the release of marker enzymes (LDH and citrate synthase) was much slower than with suspensions of freshly isolated hepatocytes. This unexpected observation led us to investigate the time course of release of such enzymes from the intact rat liver perfused with digitonin. Perfusion with high concentrations of digitonin had a remarkable effect: the liver became completely decolorized in less than 1 min of perfusion, with no apparent change in macroscopic structure of the organ. The decolorization did not occur uniformly over the length of the sinusoid, but rather progressed with time in the direction of the flow. It therefore seemed possible that the technique might be adapted to liberation of enzymes and metabolites

from specific zones within the microcirculatory unit of the liver (Rappaport, 1980). To our knowledge, digitonin perfusion of the intact organ has been reported in only one other study (Postius & Platt, 1981); in that instance, the concentration of digitonin was very low (0.1–0.3 mg/ml), and decolorization of the liver was not reported.

We present here data on the performance of the digitonin-perfusion technique in terms of the release patterns for a number of marker enzymes for cava→porta compared with porta→cava perfusions. The data indicate that cytoplasmic enzymes of periportal or perivenous origin can be separated as efficiently as by microdissection (for review, see Jungermann & Katz, 1982). Furthermore, comparison of release patterns for mitochondrial and cytosolic marker enzymes suggest that it may also be possible to study intracellular compartmentation. Thus the digitonin-perfused liver may prove to be a useful tool in the study of both intra-acinar and intracellular compartmentation of the intact perfused organ. A preliminary report on these data has been given (Quistorff *et al.*, 1984).

The term 'intra-acinar compartmentation' is used in the present paper to designate the differences found in enzyme activities and other parameters between periportal and perivenous hepatocytes of the liver. The term 'metabolic

Abbreviations used: LDH, lactate dehydrogenase (EC 1.1.1.27); ALAT, alanine aminotransferase (EC 2.6.1.2); GK, glucokinase (EC 2.7.1.2); GIDH, glutamate dehydrogenase (EC 1.4.1.3); HK, hexokinase (EC 2.7.1.1); PK, pyruvate kinase (EC 2.7.1.40).

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zation' was suggested to describe the possible functional implications of this unequal distribution (Sasse *et al.*, 1975).

Materials and methods

Reagents

Enzymes were obtained from Boehringer. Digitonin was from ICN, Merck or Sigma. Digitonin was rendered water-soluble by the following procedure (N. W. Cornell & A. M. Janski, unpublished work). Briefly, the procedure involves treating the commercial digitonin with boiling methanol, followed by precipitation and washing of the digitonin with diethyl ether. Then it was dried overnight in air. For the present experiments, the air-dried material was dissolved in water and freeze-dried for 24 h. All other reagents were of analytical grade.

Liver perfusion

Fed female Wistar rats (weight 180–210 g) were used. The liver was perfused *in situ* at 37°C, in the direction either vena porta→vena cava sup. or vena cava sup.→vena porta. The perfusion medium used was Krebs–Henseleit (1932) bicarbonate, equilibrated with O₂/CO₂ (19:1). Digitonin (2–20 mg/ml) was included in the perfusion medium after 10–20 min of porta→cava pre-perfusion in the absence of digitonin. A change in flow direction required a 2–3 s stop in perfusion. Flow through the liver was 20–25 ml/min delivered by a peristaltic pump (LKB Multi Perpex 2125). During the digitonin perfusion, the outflow from the liver was collected in fractions (1.5–2.5 ml) and the time interval for each fraction was noted. To each fraction was added 1 ml of cold homogenization buffer, to give final concentrations of 25 mM-glycylglycine, pH 7.4, 150 mM-KCl, 5 mM-MgSO₄, 5 mM-Na₂EDTA, 10 mM-mercaptoethanol and 0.2% defatted bovine serum albumin. Eluate fractions were kept at –20°C until used for enzyme-activity measurements, which were carried out on supernatants (13000g for 10 min) of these fractions. All activity measurements were carried out within 4 days; less than 10% loss of activity was observed during storage. After 180–210 s of digitonin perfusion, the experiment was completed by perfusing the liver for 20 s with cold perfusion medium without digitonin, after which a biopsy was taken and homogenized in a Potter–Elvehjem homogenizer, followed by ultrasonication (50 W for 10 s) in the homogenization buffer listed above. The supernatant (13000g for 10 min) was used for enzyme-activity measurement in the biopsy.

Measurement of digitonin

Digitonin was measured by two different methods. (1) After centrifugation (13000g for 10 min), digitonin in the eluate sample was precipitated as the cholesterol complex in ethanol/acetone/water (47:33:20, by vol.) (Sperry, 1963). The precipitate was washed with acetone and the complex dissociated by heating in dimethyl sulphoxide. Cholesterol was extracted by heptane and determined enzymically (Boehringer test-kit no. 290319). (2) Samples were evaporated to dryness, and excess of [¹⁴C]cholesterol in methanol was added. After thorough mixing, water was added (final concn. 3.3%, v/v). The digitonin–cholesterol complex was allowed to precipitate overnight and the [¹⁴C]cholesterol remaining in the supernatant was measured. The two methods gave concordant results.

Enzyme-activity measurements

All enzymes were measured at 37°C, except hexokinase and glucokinase, which was measured at 27°C. The activities of LDH, ALAT, PK, myokinase, citrate synthase and GIDH were measured by standard techniques as described in Bergmeyer (1974), except that LDH was measured with 5 mM-pyruvate (Janski & Cornell, 1980), and PK in the presence of 3.6 mM-fructose 1,6-bisphosphate. HK and GK were measured as described by DiPietro & Weinhouse (1960) in an assay mixture containing 100 mM-Tris/HCl, pH 7.4, 6 mM-MgCl₂, 5 mM-ATP, 0.5 mM-NADP⁺, 1 mM-dithiothreitol, 0.5 mM- or 100 mM-D-glucose and 0.02 unit of glucose-6-phosphate dehydrogenase. 6-Phosphogluconate dehydrogenase was omitted, since 0.02 unit did not increase the measured activity. NADPH production was read against a blank without ATP. GK and HK activities were calculated by assuming *K_m* values for glucose of 5.4 mM (Storer & Cornish-Bowden, 1976) and 0.04 mM (Ureta *et al.*, 1981) respectively and assuming ordinary Michaelis–Menten kinetics. Amyloglucosidase was measured essentially as described by Lundquist (1971), using 100 µl of eluate incubated for 60 min with 100 µl of 100 mM-sodium acetate buffer, pH 5.0, containing 100 mM-glycogen and 25 µl of 5.5% (v/v) Triton X-100. Incubations were terminated by heating to 100°C for 5 min, then glucose was measured against a blank which had been incubated similarly but with heat-inactivated eluate. It should be noted that amyloglucosidase was measured on an uncentrifuged sample of the homogenate of the liver biopsy. Glucose was measured enzymically as described by Lowry & Passonneau (1972).

The effect of digitonin on the various enzyme activities was checked on a high-speed supernatant

(31000g for 30 min) of a 10% (w/v) liver homogenate obtained by sonication (50 W for 10 s) in homogenization buffer. Assays were carried out with or without the addition of digitonin. Only GK and amyloglucosidase activities were affected. Amyloglucosidase was inhibited by 30% by the highest digitonin concentration used, 20 mg/ml, and GK was inhibited by 7%, 25%, 32% and 63% by digitonin at concentrations of 2, 6, 10 and 20 mg/ml respectively. The results presented are not corrected for this effect of digitonin.

Results and discussion

Visual appearance of the liver during digitonin perfusion

The most surprising observations in these experiments were the rapid and complete decolorization of the liver as a result of the digitonin perfusion and the marked difference between the decolorization pattern in porta→cava and cava→porta perfusions. We have followed the decolorization process photographically as shown in Fig. 1 for porta→cava and cava→porta perfusions. Fig. 1(a) shows the liver before perfusion with digitonin and Fig. 1(b) after 3 min of porta→cava perfusion with 6 mg of digitonin/ml. The white liver is the end result with both porta→cava and cava→porta perfusion. However, the intermediate decolorization process is vastly different for the two directions of perfusion. For porta→cava perfusion the decolorization process leaves regularly scattered pigmented dots on the surface of the liver, as shown in Figs. 1(d) (20 s) and 1(f) (45 s). These dots coincide with the most pigmented areas on the surface of the liver at the start of the perfusion. Since the perivenous area appears most pigmented on the surface of the haemoglobin-free perfused liver, the observed dots are likely to represent the perivenous part of the lobule (Ji *et al.*, 1980). Thus, with porta→cava perfusion, decolorization is first apparent in the periportal part of the microcirculatory unit, spreading with time along the sinusoid in the direction of flow.

Figs. 1(c) and 1(e) show similar pictures from a retrograde (cava→porta)-perfused liver. It is obvious that the reversed flow initiates decolorization at a different location in the microcirculatory unit, creating a different intermediate pattern. Instead of dark spots on a white background, one sees a negative of the previous picture. In other words, in the cava→porta perfused livers the decolorization starts in the perivenous area, leaving the confluencing periportal areas unaffected.

The time course of the colour release in the perfusate follows the visual decolorization of the liver. When most intensely coloured, the eluate is dark brown. A spectrum of the eluate shows only

one well-defined peak, namely in the Soret band at about 410 nm, making haem a likely candidate as the absorbing pigment. We have followed the elution of the 410 nm absorption for porta→cava and cava→porta perfusions and find no difference in the elution profile. Varying the digitonin concentration, however, has a decisive effect on the time course of the pigment elution. Peak absorbance was reached after 10, 50, 60 and 90 s of perfusion with digitonin concentrations of 20, 10, 6 and 2 mg/ml respectively. For 20 and 10 mg/ml the elution is virtually completed after 60 and 120 s respectively, but was still in progress after 180 s for 6 and 2 mg/ml.

Time course of uptake of digitonin by the liver

With 6 mg of digitonin/ml in the perfusate, the visually observed decolorization of the entire microcirculatory unit lasts about 1 min. The progression of the effect of digitonin along the sinusoid is thus only about 4 μ m/s (assuming an average length of the sinusoid of 250 μ m (Rappaport, 1980), in spite of a linear flow rate of the order of 300 μ m/s (Goresky, 1963), suggesting an actual titration of cholesterol along the sinusoid. We have therefore monitored the digitonin concentration in the eluate, as shown in Fig. 2. During the first 50 s of perfusion, more than 85% of the digitonin was taken up by the liver. The concentration in the eluate then increased sharply, to about 90% of the inlet concentration. In perfusions with digitonin concentrations of 10, 6 and 4 mg/ml (results not shown), the steep increase in eluate digitonin concentration was observed at approx. 25, 55 and 70 s respectively ($n = 3, 2$ and 2), corresponding to a total digitonin load of 104, 110 and 93 mg respectively. No difference was observed between porta→cava and cava→porta perfusions.

The permeabilizing effect of digitonin on cell membranes is usually attributed to the formation of a 1:1 cholesterol-digitonin complex. Since a constant digitonin load is required before significant amounts of digitonin appear in the eluate, the results above thus suggest that perfusion with digitonin is in fact a titration of cell-membrane cholesterol along the sinusoid of the liver. This conclusion is further supported by the agreement between the calculated cholesterol content of the liver and that found by direct measurement. Thus 100 mg of digitonin binds approx. 31 mg of cholesterol (M_r 1229 and 386 respectively), corresponding to about 3 mg of cholesterol/g wet wt. A value of 2.8 mg was reported, with about 70% confined to the plasma membrane (de Duve, 1971).

Release of enzymes from different intra-acinar compartments

It is known from microdissection studies that a

Fig. 1. *Decolorization of digitonin-perfused liver*

The liver was perfused with Krebs-Henseleit bicarbonate buffer, equilibrated with O_2/CO_2 (19:1), with either antegrade (p→c) or retrograde (c→p) direction of flow, at a rate of 23 ml/min. After 7–10 min of pre-perfusion, digitonin (6 mg/ml) was added to the perfusate. Photos of the liver were taken every 5 s after the addition of digitonin to the perfusate. Panels (a) and (b) show the liver at the start and after complete decolorization (3 min of perfusion) respectively. Panels (d) and (c) show the liver after 20 s of antegrade and retrograde perfusion respectively, and panels (f) and (e) similarly after 45 s of perfusion. Magnification approx. $\times 2$.

number of enzymes have an unequal distribution along the sinusoid (for review see Jungermann & Katz, 1982). Such heterogeneous distribution would be expected to show in the elution profile of the enzyme activity, i.e. if an enzyme is uniformly distributed across the microcirculatory unit its pattern of release into the perfusion fluid should not be affected by the direction of flow. However,

for enzyme activities that are zoned, release will be faster with porta→cava perfusion for enzymes with a preferential periportal location, and vice versa.

The following series of experiments was designed to study the intra-acinar resolving power of the digitonin perfusion technique. Table 1 shows the results of experiments with 6 mg of digiton-

Table 1. *Elution of cytoplasmic enzymes during digitonin perfusion*

The liver was perfused as indicated in the legend of Fig. 1 with either antegrade (P) or retrograde (C) direction of flow. Eluate fractions representing 5 s intervals were collected, and the integrals given in the Table were obtained by manual integration of the elution profiles. Results (% of total activities) are given as means ($\pm$ s.d. in parentheses) for *n* experiments.

Enzyme	<i>n</i>	Sampling interval (s)	Enzyme eluted (%)						
			0-30	30-45	45-60	60-90	90-120	120-150	150-180
LDH	P	5	20.9 (4.8)	27.6 (4.0)	22.1 (3.5)	22.6 (2.2)	5.9 (1.6)	0.7 (0.6)	0.0
	C	4	7.6 (2.2)	14.3 (2.4)	16.9 (3.3)	30.4 (3.2)	17.7 (3.8)	8.9 (2.9)	2.9 (2.2)
ALAT	P	3	21.3 (3.1)	35.3 (1.8)	20.0 (3.7)	16.4 (2.2)	5.2 (3.5)	1.3 (1.5)	0.3 (2.8)
	C	4	3.1 (1.8)	10.6 (5.1)	20.9 (3.9)	36.3 (4.8)	17.3 (4.2)	8.2 (1.4)	3.6 (1.0)
HK	P	5	22.3 (8.4)	25.0 (2.4)	22.8 (4.4)	19.2 (3.3)	5.6 (1.3)	3.2 (1.5)	1.6 (1.3)
	C	4	4.7 (2.2)	15.5 (2.4)	21.0 (4.8)	33.0 (2.8)	14.0 (2.9)	7.2 (1.1)	4.5 (0.9)
GK	P	5	19.2 (6.2)	22.4 (5.1)	16.4 (1.5)	20.6 (5.2)	13.9 (4.4)	5.0 (2.2)	1.6 (1.5)
	C	4	39.0 (22.8)	24.4 (11.0)	11.5 (7.9)	14.6 (11.4)	6.8 (4.7)	2.8 (1.9)	1.1 (1.4)
PK	P	3	15.8 (4.0)	21.5 (0.9)	21.1 (1.5)	26.7 (3.3)	9.8 (0.7)	4.1 (0.8)	1.0 (0.9)
	C	3	14.7 (2.8)	21.6 (2.4)	19.6 (3.6)	24.9 (2.9)	11.4 (2.2)	5.1 (2.0)	2.5 (1.8)

Fig. 2. *Time course of the uptake of digitonin by the liver*
The liver was perfused in the porta→cava direction as indicated in the legend of Fig. 1. Digitonin (6mg/ml) was added to the perfusion medium at zero time and eluate fraction at 6 s time intervals were collected.

in/ml for five cytoplasmic enzymes. Experiments with 2mg of digitonin/ml gave similar, although more prolonged, release patterns for porta→cava

and cava→porta perfusions. Values for the intervals 0-15 s and 15-30 s were combined in Table 1, since for all enzymes less than 5% of total activity was collected in the 0-15 s interval. For LDH, HK and ALAT it is obvious that the porta→cava perfusion elutes the activity faster than does reverse perfusion, whereas GK is eluted faster with cava→porta perfusion. Microdissection studies on the zonation of these enzymes indicate a preferential periportal location for HK, LDH and ALAT (Fischer *et al.*, 1982; Shank *et al.*, 1959; Welsh, 1972), and a perivenous location for GK (Fischer *et al.*, 1982; Trus *et al.*, 1980), predicting the elution patterns observed here. According to microdissection studies by Guder & Schmidt (1976), PK activity is zoned, with a periportal/perivenous activity ratio of about 0.5. Our studies, however, do not show significant zonation of PK (see Table 1). Even though the specific activity of HK in the liver is reported to be significantly higher in non-parenchymal cells (Dileepan *et al.*, 1979; Sapag-Hagar *et al.*, 1969), about 95% of the total activity is in the hepatocytes because of the much larger mass of these cells (90% of liver volume; Weibel *et al.*, 1969). This agrees well with the present results, which show an elution profile for HK temporally similar to that for LDH and ALAT.

There is still no clear understanding of the

physiological role of the zonation of enzyme activities in the liver parenchyma, in spite of evidence for the zonation of some metabolic pathways (Häussinger, 1983; Jungermann & Katz, 1982; Quistorff & Chance, 1984). It is likely that the observed gradients of enzyme activity play a role. It is, however, possible that it is not the absolute magnitude of the periportal→perivenous gradient of the individual enzyme that is important, but rather a mutual interdependence between enzyme gradients. The ability of the digitonin-perfusion method to differentiate between periportal and perivenous cells may therefore be more appropriately illustrated by giving the ratio of two enzyme activities for forward and reverse perfusion.

Fig. 3 is such a data presentation, which allows direct quantitative comparison with microdissection studies. Effectively, the histogram com-

pares the periportal→perivenous activity gradient of the cytoplasmic enzyme ALAT, PK, GK and HK with the corresponding gradient of LDH, chosen arbitrarily as the reference enzyme. Suppose, for the interval 15–30s, that the eluate of a porta→cava and a cava→porta perfusion represented only the cytoplasmic compartments of periportal and perivenous cells, respectively. The histogram would then indicate that the periportal→perivenous gradient of, for example, ALAT was 2.8-fold steeper than the LDH gradient. Similarly, for the same interval, the histogram indicates the HK gradient to be equal to that for LDH, whereas the PK and GK gradients are opposite, 0.4- and 0.2-fold the LDH gradient respectively.

The right-hand part of Fig. 3 shows similar data in the literature, compiled from microdissection studies. The fact that these data show virtually the same pattern of gradients as those obtained with

Fig. 3. Zonation in liver of ALAT, GK and HK relative to LDH as evaluated by digitonin perfusion. The data of Table 1 were used to generate this histogram. The enzyme activities for the different intervals indicated on the histogram were obtained by manual integration of the elution profiles. The activity gradients were calculated as:

$$\frac{(\text{porta} \rightarrow \text{cava integral X}) / (\text{cava} \rightarrow \text{porta integral X})}{(\text{porta} \rightarrow \text{cava integral LDH}) / (\text{cava} \rightarrow \text{porta integral LDH})}$$

for values ≥ 1 , and as the reciprocal values $\times -1$ for values < 1 . The column labelled 'Lit. values' shows activity gradients calculated from Fischer *et al.* (1982), Guder & Schmidt (1976), Trus *et al.* (1980) and Welsh (1972).

the digitonin perfusion for the 15–30s and 30–45s intervals supports the concept that highly zonated release of the cellular cytoplasmic material does actually occur during the digitonin perfusion and that a rather uniform perfusion of all micro-circulatory units takes place under these conditions.

Release of enzymes from different intracellular compartments

We have evaluated the ability of the digitonin-perfusion technique to resolve different intracellular compartments by following the release pattern of a number of intracellular marker enzymes. Fig. 4 shows examples of elution profiles of such marker enzymes at four different digitonin concentrations in porta→cava perfusions. With all digitonin concentrations there is a clear separation of cytoplasmic and mitochondrial marker enzymes. Although LDH and ALAT start to leak out very early, the elutions of GIDH and citrate synthase start only when LDH elution is at its peak, and, whereas the liver is almost completely emptied of LDH after 3 min of perfusion, a large part of the GIDH activity still remains in the liver at this point. The highest digitonin concentration seems to give the most complete separation of LDH and GIDH. Myokinase and amyloglucosidase (Figs. 4b and 4c) show a rather broad elution profile that has a maximum between those of LDH and GIDH and is similar to the colour-release profile. This release sequence for enzymes of the cytoplasm (LDH), the mitochondrial inter-

membrane space (myokinase) and the mitochondrial matrix (GIDH) compares well with that found with freshly isolated hepatocytes (Janski & Cornell, 1980). The dependence of the elution profile on the digitonin concentration is clearly demonstrated in Fig. 4: e.g., for GIDH activity, the maximum in the eluate is reached at 70, 90 and 120s of perfusion with digitonin concentrations of 20, 10 and 2mg/ml (Figs. 4a, 4c and 4d respectively).

Table 2 quantifies the separation obtained for cytosolic and mitochondrial marker enzymes. As judged from the LDH and GIDH activities, a good separation of mitochondrial and cytosolic compartments may be obtained. For example, with digitonin at a concentration of 10mg/ml, 57% of the total LDH activity was eluted within the first 50s of perfusion, contaminated with only 2–3% of the total GIDH activity. For the mitochondrial fraction, taking again the 10mg/ml group as an example, it may be observed that the fraction eluted at 100–180s contains 60% of the total GIDH activity with 9% LDH contamination.

It is important to note, however, that these data will be biased both by the preferential periportal enzyme release in porta→cava perfusions and by the periportal zonation of LDH (Shank *et al.*, 1959). Thus total cytoplasmic contamination in the mitochondrial fraction is not fairly judged by the LDH contamination alone. Instead, cytoplasmic contamination from periportal and perivenous hepatocytes should be considered separately, by assaying correspondingly zonated cytoplasmic en-

Fig. 4. Elution profiles for intracellular marker enzymes

The liver was perfused as indicated in the legend of Fig. 1 with antegrade flow. The digitonin concentration was varied as indicated on the Figure. Enzyme activities were measured on eluate fractions representing 5s intervals as described in the Materials and methods section. ○, LDH; □, ALAT; △, GIDH; ▽, myokinase; ▼, citrate synthase; ■, amyloglucosidase.

Table 2. *Activities of lactate dehydrogenase and glutamate dehydrogenase in perfusion medium and in liver after 3 min of perfusion with digitonin*

The liver was perfused as indicated in the legend of Fig. 1 with antegrade flow. Eluate fractions representing 5 s intervals were collected and the integrals given in the Table were obtained by manual integration of the elution profiles. At the end of each experiment a biopsy was taken allowing calculation of the total enzyme activity, in units/liver: for LDH 4649 ± 989 and for GIDH 2594 ± 652 (means $\pm$ S.D., $n = 9$).

Enzyme	[Digitonin] (mg/ml)	Enzyme activity in perfusate collected in interval (% of total)				Activity in biopsy (% of total)
		0-30s	30-50s	50-100s	100-180s	
LDH	2	14	17	32	32	5
	6	20	33	43	5	0
	10	28	29	34	9	0
	20	60	12	16	12	0
GIDH	2	0	0.4	9	29	61
	6	0.5	5	25	46	26
	10	0.3	2	34	60	4
	20	4	11	46	29	10

Table 3. *Separation of cytoplasmic and mitochondrial compartments in digitonin-perfused liver*

The liver was perfused as indicated in the legend of Fig. 1 with antegrade or retrograde flow. Eluate fractions representing 5 s intervals were collected and the integrals given in the Table were obtained by manual integration of the elution profiles. At the end of each experiment a biopsy was taken, allowing calculation of the total enzyme activity. The values are % of total liver activities.

Perfusion direction	Enzyme	Digitonin	Enzyme activity in perfusate collected in interval (% of total)			
			2 mg/ml		6 mg/ml	
			0-50s	100-180s	0-50s	100-180s
Porta→cava	LDH		31	32	53	5.1
	GK		13	36	47	15
	GIDH		0.4	29	5.5	46
Cava→porta	LDH		9.0	46	25	24
	GK		20	35	61	8.0
	GIDH		0	36	8.0	48

zymes, as shown in Table 3. Here the 0-50s fraction may be termed 'cytosolic fraction', and the 100-180s fraction may be termed 'mitochondrial fraction'. Taking the porta→cava perfusion with 6 mg of digitonin/ml as an example, the 'mitochondrial fraction' contains 46% of the GIDH activity with 5% LDH but 15% GK activity. However, by isolating the 120-180s fraction (results not shown), these values may be improved to 42%, 1% and 7% respectively.

Tables 2 and 3 show that the digitonin concentration plays an important role in the separation obtained between the mitochondrial and cytosolic compartments. At 2 mg/ml the 'mitochondrial fraction' contains almost equal contributions from both compartments. On the other hand, the 'cytoplasmic fraction' (porta→cava perfusion, Table 3), although containing less than 1% GIDH, is also

low in GK compared with LDH, suggesting that this fraction mainly represents cytoplasm from periportal cells. Upon cava→porta perfusion the reverse pattern is seen (Table 3).

Conclusion

Intracellular and intra-acinar compartmentation have long been challenging problems in the study of the liver. The digitonin-perfused liver described here adds a new and powerful method for the study of these questions. By manipulating parameters such as time, direction of flow, digitonin concentration and, perhaps, flow rate and temperature, the method seems to allow rapid and specific separation of different intra-acinar and intracellular compartments of the intact organ. Furthermore, with a high concentration of digitonin, e.g. 20 mg/ml, it is possible to empty the

liver very fast, and the technique therefore also offers an alternative to the conventional means of obtaining rapid samples of cellular material.

Lissi Immerdal and Marte Nord are thanked for skilful assistance. This research was supported by the Danish Natural Science Research Council, J.Nr. 11-3878.

References

- Bergmeyer, H. U. (1974) *Methoden der enzymatischen Analyse*, 3rd edn., Verlag Chemie, Weinheim
- de Duve, C. (1971) *J. Cell Biol.* **50**, 20D-55D
- Dileepan, K. N., Wagle, S. R., Hofmann, F. & Decker, K. (1979) *Life Sci.* **24**, 89-96
- DiPietro, D. L. & Weinhouse, S. (1960) *J. Biol. Chem.* **235**, 2542-2545
- Fischer, W., Ick, M. & Katz, N. R. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 375-380
- Goresky, C. A. (1963) *Am. J. Physiol.* **204**, 626-640
- Guder, W. G. & Schmidt, U. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1793-1800
- Häussinger, D. (1983) *Eur. J. Biochem.* **133**, 269-275
- Janski, A. M. & Cornell, N. W. (1980) *Biochem. J.* **186**, 423-429
- Ji, S., Lemasters, J. J. & Thurman, R. (1980) *FEBS Lett.* **113**, 37-41
- Jungermann, K. & Katz, N. (1982) *Hepatology* **2**, 385-395
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33-66
- Lowry, O. H. & Passonneau, J. V. (1972) *A Flexible System of Enzymatic Analysis*, pp. 174-175, Academic Press, New York
- Lundquist, I. (1971) *Enzyme* **12**, 647-657
- Postius, S. & Platt, D. (1981) *Z. Naturforsch. C* **36**, 880-883
- Quistorff, B. & Chance, B. (1984) in *Regulation of Hepatic Metabolism: Intra- and Inter-cellular Compartmentation* (Thurman, R. G., Kaufman, F. & Jungermann, K., eds.), Plenum Publishing Corp., New York, in the press
- Quistorff, B., Grunnet, N. & Cornell, N. W. (1984) *Acta Pharmacol. Toxicol.* **55**, Suppl. 1, P12
- Rappaport, A. M. (1980) *Int. Rev. Physiol.* **21**, 1-63
- Sapag-Hagar, M., Marco, R. & Sols, A. (1969) *FEBS Lett.* **3**, 68-71
- Sasse, D., Katz, N. & Jungermann, K. (1975) *FEBS Lett.* **57**, 83-88
- Shank, R. E., Morrison, G., Cheng, C. H., Karl, I. & Schwartz, R. (1959) *J. Histochem. Cytochem.* **7**, 237-239
- Sperry, W. M. (1963) *Lipid Res.* **4**, 221-225
- Storer, A. C. & Cornish-Bowden, A. (1976) *Biochem. J.* **159**, 7-14
- Trus, M., Zawalich, K., Gaynor, D. & Matschinsky, F. (1980) *J. Histochem. Cytochem.* **28**, 579-581
- Ureta, T., Radojkovic, J. & Zepeda, S. (1981) *Comp. Biochem. Physiol. B* **70**, 225-236
- Weibel, E. R., Stäubli, W., Gnägi, H. R. & Hess, F. A. (1969) *J. Cell Biol.* **42**, 68-91
- Welsh, F. A. (1972) *J. Histochem. Cytochem.* **20**, 107-111
- Zuurendonk, P. F. & Tager, J. M. (1974) *Biochim. Biophys. Acta* **333**, 393-399