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THE EFFECT OF CYTOCHALASIN B ON THE RELEASE OF LYSOSOMAL ENZYMES AND INTRA-LYSOSOMALLY-STORED POLYVINYLPYRROLIDONE IN THE ISOLATED PERFUSED RAT LIVER

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Abstract—The uptake and subcellular distribution *in vivo* and release *in vitro* of ^{125}I-polyvinylpyrrolidone (^{125}I-PVP) has been studied in rat liver.

Using the techniques of sucrose-density-gradient sedimentation and isopycnic centrifugation, it was shown that 4–15 days after injection of ^{125}I-PVP the majority of the radioactivity was still in a high-molecular-weight form and associated with the lysosomes of the liver, the size and density properties of which were not significantly altered. Practically all of the ^{125}I-PVP found in the lysosomes was free in the lumen and not associated with the lysosomal membrane, its intra-lysosomal distribution being much more similar to that of β-galactosidase than arylsulphatase or β-*N*-acetylglucosaminidase.

In an isolated recirculating perfusion system the liver released all the enzymes studied (arylsulphatase, β-galactosidase and lactate dehydrogenase) and, when previously loaded, ^{125}I-PVP was also released into the perfusate. The magnitude of the release was always in the order ^{125}I-PVP > β-galactosidase > arylsulphatase. Preloading the lysosomes *in vivo* appeared to bring about an increase in the mean levels of release of all the enzymes, but the wide spread of data made this statistically significant only for lactate dehydrogenase. The microfilament poison cytochalasin B increased the release of arylsulphatase and β-galactosidase, but not lactate dehydrogenase, in the perfused non-loaded livers, but failed to augment the release of any of the enzymes or ^{125}I-PVP in the loaded livers.

After perfusion, subcellular fractionation of the liver showed that the lysosomes had become enlarged and more fragile, especially so with those rich in ^{125}I-PVP and β-galactosidase rather than those rich in arylsulphatase and β-*N*-acetylglucosaminidase.

The role of the cytoskeleton in secretory processes has been intensively investigated using a variety of pharmacological agents. By the use of poisons such as cytochalasin B, microfilaments have been shown to be involved in many secretory processes including those exocytotic events leading to the release of lysosomal enzymes [1].

Lysosomes, as a result of both heterophagy and autophagy, accumulate a variety of indigestible or only partly digested macromolecules, a phenomenon that is accentuated in the lysosomal storage diseases. Although the role of microfilaments in the exocytosis of lysosomal enzymes is well documented, especially in polymorphonuclear leukocytes and macrophages [1], little is known about their role in the release of intralysosomal, non-enzymic macromolecules.

Polyvinylpyrrolidone is a synthetic macromolecule that has been used extensively both clinically and experimentally [2] and its use as an additive to various pharmaceutical preparations has led to the description of a PVP-storage disease, arising from the accumulation of the indigestible macromolecule inside vacuoles [3]. In a variety of cell types PVP is taken up by fluid phase pinocytosis and has been shown to be or assumed to be accumulated inside lysosomes [4–6].

In the present study we have investigated the uptake of PVP by rat liver *in vivo* and its subcellular distribution. Its subsequent release by the livers in isolated perfusion has been studied, as well as its effect on the release of lysosomal enzymes and the role of microfilaments in these processes, using the microfilament poison cytochalasin B.

MATERIALS AND METHODS

Materials

P-Nitrocatechol sulphate, p-nitrophenyl-β-D-galactopyranoside, bovine serum albumin fraction V powder, cytochalasin B, polyvinylpyrrolidone (average molecular weight 40,000) were purchased from Sigma (London) Chemical Co. Ltd., Poole, Dorset, U.K. Leibovitz-L15 medium was purchased from Flow Laboratories, Irvine, Scotland. ^{125}I-polyvinylpyrrolidone injection (specific activity 20–60 μCi/mg, average molecular weight 30,000–40,000) was purchased from the Radiochemical Centre, Amersham, Bucks., U.K. All the other chemicals came from BDH, Poole, Dorset, U.K. The experimental animals used were Sprague–Dawley male rats weighing between 400 and 500 g. They were maintained on a 'Spratts Rodent Breeding' diet, food and water being allowed *ad libitum*.

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Methods

Enzyme assays. Arylsulphatase A + B was assayed by the method of Dodgson *et al.* [7]. β-Galactosidase and β-*N*-acetylglucosaminidase were assayed by the method of Barrett and Heath [8]. Lactate dehydrogenase was assayed by the method of Plummer and Wilkinson [9]. Cytochrome-c-oxidase was assayed by a modification of the method of Cooperstein and Lazarow [10] similar to that of Appelmans *et al.* [11]. Protein was measured by the method of Lowry *et al.* [12].

Injection solution. Those animals receiving ^{125}I-polyvinylpyrrolidone (PVP) were injected through the tail vein with 100 mg/100 g body weight of PVP solution (250 mg/ml in saline) together with 65 μCi ^{125}I-PVP.

Subcellular distribution studies. After various periods of time the rats were killed by dislocation of the cervical vertebrae. The livers were then perfused *in situ* with 0.25 M sucrose to remove the blood and were then minced and homogenised in 0.25 M sucrose using three up-and-down strokes of a Potter–Elvejheim homogeniser. This step and all subsequent centrifugal steps were carried out at 4°. The homogenate was diluted with sucrose (0.25 M) to a final concentration of 1 g wet weight of liver in 10 ml (10% w/v), and was spun at 500 *g* for 10 min to give a supernatant (cytoplasmic fraction) and a pellet (cell debris fraction). A mitochondrial–lysosomal pellet was obtained by spinning the cytoplasmic fraction at 30,000 *g* for 25 min. The pellet was washed once in 26.7% (w/v) sucrose and the final pellet obtained was resuspended in the sucrose (26.7% w/v). The cytosol fraction was obtained from the supernatant by spinning the cytoplasmic fraction at 75,000 *g* for 80 min. For the sedimentation gradient centrifugation 5 ml of the cytoplasmic fraction were layered on top of a continuous sucrose gradient generated from 18 ml of 40% (w/v) and 18.5 ml of 8.5% (w/v) sucrose on top of a dense sucrose shelf (3.5 ml, 82% w/v). The sedimentation gradients were centrifuged at 1350 *g* for 120 min. For the isopycnic gradient centrifugation 3.5 ml of the mitochondrial–lysosomal preparation were layered on top of a continuous sucrose gradient generated from 25 ml of 82% (w/v) and 25.5 ml of 26.7% (w/v) sucrose solutions. The isopycnic gradients were centrifuged at 75,000 *g* for 210 min. Fractions from both gradients were collected from the bottom by piercing the bottom of the tubes.

In order to investigate the intralysosomal distribution of the lysosomal enzymes and label, a mitochondrial–lysosomal fraction (in 0.25 M sucrose) was sonicated and centrifuged at 75,000 *g* for 80 min to provide a pellet (membrane fraction) and a supernatant (luminal fraction).

Liver perfusion. The operation procedure followed for the removal of the livers was that described by Michelakakis [13]. The livers were perfused (inflow via the hepatic portal vein and outflow via the vena cava) in a cabinet maintained at 37° using recycling, oxygenated Leibovitz L-15 medium supplemented with 5% bovine serum albumin and glutamine (0.06%) and containing streptomycin and penicillin (both 100 units/ml). Cytochalasin B dissolved in dimethyl sulphoxide (100 μg/ml, 1% v/v DMSO) was added 90 min after the beginning of the perfusion. The control livers were treated with DMSO (1% v/v) only. At 30 min intervals samples of the perfusate were assayed for lysosomal enzymes, lactate dehydrogenase and radioactivity. At the end of the perfusion the liver was homogenised in sucrose (0.25 M) and the homogenate was assayed for various enzymes. In some experiments the homogenate was subcellularly fractionated as above.

Gel chromatography. Samples of the injection solutions, high-speed supernatants of sonicated mitochondrial-lysosomal fractions and perfusates were eluted through columns (1.5 × 80 cm) of Sephadex G100 with 10 mM Tris–HCl buffer pH 7.4 at 4°.

RESULTS

The total liver uptake of ^{125}I-PVP varied between 26 and 38% of the injected dose and there was no significant variation between 4, 8 and 15 days. The accumulation of PVP inside the liver caused an increase in the tissue levels of arylsulphatase A + B and β-galactosidase but had no effect on the lactate dehydrogenase levels (Table 1).

The profiles of the distribution of ^{125}I-PVP in sedimentation gradients 4, 8 and 15 days after injection were similar and closely resembled the distribution of lysosomal enzymes (Fig. 1). However, at all three time intervals a higher percentage of the recovered radioactivity than of lysosomal enzymes was associated with the top fractions of the sedimentation gradients. The same applied for the cytosol fractions obtained from loaded livers (Table 2). When the profiles of the distribution of lysosomal enzymes in sedimentation gradients (Fig. 1) and in the cell debris and cytosol fractions (Table 2) from ^{125}I-PVP preloaded livers were compared to those obtained from non-preloaded ones it was shown that the presence

Table 1. The effect of administration 4 days previously of ^{125}I-polyvinylpyrrolidone (65 μCi and 1 g/kg) on the activities of various enzymes in rat liver

	Enzyme activity (mean ± S.D.) (N)	
	Controls	Four days after PVP
Arylsulphatase	7840 ± 1180 (9)	11,400 ± 1430 (5)
β-Galactosidase	2125 ± 263 (4)	4160 ± 1040 (4)
Lactate dehydrogenase	550 ± 107 (6)	565 ± 79 (4)

Units = μg product/g liver/hour.

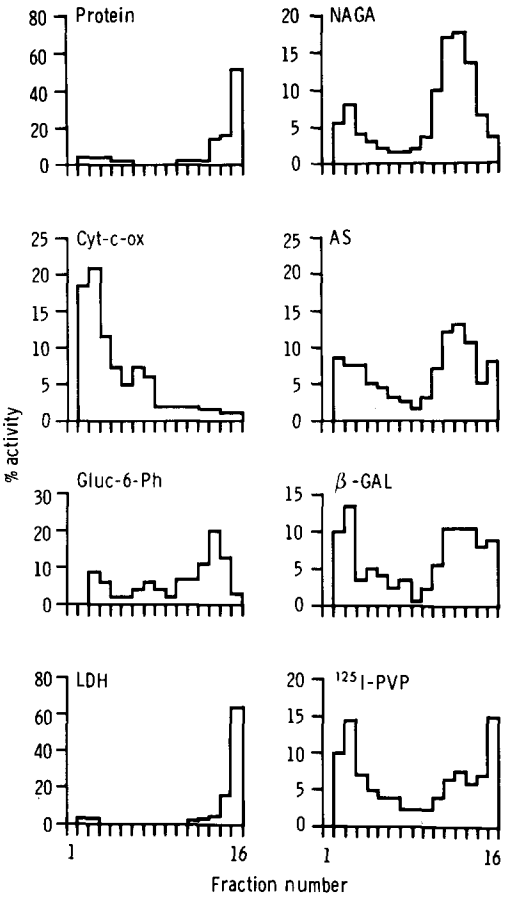

Fig. 1. The distribution profiles of protein, cytochrome oxidase (cyt-c-ox), glucose-6-phosphase (gluc-6-ph), lactate dehydrogenase (LDH), β-*N*-acetylglucosaminidase (NAGA), arylsulphatase (AS), β-galactosidase (β-GAL) and ^{125}I-polyvinylpyrrolidone (^{125}I-PVP) on an 8.5–40% (w/v) sucrose sedimentation gradient from rat livers 4 days after the injection of ^{125}I-PVP (65 μCi, 1 g/kg). The profiles 8 and 15 days after ^{125}I-PVP administration were similar, as were those from livers of non-injected animals.

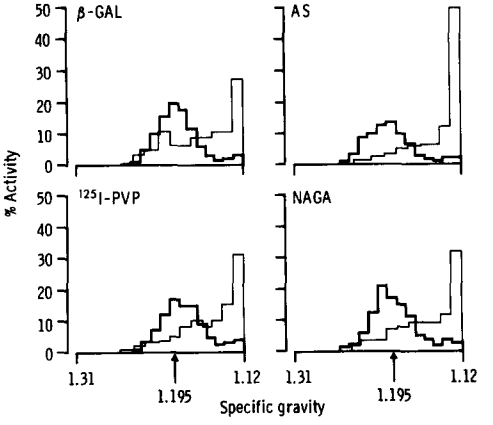

Fig. 2. The distribution profiles of β-galactosidase (β-GAL), arylsulphatase (AS), β-*N*-acetylglucosaminidase (NAGA) and ^{125}I-PVP on 26.7–82% (w/v) sucrose isopycnic gradients from rat livers 4 days after the injection of ^{125}I-PVP. The thick lines represent animals injected with ^{125}I-PVP alone and the thin lines represent animals injected with ^{125}I-PVP and Triton WR-1339 together.

of ^{125}I-PVP had not changed the sedimentation properties of lysosomes. The results obtained from the isopycnic-gradient centrifugation of resuspended mitochondrial–lysosomal pellets obtained from rat livers loaded with ^{125}I-PVP 4 days earlier is shown in Fig. 2. The profile of the distribution of ^{125}I-PVP coincided with those of the lysosomal enzymes. It appeared however that from 4 to 8 days, a shift of ^{125}I label and of the lysosomal enzymes β-galactosidase and β-*N*-acetyl-glucosaminidase to lower buoyant densities occurred. So, at 4 days the peak of ^{125}I-PVP and the three lysosomal enzymes equilibrated at a density of 1.195. At 8 days the peaks of ^{125}I-PVP, β-galactosidase and β-*N*-acetyl-glucosaminidase had shifted to less dense fractions equilibrating at a density of 1.188 while arylsulphatase remained at 1.195. When Triton WR-1339 was injected simultaneously with ^{125}I-PVP 4 days previously, it was found that the densities of both lysosomal enzymes and ^{125}I-PVP were decreased, leading to flotation to the top of the gradient (Fig. 2).

Gel-chromatography studies of the high-speed supernatant of a lysed mitochondrial–lysosomal pellet preparation showed that the accumulated label was of macromolecular nature similar to the high-molecular-weight component of the injected dose

Table 2. The activities of lysosomal enzymes, lactate dehydrogenase, the amount of protein and radioactivity expressed as the percentage of the recovered homogenate activities, found in the cell debris (A) and the cytosol (B) fractions obtained from livers loaded for 4 and 8 days with ^{125}I-PVP compared to control livers

Time after PVP injection	Activity as (%) of homogenate					
	Four days		Eight days		Control	
Fraction	A	B	A	B	A	B
Arylsulphatase A + B	40.0	1.8	40.0	1.7	38.5	0.02
β-Galactosidase	38.0	1.8	38.0	1.7	29.0	0.03
β-*N*-Acetylglucosaminidase	45.0	0.7	46.0	0.7	33.0	0.02
Lactate dehydrogenase	17.6	75.0	14.0	80.0	18.5	75.0
^{125}I-PVP	39.0	10.0	34.0	9.2	—	—
Protein	41.0	35.0	38.0	30.0	32.0	30.0

Each value is a single determination.

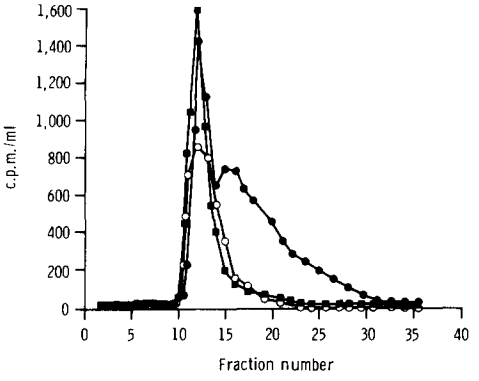

Fig. 3. Sephadex G-100 elution profiles of the ^{125}I-PVP injection solution (●——●), the high-speed supernatant of a lysed mitochondrial-lysosomal fraction (■——■) and the 6 hr perfusate without added cytochalasin B (○——○) obtained from a rat liver injected with ^{125}I-PVP 4 days earlier.

(Fig. 3). 92% of the lysosomal ^{125}I-PVP was solubilised by sonication (i.e. not membrane bound) compared with 8% for *N*-acetylglucosaminidase, 40% for arylsulphatase and 73% for β-galactosidase (Table 3).

Whether loaded with ^{125}I-PVP or not, the perfused livers lost lysosomal enzymes, lactate dehydrogenase and, in the case of loaded livers, radioactivity into the perfusate (Figs. 4 and 5). Gel chromatography of a sample of the perfusate showed that the released radioactivity was still in the form of macromolecular

Table 3. Percent activity of lysosomal enzymes and radioactivity associated with the high-speed supernatant of a sonicated mitochondrial–lysosomal pellet from non-perfused livers 4 days after the injection of ^{125}I-polyvinylpyrrolidone

	(%) Activity	
	Non-loaded	^{125}I-PVP
Arylsulphatase	58	40
β-Galactosidase	70	73
N-Acetylglucosaminidase	6	8
Radioactivity	—	92

PVP (Fig. 3). Loading the liver lysosomes with PVP appeared to increase the mean release of the enzymes 2–3 times, but because of the wide spread of values the difference was only significant for lactate dehydrogenase (Table 4). Although the spread of values was large, the extent of net release was always in the order ^{125}I-PVP > β-galactosidase > arylsulphatase. In some experiments, most notably those using PVP-loaded livers, the release of lactate dehydrogenase increased markedly towards the end of the perfusion. The effect of adding cytochalasin B to the perfusate depended on whether the livers had been preloaded or not. In the non-loaded livers the addition of cytochalasin B brought about an increase in the release of both β-galactosidase and arylsulphatase in the medium while it had no effect on the release of lactate dehydrogenase. The observed increase in the release of the lysosomal enzymes was

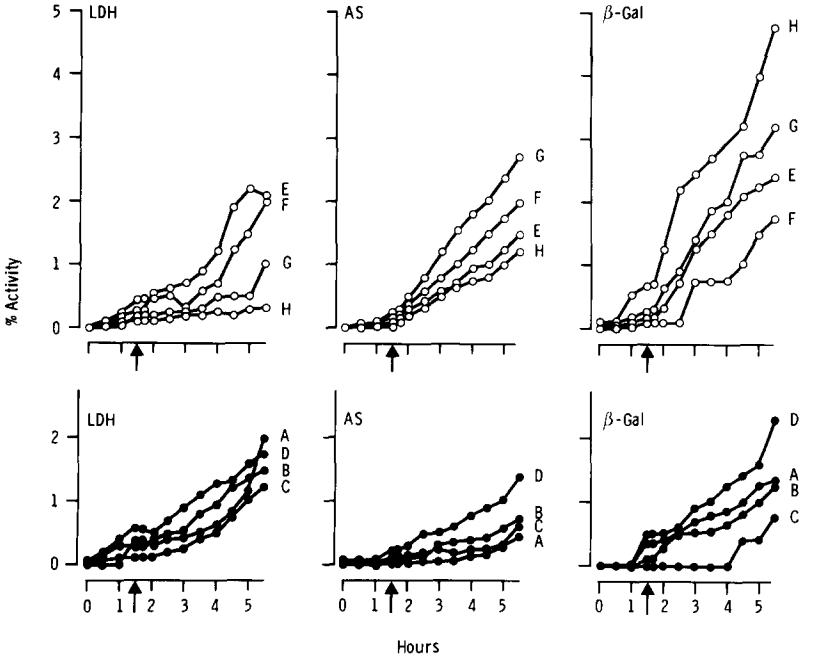

Fig. 4. The release of lactate dehydrogenase (LDH), arylsulphatase (AS) and β-galactosidase (β-gal) from controls (●——●) and cytochalasin B-treated (○——○) isolated perfused rat livers. The arrows represent the time point at which cytochalasin B or DMSO were added. The letters correspond to different experiments.

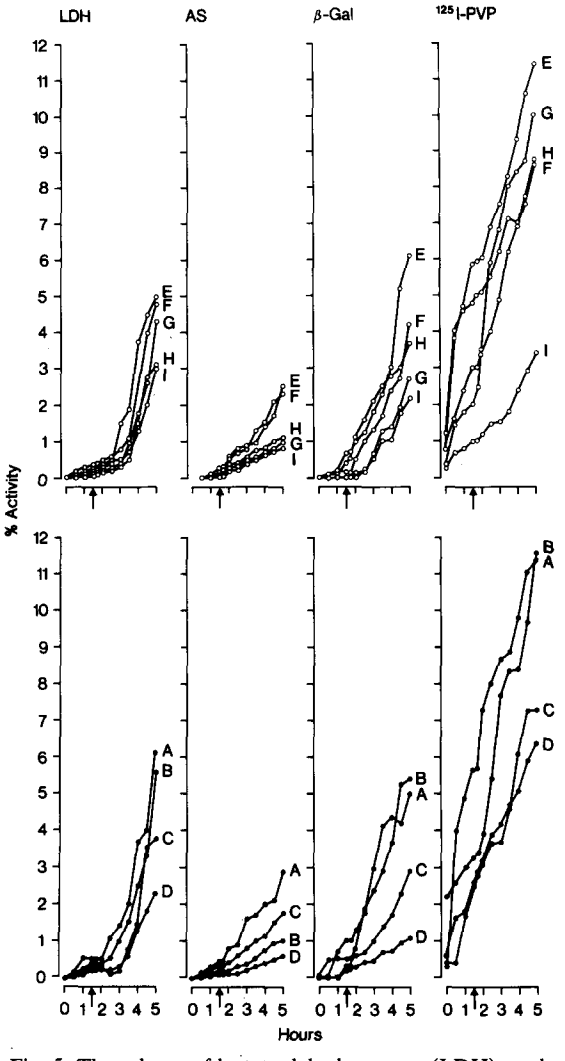

Fig. 5. The release of lactate dehydrogenase (LDH), arylsulphatase (AS), β-galactosidase (β-gal) and ^{125}I-polyvinylpyrrolidone (^{125}I-PVP) from control (●——●) and cytochalasin B-treated (○——○) isolated perfused rat livers that thad been loaded 4 days previously with ^{125}I-PVP. The arrows represent the time point at which cytochalasin B or DMSO was added. The letters correspond to different experiments.

statistically significant by Students *t*-test (Table 4). In livers loaded with ^{125}I-PVP, cytochalasin B did not augment the release of enzymes or radioactivity (Fig. 5, Table 4).

Preliminary experiments showed that the addition of the dimethyl sulphoxide resulted in the release of lower amounts of both arylsulphatase and β-galactosidase (0.7× and 0.5× respectively) into the perfusate after 5.5 hr perfusion as compared to those released in its absence. Dimethyl sulphoxide had no effect on lactate dehydrogenase release. Subcellular fractionations of loaded and non-loaded livers, either cytochalasin B-treated or controls, on sucrose sedimentation gradients were all very similar to each other, (Fig. 6) but showed considerable differences from non-perfused loaded or non-loaded livers (Fig. 1). In perfused livers there was an increase by 2–3 times in the lysosomal enzyme activities (especially β-galactosidase) and ^{125}I-PVP in the top two fractions of the sedimentation gradients which coincide with lactate dehydrogenase. After perfusion the activities of the lysosomal enzymes in the cell debris fraction approximately doubled while the cytosol (high-speed supernatant) activity increased about 10-fold. Perfusion hardly altered the ^{125}I-PVP activity associated with the cell debris fraction but increased that in the cytosol by 5–10 fold.

DISCUSSION

Polyvinylpyrrolidone appears to be taken up into a variety of cells types including rat yolk sac [4], macrophages [5] and cultured hepatocytes and Kupffer cells [6] by fluid phase endocytosis. The same also applies for rat liver uptake *in vivo* [14]. In the present study large amounts (26–38%) of PVP were taken up by the liver. This is somewhat more than that found by Munniksma *et al.* [14] but they injected several orders of magnitude less PVP than used here. The PVP that was taken up into the mitochondrial–lysosome fraction was confined to the higher molecular weights (Fig. 3). This is surprising as Munniksma *et al.* [14] found that the liver endocytosed the lower molecular weights at a greater rate than the higher ones.

The PVP-induced increase in liver lysosomal enzyme activities are in good agreement with the

Table 4. The enzyme activities and ^{125}I-PVP found in the perfusate at the end of the perfusion period in the presence or absence of cytochalasin B expressed as the percentages of total recovered activity and the statistical significance of their difference, obtained by Student's *t*-test. The livers were either not loaded or loaded with ^{125}I-PVP 4 days previously

	(%) Activity in perfusate		
	Controls (DMSO)	Cytochalasin B-treated	
Non-loaded	Mean (Range, N)	Mean (Range, N)	P Values
Arylsulphatase	0.84 (0.4–1.4, 4)	1.9 (1.3–2.7, 4)	$P < 0.025$
β-Galactosidase	1.6 (0.8–2.3, 4)	3.0 (1.8–5.0, 4)	$P < 0.05$
Lactate dehydrogenase	1.7 (1.2–2.0, 4)	1.8 (1.0–2.0, 4)	NS
PVP-loaded			
Arysulphatase	1.5 (0.6–2.9, 4)	1.5 (0.8–2.5, 5)	NS
β-Galactosidase	3.6 (1.1–5.4, 4)	3.8 (2.2–6.1, 5)	NS
Lactate dehydrogenase	4.5 (2.3–6.1, 4)	4.0 (3.0–5.0, 5)	NS
^{125}I-PVP	8.4 (6.4–11.6, 4)	9.2 (3.4–11.4, 5)	NS

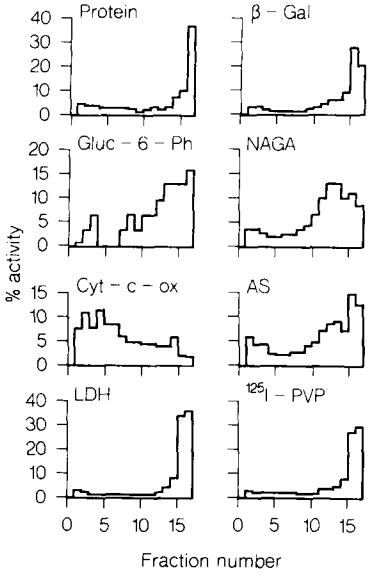

Fig. 6. The distribution of protein, glucose-6-phosphatase (Gluc-6-Ph), cytochrome-c-oxidase (cyt-c-ox), lactate dehydrogenase (LDH), β-galactosidase (β-Gal), β-*N*-acetylglucosaminidase (NAGA), arysulphatase (AS) and ^{125}I-polyvinylpyrrolidone (^{125}I-PVP) on a 8.5–40% sucrose sedimentation gradient from a rat liver that had been perfused for 6 hr in the absence of cytochalasin B. ^{125}I-PVP had been injected 4 days earlier. The profiles from perfused non-loaded livers and ^{125}I-PVP loaded livers perfused in the presence of cytochalasin B were very similar.

previous observation [15] that PVP accumulation in mouse liver was associated with increased acid phosphatase activity.

Although many workers have assumed that PVP accumulates inside the lysosomes of cells, there is little direct evidence to support this. Our sucrose sedimentation and isopycnic density gradient data (Figs. 1 and 2) show that in the liver most of the intracellular PVP is associated with the lysosomes, confirming the observation of Munniksma *et al.* [14] who used the cruder technique of differential fractionation. The excess radioactivity in the cytosol and at the top of the gradients compared with the lysosomal enzymes (Fig. 1, Table 2) could be associated with small micropinocytic vesicles devoid of lysosomal enzymes and/or it could be derived from broken vesicles which were not enriched in lysosomal enzymes. These latter vesicles could be pinocytic vesicles or old lysosomes which had lost the majority of their lysosomal enzymes. The presence of large quantities of PVP inside the liver lysosomes appeared to have little or no effect on the sedimentation characteristics of the lysosomes (size) and only a slight effect on the buoyant density at longer time intervals.

In isolated perfusion the liver lost a variety of enzymes and, when loaded, PVP was also lost. The mechanism by which the lysosomal enzymes were released into the perfusate (i.e. exocytosis or cell death) is difficult to establish as significant quantities of lactate dehydrogenase (used as a marker of cell integrity) were also released. The release of lactate dehydrogenase often increased markedly towards the end of the perfusion to reach levels in the perfusate higher than the lysosomal enzymes, but not ^{125}I-PVP. But in these cases the release of the lysosomal enzymes commenced before the release of lactate dehydrogenase. Addition of the microfilament poison cytochalasin B to the perfusate of non-loaded livers slightly but significantly elevated the net release of the lysosomal enzymes but not lactate dehydrogenase (Fig. 4, Table 4). Although cytochalasin B doubled the mean release of aryl sulphatase and β-galactosidase, the spread of values was so great that the difference was barely significant (Table 4). More obvious differences have been found in other cell types, most notably polymorphonuclear leukocytes and macrophages [16]. The difference between these and the present system may be due to lower susceptibility of liver cell, especially hepatocyte, microfilaments to disruption by cytochalasin B, the lower dependence of liver lysosomal enzyme secretion on microfilaments, or the intrinsic variability of the liver perfusion technique. In addition liver condition, as judged by lactate dehydrogenase release, deteriorated markedly after 6 hr perfusion preventing continuation of the experiments. Whereas in macrophages the maximum effect of cytochalasin B was observed after 72 h [16]. The active "Net release" of lysosomal enzymes into the perfusate is the sum of exocytosis and reendocytosis. In the present system at least part of the effect of cytochalasin B is certainly due to an inhibition of the latter process [17].

The different patterns of the release of the different lysosomal enzymes, which was also observed in the case of hepatocyte cultures [18], could be a reflection of differences in the intralysosomal localization of those enzymes. Such differences were indicated by the finding that β-galactosidase was associated mainly with the supernatant rather than with the pellet obtained from a lysed mitochondrial-lysosomal preparation, whereas β-*N*-acetylglucosaminidase was associated mainly with the pellet. Arylsulphatase was more-or-less equally distributed between the two. Hence during exocytosis, enzymes that were found mainly in the lumen of the lysosomes would be released extracellularly whereas the enzymes that were mainly membrane bound would remain attached to the lysosomal membrane, now incorporated in the plasma membrane and then be recycled back into the cell. In this respect it is interesting to note that PVP which was released to a much greater extent than either of the lysosomal enzymes was almost entirely confined to the lysosomal lumen.

Livers loaded with ^{125}I-PVP appeared to release more enzymes into the perfusate than non-loaded livers. The reason for this is not clear, but in any case it was not due to any alterations in binding of the lysosomal enzymes to the lysosomal membrane as PVP had no effect on this (Table 3).

The inability of cytochalasin B to further augment the release of lysosomal enzymes into the perfusate of livers loaded with PVP is interesting with respect to the fact that we have shown previously [18] that in ^{125}I-PVP loaded hepatocyte cultures cytochalasin B does slightly augment the release of lysosomal enzymes.

The presumably exocytotic secretion of intralysosomal ^{125}I-PVP and the elevated lysosomal enzyme secretion in the presence of ^{125}I-PVP appeared to be independent of microfilament function, as judged by the insensitivity of the process to cytochalasin B. The reason for this is unknown but may be due to altered physico-chemical properties of the lysosomal membrane caused by the accumulation of the non-digestible macromolecule.

The subcellular distribution studies showed that there was no difference in the sedimentation properties of the lysosomal marker enzymes obtained from loaded or non-loaded livers perfused in the absence or presence of cytochalasin B (Fig. 6). However compared with non-perfused livers the lysosomes appeared to have become enlarged (as shown by the increased lysosomal enzyme activity associated with the cell debris fraction) possibly through autophagy [19, 20]. Such enlargement would lead to increased fragility during homogenisation and increased activities of the lysosomal markers co-fractionating with lactate dehydrogenase. The large change in the apparent subcellular distribution of the lysosomal enzymes and PVP due to perfusion compared with the relatively small percentage release into the perfusate made it impossible to define a particular lysosomal population from which the exocytosed lysosomal contents came.

The perfusion-induced fragility of lysosomes containing β-galactosidase and ^{125}I-PVP (as shown by increased localisation at the top of the sucrose sedimentation gradients) appears to be much greater than that for lysosomes containing arylsulphatase or *N*-acetylglucosaminidase. Whether this is related to the other similarities between β-galactosidase and ^{125}I-PVP (i.e. higher rates of secretion into the perfusate and low binding to the lysosomal membrane) remains to be seen.

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