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The Cellular Distribution of some Rat-Kidney Glycosidases

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1. Free and total activities of β-glucosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase and β-glucuronidase have been determined fluorimetrically in five subcellular fractions of rat kidney. 2. The β-glucosidase activity appeared in the soluble fraction, β-glucuronidase had the distribution pattern of a lysosomal enzyme, and both β-galactosidase and *N*-acetyl-β-glucosaminidase had bimodal distributions. 3. Two types of β-galactosidase activity were found: a sedimentable type, having optimum pH 3·7, mol.wt. about 80000 and slow electrophoretic mobility at pH 7·0 in starch gel; and a soluble type of much faster mobility, having optimum pH 5·5–6·5 and mol.wt. about 40000. 4. Evidence is presented that the β-glucosidase and the soluble type of β-galactosidase are the same enzyme. 5. Most of the *N*-acetyl-β-glucosaminidase activity was in the lysosome-rich fractions, but a significant proportion occurred in the microsomal fraction in a non-latent form. 6. The use of β-galactosidase and *N*-acetyl-β-glucosaminidase as lysosomal marker enzymes is complicated by the possible presence of multiple forms, but this limitation does not apply to β-glucuronidase in the rat kidney.

In an investigation of the acid glycosidases of rat kidney, Robinson, Price & Dance (1967) demonstrated that β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) existed in at least two forms with different pH optimum, molecular weight, electrophoretic mobility and substrate specificity. One type of β-galactosidase appeared to be identical with the β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21).

From tissue-fractionation experiments, it had been concluded that the β-galactosidase of rat kidney was contained in lysosomes (Shibko & Tappel, 1965; Wattiaux-De Coninck, Rutgeerts & Wattiaux, 1965), although it had not corresponded exactly in distribution to other lysosomal enzymes. This lack of correspondence was more marked in other tissues, such as rat spleen or mouse kidney (Conchie & Hay, 1963), and histochemical studies have shown differences in the localization of β-galactosidase and other lysosomal enzymes in several organs (Rutenburg, Rutenburg, Monis, Teague & Seligman, 1958). The subcellular distribution of rat-kidney β-glucosidase had not been determined, but it appeared that its possible presence in the soluble fraction could account for the high β-galactosidase activity there. Accordingly the distribution of the β-galactosidase and β-glucosidase activities among the subcellular fractions of rat kidney have been studied.

Bimodal distributions of β-glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31) have been found in some tissues (Van Lancker, 1964), and preliminary evidence suggested that rat-kidney *N*-acetyl-β-glucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) was present in more than one form, distinguishable by gel electrophoresis. The distributions of β-glucuronidase and *N*-acetyl-β-glucosaminidase were therefore included in this study. Substrates with the same aglycone, 4-methylumbelliferone, were used in all four enzyme assays. In this way effects due to differential permeability of lysosomes to substrates carrying a variety of aglycones were reduced (Furth & Robinson, 1965; Hainsworth & Wynn, 1966). The use of substrates with a fluorescent aglycone to detect glycosidase bands on starch-gel electrophoresis has provided a simple means of allocating the different enzyme forms to the various subcellular fractions.

MATERIALS AND METHODS

Tissue fractionation. Male Sprague–Dawley rats, each weighing 200–300g. (Roebuck Farm, Welwyn, Herts.), were starved for 24hr. and then killed by a blow on the head. The kidneys were rapidly removed, weighed, sliced and suspended in 9 times their weight of ice-cold 0·45M-sucrose containing 0·68mM-EDTA and adjusted to pH 7·0 with 0·25N-NaOH. The suspension was homogenized in a smooth glass Potter–Elvehjem homogenizer by two passes of a manually rotated Teflon pestle (diameter clearance, 0·48mm.). After filtration through two layers of muslin, the homogenate was centrifuged at 4° in an MSE High

Speed 17 centrifuge fitted with an 8 × 50 ml. angle rotor (no. 69181) for 2 min. at 800*g*, 1 min. at 10000*g* and 20 min. at 12000*g* (excluding running-up and running-down times) to sediment the nuclear (N), lysosomal–mitochondrial (LM) and mitochondrial–microsomal (MM) fractions respectively. The resulting supernatant was then centrifuged in a 10 × 10 ml. angle rotor in an MSE Super Speed 50 centrifuge for 60 min. at 100000*g* to sediment the microsomal (Mic) fraction. The final supernatant was termed the soluble (S) fraction. Each sediment was resuspended in 20 ml. of the 0·45 M-sucrose medium described above. The fractionation procedure is that of Shibko & Tappel (1965).

In some experiments, the microsomal fraction was treated with sodium deoxycholate ('medium C' of Kirsch, 1962) and recentrifuged at 100000*g* for 45 min. to produce a ribosomal pellet and a supernatant fraction containing the endoplasmic reticulum and solubilized protein.

Enzyme assays. The activities of the four glycosidases were assayed by the fluorimetric procedures of Robinson *et al.* (1967), except that only 0·1 ml. of a suitably diluted sample of each fraction was incubated with 1 ml. of substrate. The reaction was terminated by the addition of 3·9 ml. of 0·5 M-glycine–NaOH buffer, pH 10·4.

The latent activities of the β-glycosidases were estimated as the difference between the free activity and the total activity, assayed under conditions identical with those used for the determination of free activity, except for the presence of Triton X-100. In determinations of free glycosidase activities, 0·1 ml. of 0·45 M-sucrose homogenates or subcellular fractions was incubated with 0·1 ml. of 0·45 M-buffered sucrose and 1 ml. of the appropriate substrate at pH 5·2, also in 0·45 M-sucrose, for 10 min. at 25°. For the assay of total activities, the buffered sucrose was replaced by 0·1 ml. of 2% (w/v) Triton X-100. In each case the addition of 3·8 ml. of glycine buffer terminated the reaction.

Acid phosphatase activity was measured by the method of Furth & Robinson (1965) and glucose 6-phosphatase activity by that of Dallner (1963), with the liberated phosphate assayed according to Allen (1940). Succinate–neotetrazolium reductase activity was assayed by the method of Slater & Planterose (1960).

Other methods. Protein was measured by the Folin method as modified for use with the Technicon Auto-Analyzer, with casein as a standard. Starch-gel electrophoresis and gel filtration through Sephadex G-200 (Pharmacia Ltd.) were carried out as described by Robinson *et al.* (1967). All salts and buffer constituents were A.R. grade.

RESULTS

Identification of subcellular fractions. The subcellular distribution patterns (Figs. 1*a*–1*c*) of typical lysosomal (acid phosphatase), mitochondrial (succinate–neotetrazolium reductase) and microsomal (glucose 6-phosphatase) enzyme activities show that the fractionation procedure is closely comparable with that of Shibko & Tappel (1965). Although the specific activity of acid phosphatase was highest in the LM fraction, significant amounts appeared in the N and MM fractions and all these fractions are probably to be regarded as containing lysosomes. Strauss (1956) has shown that kidney lysosomes occur in a wider size-range than the corresponding liver particles. The proportion of acid phosphatase (15%) appearing in the S fraction is an indication of the upper limit of lysosome breakdown occurring in the fractionation procedure. The

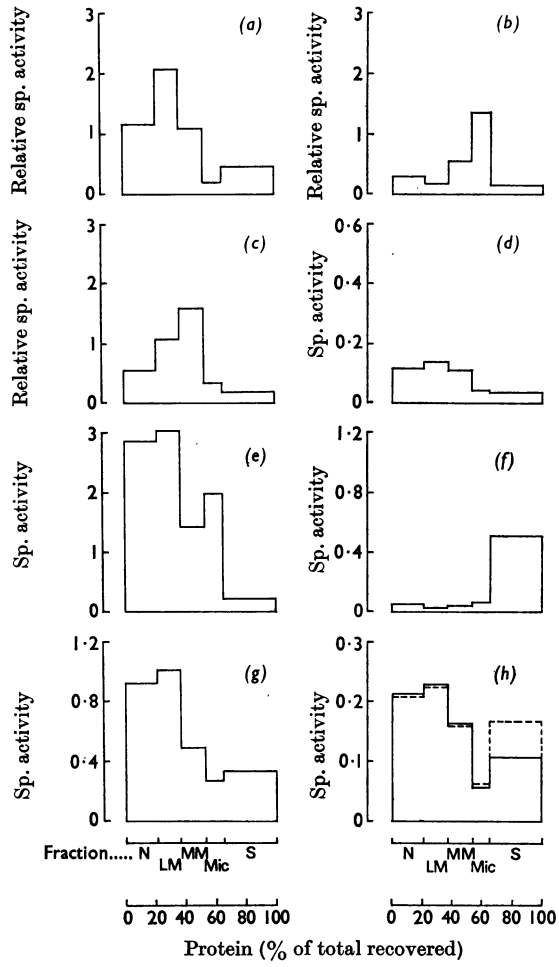

Fig. 1. Subcellular distribution of rat-kidney enzymes. Specific activities are given as μmoles/hr./mg. of protein, and the abscissae give the percentages of the total protein in each fraction in the order of isolation. N, Nuclear fraction; LM, lysosomal–mitochondrial fraction; MM, microsomal–mitochondrial fraction; Mic, microsomal fraction; S, soluble fraction. Enzymes were assayed as described in the text. (*a*) Acid phosphatase; (*b*) glucose 6-phosphatase; (*c*) succinate–neotetrazolium reductase; (*d*) β-glucuronidase; (*e*) *N*-acetyl-β-glucosaminidase; (*f*) β-glucosidase; (*g*) β-galactosidase, assayed at pH 3·7; (*h*) β-galactosidase, assayed at pH 5·5 in the presence (——) and absence (- - - -) of glucono-(1→5)-lactone (0·2%, w/v). In (*a*), (*b*) and (*c*), the relative specific activities of the fractions, not absolute values, are given.

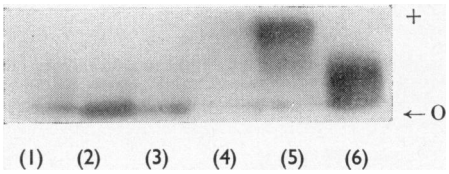

Fig. 2. Starch-gel electrophoresis of *N*-acetyl-β-glucosaminidase activity in different subcellular fractions. Electrophoresis was carried out for 3 hr. at 200v and 40 mA in sodium phosphate buffer, pH 7·0. The gel was flooded with 1 mM-4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide in sodium phosphate–citrate buffer (McIlvaine, 1921), pH 4·5. The blue fluorescent bands were photographed under u.v. illumination after 10–15 min. incubation at 37°. (1), Nuclear (N) fraction; (2), lysosomal–mitochondrial (LM) fraction; (3), mitochondrial–microsomal (MM) fraction; (4), microsomal (Mic) fraction; (5), soluble (S) fraction; (6), sucrose homogenate. O, Origin. The microsomal activity corresponded in mobility to the soluble activity, but the contrast was too low for photographic reproduction.

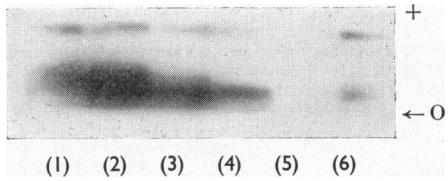

Fig. 3. Starch-gel electrophoresis of β-galactosidase in rat-kidney homogenate and subcellular fractions. Conditions were as detailed in Fig. 2, except that 1 mM-4-methylumbelliferyl β-D-galactoside in sodium phosphate–citrate buffer, pH 4·0, was used. (1), Sucrose homogenate; (2), nuclear (N) fraction; (3), lysosomal–mitochondrial (LM) fraction; (4), mitochondrial–microsomal (MM) fraction; (5), microsomal (Mic) fraction; (6), soluble (S) fraction. O, Origin.

greatest specific activity of succinate–neotetrazolium reductase was in the MM fraction, and very little appeared in the soluble fraction. The bulk of the glucose 6-phosphatase activity was in the Mic fraction. Since the N fraction has relatively high activities of all three of these enzymes, it is probably contaminated with unbroken cells and membranes, as suggested by Shibko & Tappel (1965), or by large lysosomal particles (Strauss, 1956). As this fraction is so heterogeneous its β-glycosidase content is difficult to interpret.

Subcellular distribution of glycosidases. The β-glucuronidase distribution pattern (Fig. 1*d*) was very similar to that of acid phosphatase, but it was the only glycosidase to have this distribution, considered typical of a lysosomal hydrolase (de Duve, 1964). When the activity in each fraction was compared by starch-gel electrophoresis at pH 7·0 in 20 mM-sodium phosphate buffer, a single broad band of activity migrating slowly towards the anode was found in all fractions. However, a very minor part of the soluble activity occasionally appeared as a much more rapid sharp zone.

N-Acetyl-β-glucosaminidase had a bimodal distribution (Fig. 1*e*), with a major peak of specific activity in the LM fraction and a minor peak in the Mic fraction. On starch-gel electrophoresis (Fig. 2), most of the activity of the kidney homogenate migrated as a diffuse zone very slowly towards the anode. A small variable proportion migrated as a sharp zone of much higher anodic mobility. This sharp zone (which does not reproduce well photographically) was found concentrated in the Mic and S fractions, but the slow-moving activity appeared in the more easily sedimentable fractions.

Most of the β-glucosidase (86%) was found in the S fraction (Fig. 1*f*) and, in contrast with the other β-glycosidases, thus appears to be non-sedimentable and not associated with any subcellular particle. The remaining activity was evenly distributed among the remaining fractions, and, when compared on gel electrophoresis, gave a single sharp band of activity having a fast anodic mobility, in each case corresponding exactly to the soluble activity (cf. Fig. 3). Although present in low amounts in the sedimentable fractions, the band of β-glucosidase activity was easily detected on starch-gel electrophoresis; the visualization technique appears to over-emphasize the contribution of sharp minor zones to the total activity.

The distribution of β-galactosidase activity was also bimodal, with peaks of specific activity in the LM and S fractions (Fig. 1*g*). Comparison with the distribution patterns of acid phosphatase or β-glucuronidase shows that the high activity in the S fraction could not have arisen as a result of the breakdown of lysosomes. Robinson *et al.* (1967) have shown the presence of two types of β-galactosidase activity in rat kidney, separable by starch-gel electrophoresis at pH 7·0 and by filtration through Sephadex G-200. In the electrophoresis patterns (Fig. 3) of the subcellular fractions the fast band of activity appears mainly in the S fraction, with only trace amounts sedimentable, but the slow bands are associated mainly with the heavier fractions. In agreement with the previous suggestion that both activities are associated with the same protein, the more negative β-galactosidase was found to have the same electrophoretic mobility and subcellular distribution as the β-glucosidase.

Latent activities of β-glycosidases. In an attempt to demonstrate structure-bound latency of the four glycosidase activities, they were assayed under conditions in which lysosomes are considered to be stable. From the work of Shibko & Tappel (1965),

Table 1. *Free and total specific activities and apparent latencies of some rat-kidney glycosidases in rat-kidney homogenate and subcellular fractions*

All glycosidases were assayed at 25° in 0·45M-sucrose containing the appropriate buffer, pH 5·2. Total specific activities were measured in the presence of 0·2% (w/v) Triton X-100. Full details of these assays are given in the text. (*a*), Free activity; (*b*), total activity; (*c*), percentage apparent latency. The results are means ± S.D., calculated from eight experiments, and the specific activities are expressed in mμmoles/hr./mg. of protein. The preparation of the subcellular fractions is described in the text.

	Specific activities					
		Subcellular fractions				
	Homogenate	Nuclear	Lysosomal–mitochondrial	Mitochondrial–microsomal	Microsomal	Soluble
β-Glucuronidase (*a*)	7·0 ± 2·8	3·6 ± 4·5	9·0 ± 6·0	7·0 ± 5·2	4·3 ± 4·8	8·4 ± 5·8
(*b*)	16·3 ± 6·0	19·2 ± 12·1	28·2 ± 11·8	27·1 ± 12·9	10·6 ± 9·7	10·6 ± 5·9
(*c*)	60·9 ± 9·9	81·2 ± 22·7	75·4 ± 15·8	76·5 ± 11·5	41·6 ± 35·6	9·6 ± 11·2
N-Acetyl-β-glucosaminidase (*a*)	114 ± 59	166 ± 76	216 ± 105	158 ± 82	325 ± 85	51 ± 25
(*b*)	313 ± 106	521 ± 185	680 ± 194	327 ± 117	326 ± 100	52 ± 24
(*c*)	60 ± 13	69 ± 7·0	68 ± 5·8	50 ± 18	6·1 ± 9·0	4·8 ± 9·6
β-Glucosidase (*a*)	59 ± 29	20 ± 8·9	15 ± 8·0	13 ± 7·7	14 ± 7·0	149 ± 51
(*b*)	95 ± 48	65 ± 32	29 ± 15	33 ± 17	28 ± 8·7	196 ± 71
(*c*)	39 ± 7·2	70 ± 9·6	53 ± 11	62 ± 8·5	54 ± 4·8	20 ± 15
β-Galactosidase (*a*)	91 ± 40	79 ± 38	90 ± 36	51 ± 18	47 ± 26	155 ± 62
(*b*)	203 ± 79	258 ± 112	290 ± 94	153 ± 53	82 ± 43	214 ± 78
(*c*)	57 ± 7·0	70 ± 7·4	68 ± 3·9	68 ± 7·2	43 ± 8·8	31 ± 8·3

it was apparent that kidney lysosomes would suffer only negligible breakdown in a 10min. incubation at 25° and pH 5·2 in 0·45M-sucrose, and therefore free activities were measured under these conditions. The total activities were measured under the same conditions in the presence of 0·2% (w/v) Triton X-100. Because of the high sensitivity of the fluorimetric assay, dilute enzyme solutions could be used and precipitation of proteins was not necessary. The presence of Triton X-100 did not interfere with the assays. The high sucrose molarity causes a slight inhibition (less than 5%) of β-glycosidase activity, which has been ignored in the calculation of the free and total activities shown in Table 1.

In the presence of Triton X-100, the subcellular distribution of β-glucuronidase activity was very similar to Fig. 1(*d*), though activities were lower because of the suboptimum assay conditions. Free β-glucuronidase activities were significantly less than the total activities only in the N, LM and MM fractions, in which this enzyme appears to be about 75% latent.

Only in the LM fraction was the latency of *N*-acetyl-β-glucosaminidase closely similar to that of β-glucuronidase. In contrast with the other β-glycosidases, the highest free activity of *N*-acetyl-β-glucosaminidase was in the Mic fraction. About 15% of the total activity sediments in this fraction, apparently in a non-latent form.

Determination of the specific free and total activities of β-glucosidase confirmed that it is principally a soluble enzyme (Table 1). The activities in the LM, MM and Mic fractions show an apparent latency appropriate to a lysosomal enzyme, but there is also an anomalous latency (24%) in the S fraction. The possibility that β-glucosidase is activated by Triton X-100 is under investigation.

When β-galactosidase is assayed under conditions in which lysosomes retain their structure, the contribution of the soluble β-galactosidase (with its higher pH optimum) is emphasized (Table 1). Thus the S fraction has the highest free specific activity and a total specific activity only a little less than that of the LM fraction. Because of the presence of two types of β-galactosidase the distribution pattern differs from that regarded as typical of a lysosomal enzyme. The latency of the β-galactosidase activity in the sedimentable fractions is compatible with a lysosomal origin, but a similar anomalous apparent latency was found in the S fraction as in the case of β-glucosidase. Since the soluble type of β-galactosidase is completely inhibited by 0·2% glucono-(1→5)-lactone (Robinson *et al.* 1967), the distribution of the lysosomal type could be established by assaying the enzyme in the presence of this inhibitor (Fig. 1*h*). Little difference was found in the heavier fractions, but the activity of the S fraction was lower by 35%. When the β-galactosidase activity of the S fraction was developed after starch-gel electrophoresis in the presence of glucono-(1→5)-lactone, the fast band of activity (cf. Fig. 3) did not appear and the slow band was unaffected. Thus the fast band of activity apparently amounts to 35% of the soluble β-galactosidase activity when assayed at pH 5·2.

Studies on partially purified β-glycosidases. The elution patterns of the β-galactosidase and β-glucosidase activities of the S fraction on filtration through Sephadex G-200 columns are shown in Fig. 4. Assayed at pH 3·7, β-galactosidase appeared in a single peak well separated from the β-glucosidase

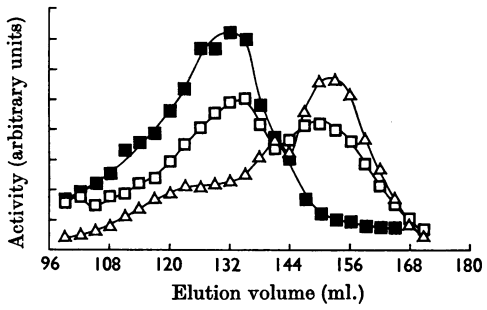

Fig. 4. Gel filtration of rat-kidney soluble fraction through a Sephadex G-200 column (36cm.×3cm. diam.). The soluble fraction from rat kidney (3ml.) was eluted with 0·01M-sodium phosphate buffer, pH7·6, containing 0·4M-NaCl and 10mM-EDTA (flow rate 15ml./hr.). Fractions (3ml.) were collected and 0·1ml. of each fraction was assayed for β-galactosidase activity with 1mM-4-methylumbelliferyl galactoside in sodium phosphate–citrate buffer (McIlvaine, 1921), pH3·7 or 5·5, with a 20min. incubation at 37°. β-Glucosidase was assayed at pH5·5 under the same conditions. □, β-Galactosidase activity at pH5·5; ■, β-galactosidase activity at pH3·7; △, β-glucosidase activity at pH5·5. Void volume (V_0), 72ml.; internal volume (V_i), 201ml.

activity (assayed at pH5·5). When β-galactosidase was re-assayed at pH5·5 a second peak was found, having about 30% of the total activity and coinciding with the β-glucosidase peak. The elution volumes of the peaks corresponded to molecular weights of about 80000 and 40000 respectively. Similar values were obtained in experiments with the whole homogenates (Robinson *et al.* 1967). The 'heavy' β-galactosidase of the S fraction was indistinguishable from the β-galactosidase of the LM fraction with respect to filtration rate through Sephadex G-200, electrophoretic mobility on starch gel in sodium phosphate buffer, pH7·0, and pH optimum in sodium phosphate–citrate buffer. The 'light' β-galactosidase had the same fast electrophoretic mobility as the soluble type. Thus both lysosomal and soluble types of β-galactosidase are found in the S fraction, and the second accounts for about 30–35% of the total soluble activity when assayed under conditions suitable for the study of latent enzyme activities of lysosomes. The two tubes containing the highest β-glucosidase activity were pooled and diluted to 50ml. with distilled water. The diluted enzyme sample was assayed with 4-methylumbelliferyl β-D-glucoside, 4-methylumbelliferyl β-D-galactoside or an equimolar mixture of the two, over a final concentration range 0·06–0·5mM. If the two substrates are hydrolysed at the same active site then the K_m values for the two activities should obey the equation:

$$\frac{K_m\text{ (glucoside)}}{K_m\text{ (galactoside)}} = \frac{V_{\text{glucoside}} - V_m}{V_m - V_{\text{galactoside}}}$$

where $V_{\text{glucoside}}$ and $V_{\text{galactoside}}$ are maximum velocities with glucoside and galactoside as substrate and V_m is the maximum velocity with an equimolar mixture of the two substrates (Foster & Niemann, 1959). From the Lineweaver & Burk (1934) plots, the ratio of the K_m values was 1·34 and the expression on the right-hand side of the equation had a value of 1·24. These kinetic data appear to confirm, within the limits of the technique, that a single active site is involved in the hydrolysis of the substrates. The average K_m values from six results for β-glucosidase and β-galactosidase were 0·35mM and 0·24mM respectively.

Microsomal N-*acetyl*-β-*glucosaminidase*. The determination of free and total activities of *N*-acetyl-β-glucosaminidase suggested that the Mic fraction contained an enzymic form specific to that fraction, possibly identifiable by its higher mobility on starch-gel electrophoresis (Fig. 2). The microsomal enzyme could not be distinguished by its molecular weight, for, when either the LM fraction (3ml.) or the Mic fraction (3ml.) was filtered through Sephadex G-200, the *N*-acetyl-β-glucosaminidase activity emerged with the same elution volume as rabbit-muscle aldolase (mol.wt. 140000–150000; Stellwagen & Schachman, 1962).

The microsomal pellet was resuspended in a medium containing sodium deoxycholate (Kirsch, 1962) and recentrifuged at 100000*g* for 45min. The resulting pellet, consisting mainly of ribosomes, contained only 13% of the *N*-acetyl-β-glucosaminidase activity; the remainder was contained in the solubilized protein and membrane fragments in the supernatant. When the supernatant activity was subjected to starch-gel electrophoresis, it no longer migrated as a sharp zone (cf. Fig. 2) but as a broad band stretching from the origin to beyond the original sharp zone.

DISCUSSION

The sedimentability and structure-linked latency attributed to lysosomal enzymes have been demonstrated for a number of mammalian glycosidases (de Duve, 1963). In particular, β-galactosidase and β-glucuronidase were found mainly in the lysosomes of rat kidney, though significant amounts were in the soluble fraction (Wattiaux-De Coninck *et al.* 1965; Shibko & Tappel, 1965). Rat-liver *N*-acetyl-β-glucosaminidase is also lysosomal (Sellinger, Beaufay, Jacques, Doyen & de Duve, 1960), but its localization in the rat kidney had not been established. In appearing predominantly in the soluble fraction, rat-kidney β-glucosidase therefore presents a marked contrast with the other

glycosidases that have been studied, even though its pH optimum places it in the class of acid hydrolases. Only 14% of the total β-glucosidase activity was contained in, or absorbed on, sedimentable material. There was no evidence for even a minor β-glucosidase component specific to the lysosomes, as the same single sharp zone of activity was found on starch-gel electrophoresis in each fraction.

A distinguishing feature of the soluble β-glucosidase is its wide specificity towards 4-methylumbelliferone substrates in comparison with lysosomal glycosidases. It hydrolyses the β-D-galactoside at about one-third of the rate of the β-D-glucoside. That this enzyme is also active as an α-L-arabinosidase was reported by Robinson *et al.* (1967) on the basis of the coincidence of β-D-glucosidase, β-D-galactosidase and α-L-arabinosidase zones on starch-gel electrophoresis and the inhibition of all three activities in this zone by glucono-(1→5)-lactone. The same technique shows that this enzyme can also hydrolyse 4-methylumbelliferyl β-D-xyloside. The soluble glycosidase appears therefore to be non-specific with regard to the C-4 and C-6 configuration of the glycoside. However, it will not hydrolyse β-glucuronides or *N*-acetyl-β-glucosaminides. A glycosidase of wide specificity has been found in the kidneys of a number of mammals including man (R. G. Price & N. Dance, unpublished work), and the corresponding enzyme in pig kidney is at present under investigation in these Laboratories (Robinson & Abrahams, 1967). Ox kidney also contains an enzyme of this type, apparently identical with that observed in ox liver by Chytil (1965). On the other hand, our preliminary experiments show that rat liver does not contain a similar enzyme. Clearly, rat kidney contains a non-particulate glycosidase able to hydrolyse a number of synthetic glycosides. It would be wrong to assume that the relative activities towards 4-methylumbelliferone substrates reflect the activities towards the normal substrates of this enzyme, the nature of which is, at present, obscure.

The presence of a relatively non-specific soluble glycosidase leads to a bimodal distribution of β-galactosidase activity among the subcellular fractions of rat kidney, with the greatest activity in the lysosome-rich and soluble fractions. The proportion of the total β-galactosidase activity appearing in the soluble fraction will depend on the ratio of lysosomal β-galactosidase (optimum pH 3·7) to soluble β-galactosidase (optimum pH 5·5). As a high pH is required to maintain the integrity of the lysosomal structure, the contribution of the soluble enzyme will be emphasized in tissue-fractionation studies. The report (Levvy, McAllan & Hay, 1962) that 80% of the β-galactosidase activity of an ox-liver sucrose homogenate was in the soluble fraction might be explained by the high activity of the soluble type of β-galactosidase in that tissue. In general the presence of the soluble β-galactosidase will obscure the existence of a specifically lysosomal β-galactosidase unless the various fractions are monitored by starch-gel electrophoresis or other suitable method of protein separation. In comparisons of β-galactosidase with other enzymes appearing in the lysosome it is therefore necessary to eliminate the contribution of the soluble glycosidase to the total activity, for example by the inclusion of glucono-(1→5)-lactone in the assay solutions.

Rat-kidney *N*-acetyl-β-glucosaminidase also showed a bimodal distribution; the activities were greatest in the lysosomal and microsomal fractions. The major part was in the lysosomal fraction, and displayed the same degree of latency as β-galactosidase and β-glucuronidase, but a higher proportion sedimented in the microsomal fraction than would be expected by analogy with the distribution of these two enzymes. Further, the *N*-acetyl-β-glucosaminidase activity is not latent in the microsomal fraction to the same extent as the other glycosidases. About 15% of the total activity of rat kidney occurs in a non-latent form associated with the endoplasmic reticulum. In some tissues the microsomal contribution may predominate as was found by Conchie & Hay (1963) in mouse kidney, spleen and T2146 tumour.

The dual localization of β-glucuronidase in the endoplasmic reticulum and the lysosomes of mouse liver and rat preputial gland has been demonstrated by a histological technique (Fishman, Goldman & DeLellis, 1967). However, in our experiments the subcellular distribution of rat-kidney β-glucuronidase was compatible with a purely lysosomal origin.

de Duve (1964) lists a number of enzyme activities with bimodal subcellular distributions arising from the superimposition of the unimodal distributions of two distinct homologous enzyme forms. Included in this group are rat-liver deoxyribonuclease, arylsulphatases and phosphatases. To these enzymes may now be added rat-kidney β-galactosidase and *N*-acetyl-β-glucosaminidase, though the presence of two distinct forms of the latter remains to be shown. As the relative proportions of homologous enzymes may vary between different tissues it would be dangerous to compare one tissue with another without first characterizing the cellular distribution of all the homologous forms.

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