Rabbit β -Glucuronidase

SUBCELLULAR DISTRIBUTION AND IMMUNOCHEMICAL PROPERTIES

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1. The subcellular distribution of β -glucuronidase and other hydrolases in rabbit liver was investigated. β -Glucuronidase was found in both microsomal and lysosomal fractions. 2. Multiple forms of β -glucuronidase were present in extracts of microsomal and lysosomal fractions. All forms were common to both fractions. 3. A specific antiserum against β -glucuronidase was raised, and characterized by immunoprecipitation and affinity-chromatography procedures. 4. The immunological identity of the multiple forms in the pure β -glucuronidase preparation, and the immunological identity of the β -glucuronidase complement of lysosomal extracts with that of microsomal extracts, were demonstrated by means of the antiserum. The presence of inactive enzyme in various enzyme preparations was shown.

In view of the unusual subcellular distribution of β -glucuronidase in many tissues, as discussed in the preceding paper (Dean, 1974), the subcellular localization of β -glucuronidase and other hydrolases in rabbit liver is described in the present paper.

The production of a specific antiserum to β -glucuronidase allows for the first time an immunochemical investigation of the structural relationship between β -glucuronidase molecules in the lysosomal and microsomal fractions, and between the multiple forms described previously (Dean, 1974). The antiserum also makes available methods for the simplified isolation of β -glucuronidase, and one such method, with (anti- β -glucuronidase) IgG†-Sepharex, is discussed.

Some of this work has been briefly reported previously (Dean, 1973).

Materials and Methods

These were described by Dean (1974) with the following additions. New Zealand White rabbits (6–8 weeks old) were obtained from laboratory stock. Pooled normal pig blood was obtained from a local slaughterhouse. *p*-Nitrophenyl glycoside substrates for the assay of β -glucosidase, β -galactosidase and β -*N*-acetylglucosaminidase, were obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames KT2 7BH, Surrey, U.K. CNBr was from Koch–Light Laboratories Ltd., Colnbrook SL3 0BZ, Bucks., U.K., and chloramine-T from British Drug

Houses, Poole BH12 4NN, Dorset, U.K. Carrier-free $K^{125}I$ was bought from The Radiochemical Centre, Amersham, Bucks., U.K. Antisera to pig serum proteins and to pig immunoglobulin G were prepared by Dakopatts, Copenhagen, Denmark, and purchased from Mercia Diagnostics Ltd., Watford, Herts., U.K.

Enzyme assays

Glycosidase assays were performed as described for β -glucuronidase (Dean, 1974) in 100 mM-sodium acetate buffer with the appropriate *p*-nitrophenyl glycoside as substrate. pH values used were 5.6 for β -glucosidase and 4.0 for β -galactosidase. β -*N*-Acetylglucosaminidase was assayed at pH 4.0 in 0.1 M-sodium citrate buffer. The pH values used were found to be optimal in preliminary experiments. Triton X-100 (0.1%) was normally included.

Cathepsin D was assayed as described by Barrett (1970) with haemoglobin as substrate.

Short assays were performed in the presence of 0.25 M-sucrose, with and without 0.1% Triton X-100, to demonstrate latency of lysosomal enzymes. However, since little latency was detected (total activity in the presence of Triton X-100 was no more than 150% of that in its absence), sedimentability of activity was also checked. It may be that the conditions required for cell breakage in rabbit liver inevitably result in damage to lysosomes which increases their permeability to substrates.

Glucose 6-phosphate was assayed as described by de Duve *et al.* (1955).

Enzyme activities are expressed as μ mol of substrate transformed/min.

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† Abbreviation: IgG, immunoglobulin G.

Subcellular fractionations

In initial experiments, homogenization in 0.25M-sucrose-50mM-Tris-HCl, pH 7.4, was with the automatic homogenizer described by Dingle & Barrett (1969a), but fluorescence microscopy with Acridine Orange (Dingle & Barrett, 1969a) showed very incomplete cell breakage, even with many passes of the pestle. This homogenizer gives very satisfactory cell breakage in a few passes with rat liver, and the difference is perhaps due to the presence of a greater amount of connective tissue in rabbit liver.

Subsequently a Silverson homogenizer was used, and cell breakage assessed by Acridine Orange staining. Fair homogenization was obtained with six 15s bursts at top speed with the medium grid, at 4°C, but even so considerable amounts of activity were sedimented with nuclear-debris pellets.

Fractionation was as described by de Duve *et al.* (1955) at 4°C, except that the nuclear-debris fraction was not rehomogenized and re-centrifuged. An MSE 17 centrifuge was used for all except the last centrifugation, which was performed in an MSE Superspeed 65, with an 8 × 50ml angle rotor. Subcellular fractions were also viewed by fluorescence microscopy and the presence of lysosomes and mitochondria in the two lysosomal-mitochondria fractions observed. Few lysosomes were seen in the microsomal fraction.

In the preparation of microsomal and lysosomal fractions for analysis of multiple enzyme forms, the second centrifugation step was omitted, and thus the 'heavy mitochondrial' was combined with the 'light mitochondrial' fraction as a total 'lysosomal-mitochondrial' fraction.

Sedimentability was assessed by centrifugation in the homogenization medium for 1h at 100000g in an MSE Superspeed 65 centrifuge with an 8 × 50ml angle rotor at 4°C.

Sucrose density gradients from 20 to 65% (w/v) sucrose in 50mM-Tris-HCl buffer, pH 7.4, were run at 4°C in an MSE 65 ultracentrifuge, with a 3 × 23ml swing-out rotor, as indicated in the Results section.

Production of a specific antiserum to β -glucuronidase

By running β -glucuronidase from the sedimentation stage of the purification procedure (Dean, 1974), in gel diffusion against the polyspecific guinea-pig antisera (containing antibodies to β -glucuronidase and other proteins), with antigen and antibodies in alternate troughs on phosphate-buffered plates (as described by Weston, 1969), precipitin lines containing β -glucuronidase were obtained. After washing with phosphate-buffered saline [0.80% NaCl, 0.02% KCl, 0.02% KH_2PO_4 and 0.12% Na_2HPO_4 containing 1% (v/v) butanol] for 4 days, ten such lines were cut out, and emulsified in 0.5ml

of phosphate-buffered saline and 0.5ml of Freund's complete adjuvant by use of a small Ultra-Turrax homogenizer in 15s bursts.

A pig was injected intramuscularly with ten such lines on days 0, 15 and 29, and bled on day 39. The blood was allowed to clot for 24h and the serum was separated by centrifugation.

Purification of immunoglobulin G

Pig immunoglobulin G was purified as described by Starkey & Barrett (1973) by means of $(\text{NH}_4)_2\text{SO}_4$ precipitation and DEAE-cellulose chromatography. Purity was confirmed in immunoelectrophoresis against antisera to pig IgG and to pig serum proteins.

Immunoprecipitation

This was done at pH 7.8, in 50mM-Tris-HCl buffer (with 0.5% sodium deoxycholate for microsomal extracts) in 'Microfuge' tubes (final volumes up to 450 μl). After mixing, sera (or IgG) and antigens were incubated for 1h at 37°C and then overnight at 4°C. They were subsequently spun for 5min in a Microfuge (haematocrit centrifuge; Gelman-Hawskley, Lancing, Sussex, U.K.), before careful separation of supernatant and pellets, and resuspension of the pellets in the medium used initially.

Electrophoresis of antigens into agarose containing antibodies

These plates were run as described by Laurell (1972), at pH 8.6, sometimes in the presence of 0.1% octyl sulphate.

Preparation of IgG-Sepharose

Sephacrose 4B was activated by treatment for 10min at pH 11, with 50mg of CNBr/g of Sepharose (Axen & Ernback, 1971). Then the gel was rapidly washed with a large volume of 0.1M- K_2HPO_4 - KH_2PO_4 , pH 6.5, at 4°C, suspended in the same buffer and stirred overnight with IgG, at 4°C, as recommended by Cuatrecasas & Anfinsen (1971). The gel was washed again until the E_{280} of the washes approached zero, and was stirred with 0.2M-glycine overnight at 4°C to block any remaining activated sites. After further thorough washing the gel was packed in a chromatographic column for use.

Radioactive labelling of β -glucuronidase with ^{125}I

Purified enzyme (100 μg) in 50mM- K_2HPO_4 - KH_2PO_4 , pH 7.0, was mixed at 4°C with 0.5mCi of carrier-free K^{125}I . Chloramine-T (100 μg) in the same buffer was added, the solution rapidly mixed, and reaction allowed to proceed for 2min. At the end of this period 100 μg of sodium metabisulphite in the same buffer was added and the solution again mixed

Table 1. *Subcellular distribution of various enzymes in rabbit liver*

Results are expressed as percentage of total activity or protein in the homogenate present in each fraction (%) and as the ratio of the specific activity (in units/mg of protein) in a fraction to that in the homogenate (relative sp. activity).

Fraction	Protein %	β -Glucuronidase		β -N-Acetylglucosaminidase		β -Galactosidase		β -Glucosidase		Cathepsin D		Glucose 6-phosphatase	
		%	Relative sp. activity	%	Relative sp. activity	%	Relative sp. activity	%	Relative sp. activity	%	Relative sp. activity	%	Relative sp. activity
Nuclear debris	60	35	0.58	34	0.56	38	0.63	38	0.63	40	0.66	40	0.66
Heavy mitochondria	11	25.5	2.3	38	3.4	22	2.0	5.5	0.50	30	2.7	5	0.45
Light mitochondria	8	11.0	1.4	15	1.9	10	1.25	2.9	0.36	10	1.25	6	0.75
Microsomal	4	15.0	3.7	2	0.5	4	1.0	2.2	0.55	3	0.75	40	10.0
Supernatant	14	3.0	0.21	4	0.28	26	1.9	50	3.6	6	0.42	4	0.28
Total	97	90		94		100		98		90		95	
Absolute values (units/g of liver or mg/g of liver)	330	0.52		1.21		0.34		1.78		22		12.3	

to stop the reaction. The product was dialysed against 2.5 litres of phosphate-buffered saline until radioactivity ceased to appear outside the dialysis bag. A low recovery of enzyme activity was obtained (5%).

Results

Subcellular fractionations

The results of a representative subcellular fractionation are presented in Table 1. Two such identical fractionations were performed, and they gave very similar results. Cathepsin D and *N*-acetylglucosaminidase were mainly in the lysosomal-mitochondrial fractions, whereas β -galactosidase and β -glucosidase showed considerable activity in the supernatant fraction. β -Glucuronidase was present in microsomal and lysosomal-mitochondrial fractions. Separate sedimentability measurements confirmed that the various enzymes in the lysosomal-mitochondrial fractions, and β -glucuronidase in the microsomal fraction, were largely particle-bound. Presumably the amount of material obtained in the nuclear-debris fraction could be decreased by repeated homogenization and centrifugation.

The problems of lysosome preparation in a very tough tissue have been studied in detail by Canonico & Bird (1969) with rat skeletal muscle. They have shown that in media of low ionic strength, such as used here, considerable amounts of lysosomal material may adhere to nuclear pellets. Although few free lysosomes were seen by Acridine Orange fluorescence in nuclear pellets prepared as described above, much lysosomal material may well have been present. But in a single rabbit liver fractionation in 0.15M-KCl (as suggested by Canonico & Bird, 1969) as the homogenization medium, results similar to

those with the normal homogenization medium were obtained.

125 I-labelled β -glucuronidase added to a homogenate before differential centrifugation was adsorbed on to particulate material and distributed throughout the fractions obtained. Thus it seemed unlikely that a definitive quantitative subcellular localization of this enzyme could be obtained by such fractionations. However, it should be noted that the largely inactivated labelled enzyme may not fairly represent the adsorption of soluble native enzyme. Similar problems of adsorption of solubilized enzymes have been documented by Baccino & Zuretti (1971) and Baccino *et al.* (1971).

The supernatant β -glucosidase activity was associated with a molecule of similar elution position to ovalbumin (mol.wt. 43000) in gel filtration on Sephadex G-100. These results are in agreement with those of Mellor & Layne (1971) on steroid glucosidase in rabbit liver. Thus the situation in rabbit liver is probably like that in rat kidney (Robinson *et al.*, 1967; Price & Dance, 1967) where an enzyme of mol.wt. 40000 is responsible for both β -glucosidase and β -galactosidase activity in the supernatant fraction, and a separate β -galactosidase of mol.wt. 80000 is present in lysosomes.

Possibly the most satisfactory way of deciding the subcellular localization of β -glucuronidase is by enzyme histochemistry, and some evidence for the presence of microsomal and lysosomal enzyme in various rat tissues has been presented by light-microscopy (Fishman *et al.*, 1967), and by electron microscopy (Smith & Fishman, 1969).

Sucrose-density-gradient separations of the pooled lysosomal-mitochondrial fraction gave a major peak of activity for β -glucuronidase, *N*-acetylglucosaminidase and cathepsin D at densities between

1.21 and 1.23 g/ml. Shoulders were sometimes seen, but since the patterns were affected by the position in the gradient at which samples were loaded, in runs of 3 or 6 h their significance was unclear. The sedimentability of enzymes in these fractions from gradients was good at 6 h (80–90%). As patterns with a given sample and mode of loading were similar at both times isopycnic conditions were probably reached, and thus the final equilibrium densities of particles were affected by the mode of loading the samples. Nevertheless, the results suggested that the enzymes were associated with a fairly uniform set of particles. No evidence was obtained for very small Acridine-Orange-staining particles at the top of gradients, but some non-sedimentable activity was present in this position. In contrast, Dingle & Barrett (1969b) observed such small particles in experiments with rat kidney.

Extraction of β -glucuronidase from particulate fractions

Most β -glucuronidase in the total lysosomal-mitochondrial fraction was rendered non-sedimentable by homogenization with an Ultra-Turrax homogenizer (4×30 s) with cooling, followed by addition of Triton X-100 to 0.1%, and the supernatant obtained after sedimenting such extracts at 100000g for 1 h was used for immunoprecipitation and gel electrophoresis and electrofocusing.

Treating the microsomal fraction similarly only released 40–50% of the enzyme. Of a variety of treatments used, 0.5% sodium deoxycholate and 0.1% octyl sodium sulphate were most effective, and both rendered 85–90% of the enzyme non-sedimentable. Deoxycholate solubilization of β -glucuronidase has been used previously by Kato *et al.* (1972), and octyl derivatives have been suggested as very effective alternatives (Tanford, 1972). Ribonuclease digestion, as used by Van Lancker & Lentz (1970), was not necessary. The nature of the testicular-hyaluronidase digestion used by Ide & Fishman (1969) to release mouse microsomal β -glucuronidase is unknown, since the commercial enzyme preparation used probably contained a large number of enzyme activities, including various proteinases (Morton, 1973; D. B. Morton, personal communication).

For immunoprecipitation of microsomal enzyme, deoxycholate extracts were used directly, and the deoxycholate concentration maintained at 0.5%, since immunoprecipitation is not significantly affected by these conditions (Crumpton & Parkhouse, 1972). For gel electrophoresis and isoelectric focusing, small samples of the deoxycholate extracts were used directly, since β -glucuronidase patterns obtained with completely soluble samples from the purification procedures (and with Triton X-100-solubilized lysosomal extracts) were not affected by the addition

of deoxycholate to 0.5%. Prior removal of small amounts of enzyme solubilized from microsomal fractions by hypo-osmotic treatment (and thus presumably in contaminating lysosomes) had no effect on the β -glucuronidase patterns in either technique.

Crude extracts of β -glucuronidase from rabbit liver, kidney, skin and brain were made by homogenization in 50mM-Tris-HCl buffer, pH 7.4 at 4°C, with the Ultra-Turrax homogenizer and addition of sodium deoxycholate to 0.5%. The supernatant from a subsequent centrifugation at 100000g for 1 h was then used. Some 85–95% of the total activity of the homogenates was present in such extracts.

Characteristics of microsomal and lysosomal β -glucuronidase

pH optima and K_m values determined on microsomal and lysosomal extracts were very similar to those for the purified enzyme (Dean, 1974). Both extracts gave single β -glucuronidase bands in gel electrophoresis and no clear distinction between their rates of migration was observed. Possible minor distinctions in migration under these conditions have been reported by Kato *et al.* (1972) and Mameli *et al.* (1972), and clearer differences were observed by Goldstone *et al.* (1973) and Goldstone & Koenig (1973) in extracts of rat kidney and by Dofoku *et al.* (1971) in extracts of mouse liver.

In analytical isoelectric focusing, in the pH range 3–10, multiple forms of β -glucuronidase from rabbit liver microsomal fractions and lysosomes were again very similar to those seen with purified preparations (Dean, 1974), and no distinctions between the microsomal and lysosomal complements could be made, over a large number of runs. This is in agreement with the results of Coutelle (1971) with microsomal and lysosomal extracts from mouse brain and Ehrlich ascites-tumour cells.

Since the work of Paigen (1961), who showed that heat inactivation of mouse liver β -glucuronidase varies markedly with the strain of mouse, and the sensitive discrimination between mutants of *Escherichia coli* β -galactosidase (Langridge, 1968) by heat-inactivation studies, much attention has been paid to this technique as a means of distinguishing microsomal and lysosomal β -glucuronidase. The time-courses of heat inactivation of various extracts were determined. Since plots of log (% activity remaining) versus time for crude extracts were not linear, and thus the inactivation did not appear to be a unimolecular reaction, comparison between lysosomal and microsomal extracts was not satisfactory. Other attempts to distinguish enzyme in such crude extracts have usually failed (Canonico & Bird, 1969; Ide & Fishman, 1969; Kato *et al.*, 1972) and the results of the latter group indicate that rates of

inactivation are very much influenced by ionic environments. They are also probably very sensitive to factors such as enzyme-membrane binding (Ide & Fishman, 1969) and other micro-environmental effects; these considerations may apply in solubilized enzyme extracts if the enzymes are still bound to membrane (or detergent) micelles. Some of these factors probably affect the other parameters discussed earlier in this section.

Thus more direct means of investigating the differences between the enzyme molecules themselves were adopted in the present work. These involved the use of a specific antiserum to β -glucuronidase, prepared as described in the Materials and Methods section.

Characterization of the pig antibodies to rabbit β -glucuronidase

The antiserum, concentrated fivefold, was tested as described (Dean, 1974) for the purified enzyme, against concentrated liver extracts, against samples of each of the purification stages and against rabbit serum. No line lacking enzyme activity was obtained. It was also tested against purified rabbit ferritin (Dean, 1974) and gave no reaction. The antiserum was therefore specific for β -glucuronidase.

Specific antibodies may be useful in studying the biosynthesis of a protein, since they can be used in very sensitive isolation procedures, for instance by immunoprecipitation (Palmiter *et al.*, 1972; Beck *et al.*, 1972). Such a use of the antibodies in affinity chromatography was assessed. β -Glucuronidase activity in crude extracts (specific activity 0.004 units/mg) in 50 mM-Tris-HCl (pH 7.8)-0.2 M-NaCl-0.1% octyl sodium sulphate, was retained by a 5 ml (anti- β -glucuronidase) IgG-Sepharose 4B column during elution with 5 bed vol. of the same solution, whereas most of the protein was unretarded. On addition of 5 M-urea to the eluting solution, 35-40% of the adsorbed activity emerged, at a specific activity of 0.5 units/mg. Elution of the activity by 100 mM-sodium carbonate buffers at pH 10.0 or 10.5, in place of the urea solution, gave rather lower recoveries. At pH 3.0, enzyme activity was lost rapidly. The addition of 1% bovine serum albumin to the eluting solutions only slightly improved recoveries. Thus the normal means of disruption of immunoprecipitates (extreme pH values) were less effective than denaturation with urea in this case, and this latter procedure was adopted. Gel electrophoresis of the eluted antigen gave a single protein band possessing enzymic activity at loadings of 20 μ g/gel. The column retained its binding capacity after re-equilibration at pH 7.8. No activity was retarded under the same conditions on a Sepharose 4B column that had been activated with CNBr and then treated with glycine.

The explanation for the relatively low specific activity obtained was unclear. Possibly there was non-specific IgG-protein interaction; this might be decreased by use of fragment Fab' conjugated to Sepharose. Secondly, since the antiserum may react with incomplete or inactive polypeptide chains (Palmiter *et al.*, 1972), considerable numbers of inactive β -glucuronidase molecules may have been present.

The presence of such inactive, or partially inactivated, enzyme molecules in various enzyme preparations was demonstrated by use of the electroimmunoassay, as described by Laurell (1972) for the quantitative measurement of antigens. The agarose plate contained 1% antiserum to β -glucuronidase; several dilutions of purified enzyme, various pools from the affinity column and other soluble extracts were electrophoresed into the plate to form rocket-shaped precipitin lines. Values for amounts of antigen obtained were by no means at a constant ratio to activity, thus showing that some preparations, including those from the affinity column, contained inactive enzyme, and that the antibodies reacted with at least some incompletely active molecules.

The antiserum was capable of quantitatively precipitating purified enzyme and enzyme activity in solubilized extracts of homogenate, microsomal fraction and lysosomes. IgG preparations from the antiserum were also potent (Fig. 1). Control non-immune pig sera and IgG were non-precipitating. No inhibition of activity was observed, presumably because the small substrate used suffered little steric hindrance from the bound antibodies, none of which reacted directly with the active site, as is usual (Cinader, 1967).

Fig. 1. Immunoprecipitation of β -glucuronidase by (anti- β -glucuronidase) IgG preparation

Various amounts of enzyme activity in a crude extract in the presence of 0.5% sodium deoxycholate were added to 20 μ l of IgG (48 mg/ml) at pH 7.8. Results are means of duplicate preparations. No activity was precipitated in tubes with 20 μ l of non-immune IgG (50 mg/ml), or without IgG, treated similarly. O, Enzyme activity in supernatant; Δ , enzyme activity in pellet.

β -Glucuronidases in extracts from rabbit kidney, skin, liver and brain were immunologically identical in Ouchterlony gel diffusion, and all the enzyme activity in the extracts was precipitable by the antiserum.

Immunological relationship between lysosomal and microsomal β -glucuronidase and between the multiple forms

Successive fractions from preparative isoelectric focusing of purified enzyme were immunologically identical in Ouchterlony gel diffusion (Plate 1), as were renatured fractions from preparative isoelectric focusing in the presence of 8M-urea. Neuraminidase-digested molecules were also immunologically identical with undigested molecules, which shows that the sialic acid residues were not antigenic. Similar results of the relationship of β -glucuronidase components were obtained with the two polyspecific antisera (Dean, 1974). No immunochemical differences were detected between autolysed and non-autolysed molecules. Thus it seemed that sequence differences between the polypeptides must be extremely small, as discussed previously (Dean, 1974). However, more sensitive immunological techniques, such as micro-complement fixation, might demonstrate differences.

In view of these results, it is not surprising that microsomal and lysosomal extracts gave β -glucuronidase precipitin lines showing reactions of complete identity. Differences between an intrinsic population of microsomal β -glucuronidase and one in transit can thus originate only in membrane binding and possibly in the biosynthetic mechanism, and not in the molecules themselves.

Discussion

The immunochemical studies described show clearly that a very closely related set of polypeptides is responsible for all the β -glucuronidase activity in rabbit liver. Okochi *et al.* (1968) have shown immunological cross-reactivity by immunoprecipitation of antisera against different β -glucuronidase fractions from DEAE-cellulose chromatography of a crude rat liver extract. However, they also obtained some non-cross-reacting antibodies, suggesting that at least some antigenic determinants are not common to all β -glucuronidase molecules in rat liver, although interpretation of these results is difficult because of the use of crude mixtures of antigens and uncharacterized antisera.

The two major forms of *N*-acetylglucosaminidase have been shown to be immunologically identical (Carroll & Robinson, 1972, 1973; Srivastava & Beutler, 1972). One form is absent, both as an

enzyme and as an antigen, in patients with Tay-Sachs disease, and in spite of the immunological identity of the forms, certain catalytic properties are apparently lacking, with the result that storage product accumulates. A contrary case is provided by the recent evidence of α -galactosidase (Beutler & Kuhl, 1972): here immunologically distinct forms are found. The immunochemical characteristics of the recently described β -glucuronidase deficiency (Hall *et al.*, 1973) will be of interest.

Lanzerotti & Gullino (1972) have reported the production of antisera against rat β -glucuronidase, but they have not studied the properties of these antisera in detail. Ohishi & Shioya (1971) have shown that an antiserum to rabbit liver β -glucuronidase can completely precipitate enzyme in extracts from various other rabbit sources, including serum, as has been shown here for certain tissues. They have also measured the immunoprecipitation of β -glucuronidase in liver of other species by their rat antiserum. Much precipitation was obtained with mouse, guinea-pig and dog enzymes, but little with that of calf.

From the subcellular-fractionation studies, it seems likely that β -glucuronidase is bimodally distributed in rabbit liver as in other tissues (de Duve *et al.*, 1955; Canonico & Bird, 1969; Fishman *et al.*, 1967). The immunochemical evidence presented here shows that lysosomal and microsomal β -glucuronidase molecules are very similar, and thus differences between intrinsic microsomal enzyme (if present) and enzyme in transit to lysosomes must also be extremely restricted.

Evidence found by Mameli & Gianetto (1973) implies that all the β -glucuronidase in rat liver microsomal fractions is membrane-bound, and shows that it can be almost entirely released by sonication without much leakage of intracisternal protein. The evidence presented does not permit a decision as to whether the enzyme has more than one mode of binding to the microsomal membranes, although the binding is clearly distinguishable from that of glucose 6-phosphatase. Thus the existence of 'intrinsic' microsomal β -glucuronidase remains unproved, and the role of microsomal enzyme and the mode of action of Eg^+ allele in mouse liver (Paigen, 1971), which determines the microsomal incorporation of β -glucuronidase, are still open questions.

The specific antiserum was used to demonstrate the presence of incompletely active enzyme in various enzyme samples. Variation in content of such molecules may explain in part the low specific activities per mg of protein in some procedures which favour preservation of inactive enzyme (such as the immuno-adsorbent column) rather than its loss (at least some normal purification steps). The incompletely active enzyme may be partially denatured or inhibited (possibly by bound molecules such as cholesterol;

EXPLANATION OF PLATE I

Gel-diffusion analysis of the multiple forms of rabbit liver β -glucuronidase

The central well contained the specific antiserum to β -glucuronidase. Proceeding anticlockwise from the bottom right well, the outer wells contained fractions of progressively increasing isoelectric point from preparative isoelectric focusing, until the final well, which was empty.

Tappel & Dillard, 1967), or may represent pro-enzyme awaiting activation or incomplete enzyme molecules. These possibilities are now open to investigation.

It is hoped that studies of the biosynthesis and translocation of β -glucuronidase in comparison with that of other lysosomal enzymes will clarify somewhat the role of microsomal β -glucuronidase and the mechanism of translocation of lysosomal enzymes.

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