Mammalian Glycosidases

4. THE INTRACELLULAR LOCALIZATION OF β -GALACTOSIDASE, α -MANNOSIDASE, β -N-ACETYLGLUCOSAMINIDASE AND α -L-FUCOSIDASE IN MAMMALIAN TISSUES*

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The lysosome theory, whether it is true or not, embodies biological features of general importance. In its original form (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955) this theory favours the view that 'lysosomes form a single population of enzymically homogeneous granules', within which the hydrolytic enzymes display structure-linked latency, becoming active only on the death of the cell. This theory has become considerably more elastic, as for instance in the statement that 'individual lysosomal particles may differ quite widely from each other in a number of properties such as size, structure, relative enzymic equipment, density in various media and sedimentation coefficient' (de Duve, 1962). Nevertheless, cytologists have used acid-phosphatase activity as the sole and sufficient enzymic criterion in studies in which they claim to observe lysosomes as a new type of subcellular particle (e.g. Holt, 1959; Novikoff, 1961; Holt & Hicks, 1962; Bitensky & Gahan, 1962). So far as histochemical work is concerned, another important factor has been ignored in attempts to study the lysosome, namely that the original work on which the definition is based dealt with gross differences in subcellular localization as measured by centrifugal fractionation. Fractions containing small amounts of enzyme activity were ignored. Such fractions, however, may represent the only 'free' or easily accessible enzyme in the cell, and would thus be the most readily identified by histochemical techniques. The lysosomal enzyme by definition is not readily accessible to substrate.

Rodent liver was used almost exclusively in developing the lysosome theory. However, the intracellular localization of β -glucuronidase in other mammalian tissues has been investigated (Conchie, Hay & Levvy, 1961). In most of the tissues examined, the bulk of the enzyme was associated with the cytoplasmic granules, within which, in sucrose homogenates, only a fraction of the potential activity was observed: the actual degree of latency displayed by the particulate enzyme varied from tissue to tissue. Mouse spleen and

certain mouse tumours, however, had a considerable proportion of their β -glucuronidase activity in the soluble fraction. No appreciable fraction of the enzyme activity appeared to be in the nucleus.

The observation that β -glucuronidase in animal tissues is not always particle-bound, or if particle-bound is not necessarily latent to any marked degree, has led to the present investigation on the intracellular localization of those mammalian glycosidases associated with β -glucuronidase, namely, β -galactosidase, α -mannosidase, β -N-acetylglucosaminidase and α -L-fucosidase (Conchie, Findlay & Levvy, 1959; Levvy & McAllan, 1961; Levvy, McAllan & Hay, 1961). It was considered that such a study would prove of value in further elucidating the properties and function of the lysosomal enzymes.

The intracellular localization of β -galactosidase and β -N-acetylglucosaminidase in rat liver has also been studied by Sellinger, Beaufay, Jacques, Doyen & de Duve (1960), who also mentioned a few preliminary experiments with α -mannosidase.

EXPERIMENTAL

Substrates

o-Nitrophenyl β -D-galactoside, prepared by the method of Seidman & Link (1950), had m.p. 193° and $[\alpha]_D^{25} -51^\circ$ (c 1.0) in water. *p*-Nitrophenyl α -D-mannoside was prepared as described by Conchie *et al.* (1959). *p*-Nitrophenyl *N*-acetyl- β -D-glucosaminide was prepared as described by Findlay, Levvy & Marsh (1958). *p*-Nitrophenyl α -L-fucoside was prepared as described by Levvy & McAllan (1961). The yield from the tri-*O*-acetate was greatly improved by using the method of deacetylation described by Leaback (1960).

Preparation of homogenates

Tissues were homogenized in a cooled Potter-Elvehjem all-glass homogenizer, with a diameter clearance of 0.23–0.43 mm., driven by an electric motor (average speed 1500 rev./min.). For the preparation of sucrose homogenates, tissues were washed with ice-cold 0.25 M-sucrose, pressed between pieces of hardened filter paper, weighed and homogenized in 0.25 M-sucrose. Periods of homogenizing were as stated in the text. Enzyme concentrations were adjusted so that not more than 10% of each substrate was hydrolysed during the period of assay.

* Part 3: Conchie, Hay & Levvy (1961).

Fractionation of homogenates

Homogenates were fractionated as described by Conchie *et al.* (1961). Fraction I, obtained by layering the 0.25 M-sucrose homogenate over 0.34 M-sucrose and centrifuging at 700g for 10 min., consisted mainly of unbroken cells and nuclei. Fraction II, obtained by centrifuging the supernatant and washings from fraction I at 5000g for 10 min., contained the larger cytoplasmic particles. Fraction III, which contained the small cytoplasmic particles, was obtained by centrifuging the supernatant from fraction II at 70000g for 1 hr. In some experiments (see below) acetic acid-NaOH buffer, pH 5.2, was used to obtain fraction III. The final supernatant and washings, which were particle-free, formed fraction IV.

Enzyme assays

In experiments involving the measurement of enzyme activity of untreated homogenates in 0.25 M-sucrose, the incubation buffers for all assays contained 0.25 M-sucrose (final concentration). In osmotic-activation experiments, sucrose of the appropriate concentration was present in the incubation buffers. For measurement of the total activity of sucrose homogenates Triton X-100 (0.1%, w/v) was included (Walker, 1952).

α -Mannosidase and β -galactosidase. Assay conditions were as described by Conchie & Hay (1959), but to diminish enzyme blanks assays were stopped by the addition of 2 ml. of 5% (w/v) trichloroacetic acid. The mixture was centrifuged at 1500g for 10 min., 4 ml. of the supernatant was added to 4 ml. of 0.4 M-glycine-NaOH buffer, pH 11.2, and the colour intensity of the liberated nitrophenol was measured on the Spekker photoelectric absorptiometer with Ilford no. 601 violet filters (peak transmission 430 m μ). When the β -galactosidase activity of sucrose homogenates was being measured, assays were done at pH 5, since at pH 3 there was loss of latency (see below).

β -N-Acetylglucosaminidase. Liberation of *p*-nitrophenol from *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide was measured as described by Findlay *et al.* (1958).

α -L-Fucosidase. Assay conditions were as described by Levvy & McAllan (1961), but the reaction was stopped by the addition of trichloroacetic acid and the colour of the liberated *p*-nitrophenol developed as described above for α -mannosidase.

RESULTS

Distribution of glycosidase activities after fractionation. Studies on the intracellular localization of β -glucuronidase had shown that to compare the distribution of enzyme activity in the fractions obtained from various tissues it was necessary to control the clearance in the homogenizer and the period of homogenization (Conchie *et al.* 1961). The most satisfactory procedure for β -glucuronidase and the related glycosidases studied in the present work was to homogenize four times for 15 sec. This gave maximum disintegration of the cells with minimum disruption of the cell particles. The distribution of β -galactosidase, α -mannosidase and β -N-acetylglucosaminidase obtained by this pro-

cedure for a number of mouse and rat tissues is shown in Table 1.

Fraction I comprises mainly unbroken cells and nuclei, fraction II the usual mitochondrial fraction (large granules), and fraction III the microsomal fraction (small granules). Fraction IV, the clear supernatant, is defined as soluble enzyme. For assay, all fractions were suspended in 0.25 M-sucrose, and enzyme activities were measured in the presence of Triton X-100, the results being expressed as percentages of the activities of the whole homogenate in 0.25 M-sucrose, also assayed in the presence of Triton X-100. In many cases all three enzymes were assayed in the same enzyme preparation. Glycosidase activities of sucrose homogenates in the presence of Triton X-100, after correction for any small inhibition caused by sucrose, were always equal to those of the corresponding water homogenates. Sucrose never caused more than 10% inhibition of any enzyme.

The distribution of β -galactosidase, α -mannosidase and β -N-acetylglucosaminidase activities in mouse liver was similar to that observed for β -glucuronidase in that the bulk of the enzyme activity was confined to the two granular fractions. Among the different enzymes there was evidence of small variations. The same general pattern was also observed in rat liver and in mouse kidney. A different pattern was observed in mouse spleen and certain mouse tumours, and qualitative differences between enzymes were marked. In both of the latter types of tissue there was a large proportion of β -galactosidase and α -mannosidase activities in the soluble enzyme fraction, with very little activity in the mitochondrial fraction. In contrast, β -N-acetylglucosaminidase displayed little soluble activity in mouse spleen and tumours, and the bulk of the enzyme was in the microsomal fraction. Ehrlich tumours gave results similar to those shown in Table 1 for T 2146 tumours. Rat spleen resembled mouse spleen, except in that it lacked soluble α -mannosidase. [β -Glucuronidase in rat spleen followed the pattern described for mouse spleen by Conchie *et al.* (1961), being divided mainly between the microsomal and soluble fractions.] Enzyme activities and distribution patterns for the livers of C3H mice, previously examined because of their low β -glucuronidase activities, resembled those for white mice in the present work.

In some further fractionations of rat-liver homogenates, the particulate glycogen was separated by the procedure of Drochmans (1962). Fraction III was obtained by adding acetic acid-sodium hydroxide buffer, pH 5.2, and centrifuging at 1100g for 5 min. Particulate glycogen was then separated from the supernatant by centrifuging at 20000g for 50 min. None of the glycosidases showed any activity in the glycogen fraction.

Table 1. *Distribution of glycosidase activities in sucrose homogenates of mammalian tissues*

Details of homogenization, fractionation and assays are given in the text. Results are expressed as percentages of the activity of a whole sucrose homogenate: all assays were done in the presence of Triton X-100 (0.1%). Typical total activities for the various tissues are shown in Tables 3-5. The values in parentheses were obtained in experiments in which fraction III was agglutinated with 0.1 M-acetic acid-NaOH buffer, pH 5.2, and sedimented at 1500g for 10 min. The fractions had the following compositions: I, unbroken cells and nuclei; II, mitochondria; III, microsomes; IV, clear supernatant (soluble enzymes).

	Fraction	β -Galactosidase*	α -Mannosidase	β -N-Acetyl-glucosaminidase
Mouse liver	I	0 (0)	1 (1)	2 (2)
	II	61 (61)	37 (37)	56 (58)
	III	23 (23)	47 (47)	31 (28)
	IV	9 (9)	14 (14)	6 (8)
	Recovery	93 (93)	99 (99)	95 (96)
Mouse kidney	I	9 (3)	11 (11)	11 (5)
	II	39 (45)	41 (41)	37 (41)
	III	22 (33)	44 (28)	45 (33)
	IV	22 (16)	16 (23)	6 (20)
	Recovery	92 (97)	112 (103)	99 (99)
Mouse spleen	I	7 (3)	9 (0)	21 (1)
	II	10 (13)	20 (8)	15 (6)
	III	17 (29)	32 (51)	46 (79)
	IV	72 (50)	44 (35)	12 (12)
	Recovery	106 (95)	105 (94)	94 (98)
Mouse T2146 tumour	I	11 (2)	13 (9)	15 (5)
	II	12 (5)	15 (9)	11 (6)
	III	39 (43)	34 (34)	48 (74)
	IV	42 (46)	44 (49)	18 (11)
	Recovery	104 (96)	106 (106)	92 (96)
Rat liver	I	2 (1)	1 (1)	2 (2)
	II	53 (64)	41 (45)	52 (62)
	III	34 (27)	50 (48)	32 (36)
	IV	6 (12)	8 (7)	4 (3)
	Recovery	95 (104)	100 (101)	90 (103)
Rat spleen	I	1 (4)	10 (3)	17 (14)
	II	8 (16)	27 (13)	33 (23)
	III	36 (37)	50 (71)	37 (49)
	IV	52 (41)	0 (1)	10 (8)
	Recovery	97 (98)	87 (88)	97 (94)

* Assays at pH 3.0.

The distribution of α -L-fucosidase in rat liver and rat spleen is given in Table 2; it resembled that of β -galactosidase in the rat. Studies of α -L-fucosidase in the mouse were not possible owing to its low activity in mouse tissues.

Use of acetate for fractionation. In initial experiments, agglutination by means of acetic acid-sodium hydroxide buffer (Conchie *et al.* 1961) was used, both for complete fractionations and for obtaining non-sedimentable fractions from sucrose and water homogenates. This method is convenient, and in general gives results identical with those obtained by the high-speed-centrifuging procedure (see Table 1). In a few specific cases, however, higher soluble activities were obtained by the acetate separation method, as a result of extraction of particle-bound enzyme by the buffer in sucrose or water homogenates.

Glycosidase activities in sucrose homogenates. Tissue homogenates in 0.25 M-sucrose display only

Table 2. *Distribution of α -L-fucosidase activities in sucrose homogenates of rat liver and rat spleen*

Details of homogenization, fractionation and assays are given in the text and Table 1. Results are expressed as percentages of the activities of a sucrose homogenate. All assays were done in the presence of Triton X-100 (0.1%). Total activities, expressed as μ g. of *p*-nitrophenol liberated/g. wet wt. of tissue/hr., were: liver, 1190; spleen, 2130.

Fraction	Activity (%)	
	Rat liver	Rat spleen
I	0	3
II	50	17
III	33	45
IV	10	34
Recovery	93	99

a fraction of their total hydrolase activity as measured in the corresponding water homogenates (Berthet & de Duve, 1951; Walker, 1952; Gianetto

& de Duve, 1955; Conchie *et al.* 1961). Further, though the water homogenates are fully active, only a proportion of the enzyme activity is non-sedimentable, the remainder still being bound to the cell fragments. Analysis of these variables was made for the glycosidases and tissues studied in the present work. Tables 3-5 show the enzyme activities of iso-osmotic (0.25M) sucrose homogenates of some mouse and rat tissues expressed as percentages of their total activities, together with the percentage activities in the soluble fractions from both sucrose and water homogenates.

The optimum pH of β -galactosidase in the tissues studied is 3.0. At this pH, however, the β -galactosidase activities of iso-osmotic sucrose homogenates were equal to those of corresponding water homogenates, i.e. there was instantaneous and complete activation of the sucrose homogenates at this pH.

When assays were done at pH 5.0, β -galactosidase resembled the other glycosidases in that the activities of iso-osmotic sucrose homogenates were only a fraction of those of corresponding water homogenates measured at pH 5.0. Accordingly, all β -galactosidase assays concerned in Table 3 were carried out at pH 5.0. Tissue activities at pH 3.0 are given by Conchie *et al.* (1959).

In all cases the percentage enzyme activities observed in whole iso-osmotic sucrose homogenates were considerably greater than those in the derived soluble fractions. The difference between the two values measures the activity of the enzyme within the granules, together with the contribution of any soluble enzyme adsorbed on their surface. These differences, which are an inverse measurement of latency, varied widely from tissue to tissue with each enzyme.

Table 3. β -Galactosidase activities of tissue homogenates in 0.25M-sucrose, and of the soluble fractions from sucrose and water homogenates

Tissues were homogenized for the single periods shown. The whole sucrose homogenates were assayed in the absence of Triton X-100, and the results are expressed as percentages of the total activities determined in the presence of Triton X-100. The soluble fractions were prepared by removing all particulate matter by centrifuging at 70000g for 1 hr. Results for the sucrose-homogenate soluble fractions are expressed as percentages of the activities of the whole homogenates when treated with Triton X-100. Those for the water-homogenate soluble fractions are expressed as percentages of the activities of the whole water homogenates. Total activities are expressed as μ g. of *o*-nitrophenol liberated/g. wet wt. of tissue/hr. Details of assay conditions are given in the text.

	Period of homogenizing (sec.)	Activity (%)			Total activity* (μ g./g./hr.)
		Sucrose homogenate		Water homogenate.	
		Whole homogenate	Soluble fraction	Soluble fraction	
Mouse liver	15	71	9	51	588
Mouse kidney	30	74	18	51	2525
Mouse spleen	15	80	52	82	2810
Mouse T2146 tumour	30	85	41	73	1490
Rat liver	15	29	9	70	2800
Rat spleen	15	74	53	73	4840

* Measured at pH 5.0.

Table 4. α -Mannosidase activities of tissue homogenates in 0.25M-sucrose, and of the soluble fractions from sucrose and water homogenates

The preparation of homogenates and fractions was as given in Table 3. Total activities are expressed as μ g. of *p*-nitrophenol liberated/g. wet wt. of tissue/hr. Details of assay conditions are given in the text.

	Period of homogenizing (sec.)	Activity (%)			Total activity (μ g./g./hr.)
		Sucrose homogenate		Water homogenate.	
		Whole homogenate	Soluble fraction	Soluble fraction	
Mouse liver	15	68	15	55	5440
Mouse kidney	30	73	10	19	7280
Mouse spleen	15	80	47	57	4660
Mouse T2146 tumour	30	80	36	49	2900
Rat liver	15	61	12	18	5210
Rat spleen	15	72	13	23	1840

Table 5. *β -N-Acetylglucosaminidase activities of tissue homogenates in 0.25M-sucrose, and of the soluble fractions from sucrose and water homogenates*

The preparation of homogenates and fractions was as given in Table 3. Total activities are expressed as μg . of *p*-nitrophenol liberated/g. wet wt. of tissue/hr. Details of assay conditions are given in the text.

	Period of homogenizing (sec.)	Activity (%)			Total activity ($\mu\text{g./g./hr.}$)
		Sucrose homogenate		Water homogenate. Soluble fraction	
		Whole homogenate	Soluble fraction		
Mouse liver	15	68	3	13	33000
Mouse kidney	30	88	5	5	100400
Mouse spleen	15	89	20	34	135300
Mouse T2146 tumour	30	84	11	16	37200
Rat liver	15	82	1	7	46000
Rat spleen	15	79	7	16	22500

Table 6. *Glycosidase activities of tissue homogenates in 0.25M-sucrose after incubation with substrate for various periods*

Tissues were homogenized in 0.25M-sucrose for the single periods shown. Activities of homogenates were measured after incubation with substrate for the periods shown and results are expressed as percentages of the total activities determined by assaying in the presence of Triton X-100. Details of other assay conditions are given in the text.

	Period of homogenizing (sec.)	Duration of assay (min.)		
		15	30	60
α -Mannosidase				
Mouse liver	15	67	70	72
Mouse T2146 tumour	30	69	73	86
β -Galactosidase				
Mouse kidney	30	43	55	73
Mouse spleen	15	51	79	88
β -N-Acetylglucosaminidase				
Mouse spleen	15	75	84	85
Mouse T2146 tumour	30	56	78	83

All results quoted are based on 1 hr. assay values, and some activation of the homogenates during the period of assay must be expected. For assay periods of less than 1 hr., values lie on a non-linear portion of the velocity curve, and are more liable to error the shorter the period of incubation. After 1 hr. the slope of the curve becomes fairly constant, the absolute experimental error is smaller, and comparison between different tissues can be made more accurately. The effect of activation in the assay is illustrated in Table 6, and has been taken into account in drawing comparisons between different enzymes and tissues on the basis of 1 hr. assay values. Errors from activation in the assay enter only into measurements of latency, not of distribution.

The fact that the percentages of non-sedimentable β -N-acetylglucosaminidase (Table 5) in water homogenates of all the tissues were appreciably lower than the corresponding values for the other glycosidases (Tables 3 and 4) could conceivably have been due to the use of citric acid-sodium hydroxide buffer in the assay instead of acetic acid-sodium hydroxide buffer. There was, however, no change in the percentages of non-sedimentable β -galactosidase and α -mannosidase in sucrose homogenates when assays were done in 0.2M-disodium hydrogen phosphate-citric acid buffer.

Effect of increasing substrate concentration on β -galactosidase activity. Walker (1952) found that the β -glucuronidase activity of iso-osmotic sucrose homogenates of mouse liver could be increased by increasing the substrate concentration beyond the optimum for the enzyme, although the activated enzyme is subject to inhibition by excess of substrate. A similar effect was observed when the β -galactosidase activity of an iso-osmotic sucrose homogenate of rat liver was measured with increasing concentrations of *o*-nitrophenyl β -galactoside (Table 7). At a substrate concentration of 30 mM, which caused considerable inhibition in an activated preparation, there was greatly increased activity of the untreated iso-osmotic sucrose homogenate. It was evidently possible to increase the access of the enzyme to the substrate by increasing the substrate concentration.

Owing to the limited solubility of *p*-nitrophenyl α -mannoside, *p*-nitrophenyl *N*-acetyl- β -glucosaminide and *p*-nitrophenyl α -L-fucoside, this type of experiment could not be repeated with these substrates.

Activation of sucrose homogenates. Iso-osmotic sucrose homogenates of mouse and rat tissues showed a progressive activation of all four enzymes (β -galactosidase, α -mannosidase, β -N-acetylglucosaminidase and α -L-fucosidase) as the suspending

medium was made increasingly hypo-osmotic. The osmotic activation of a rat-liver homogenate is shown in Fig. 1.

As observed above, Triton X-100 (0.1 %) added to iso-osmotic sucrose homogenates made them fully active. It did not, however, make the enzymes

completely soluble, the proportion of soluble enzyme being in general only a little higher than that in a water homogenate; neither did treatment of a water homogenate with Triton X-100 make the enzymes fully soluble.

DISCUSSION

The hydrolytic enzymes discussed in the present paper all belong to the group known as 'lysosomal enzymes'. All of them are to some extent particle-bound in the tissues examined, though there is considerable variation in the actual pattern, depending on the enzyme and the tissue. Under the experimental conditions described, some enzyme activity is always found in the final supernatant after fractionation, although this fraction is frequently small. Though such activity is generally regarded as coming from the extragranular cytoplasm, it is possible that some of the activity was originally present behind highly permeable membranes, which may or may not rupture when the cell is homogenized, but which allow immediate diffusion, to reach equilibrium with the artificial medium. Conversely, there will be immediate diffusion of substrate through such membranes. Consequently, it is just such a 'cytoplasmic' fraction that would be prominent in a histochemical study. It has been shown that liver nuclei prepared in sucrose solution by several methods are devoid of certain water-soluble enzymes, such as adenosine deaminase and nucleoside phosphorylase, which are concentrated in nuclei prepared by non-aqueous methods (Stern & Mirsky, 1953; Stern, Allfrey, Mirsky & Saetren, 1952; Allfrey, Stern, Mirsky & Saetren, 1952).

For any given tissue, the differences in the patterns of distribution for the glycosidases could be considerable. Since the enzymes segregate differently, the particles with which they are associated must be heterogeneous both in function and size. Previous workers have observed that there is considerable β -glucuronidase in the microsomal fraction of liver, as well as in the 'mitochondrial' or 'lysosomal' fraction. It has been argued by de Duve (1962) that, compared with other members of the lysosomal group, β -glucuronidase is atypical in this respect. Examination of the results described above for other glycosidases, however, suggests that such a distribution may be the rule rather than the exception. Other workers have also commented on the heterogeneity both in enzymic composition and size of the particles with which the lysosomal enzymes are associated (Whittaker, 1959; Roy, 1960; Franklin, 1962; Zalkin *et al.* 1962). Moreover, when acid-phosphatase activity in fractions from mammalian-liver sucrose homogenates is assayed with substrates

Table 7. *Effect of increasing substrate concentrations on the β -galactosidase activity of a rat-liver homogenate in 0.25M-sucrose, before and after activation with Triton X-100*

The tissue was homogenized in 0.25M-sucrose for a single period of 15 sec. Details of the conditions of assay are described in the text, the pH of assay being 5.0 and the substrate concentrations as shown in the Table. Activation was obtained by assaying in the presence of Triton X-100 (0.1 %). Activities are expressed as μ g. of *o*-nitrophenol liberated/g. wet wt. of tissue/hr.

Concn. of substrate (mM)	Sucrose homogenate activity (μ g./g./hr.)	
	Before activation	After activation
2.5	992	2515
7.5	1155	2575
15.0	1248	2310
30.0	1410	2070

Fig. 1. Enzyme activities in a rat-liver homogenate suspended in various concentrations of sucrose. $\circ$, β -Galactosidase; $\bullet$, α -mannosidase; Δ , β -N-acetylglucosaminidase; $\blacktriangle$, α -L-fucosidase. Activities are expressed as percentages of the total activities measured in a water homogenate of the liver. Details of the conditions of assay are given in the text. Total activities, expressed as μ g. of *o*- or *p*-nitrophenol liberated/g. wet wt. of tissue/hr., were: β -galactosidase, 3110; α -mannosidase, 4500; β -N-acetylglucosaminidase, 34700; α -L-fucosidase, 1130.

other than β -glycerophosphate, such as *p*-nitrophenyl phosphate, the distribution pattern is markedly different, there being considerable activity in the final supernatant (Neil & Horner, 1962); nitrophenol derivatives are often used in lysosomal studies. There appears to be no justification for the use of acid phosphatase, with β -glycerophosphate as the only substrate, as the marker enzyme in tissue sections for identifying unknown organelles that are assumed to be the sole depot, not only of acid phosphatase, but also of certain other hydrolytic enzymes (see above).

Under suitable conditions all of the glycosidases investigated exhibited the phenomenon of latency in sucrose homogenates of all the tissues examined, though to different degrees, whereas water homogenates displayed full activity. Sometimes the degree of latency displayed by a particle-bound enzyme was small, i.e. the activity in the sucrose homogenate was too high to be explained by the soluble enzyme present. In certain tissues, such as spleen and tumours, some enzymes displayed considerable activity in the supernatant fraction from the sucrose homogenates. In ox liver, 80% of the β -galactosidase activity was found to be soluble (i.e. 'cytoplasmic') on centrifugal fractionation of homogenates made in 0.25 M-sucrose solution (Levy, McAllan & Hay, 1962).

Such variability in the distribution of acid-hydrolase activity in the examples discussed above makes it difficult to accept the concept of the lysosome as originally envisaged by de Duve. If the enzymes dealt with in the present study are situated in special particles distinct from mitochondria and microsomes, then individually they must differ in sedimentation properties and stabilities in different tissues, and must differ between each other in stability and situation within a single tissue. What is required is a new definition of the term that will embrace all the different shades of meaning and reservations attached by different authors to the term. At one extreme of opinion, the mere suggestion that the enzyme has an autolytic or catabolic function is enough to label it 'lysosomal'; at the other, the original 'lethal-bag' theory is adhered to strictly. The lysosome theory does embrace phenomena of great importance in the regulation of cell metabolism, but it may be that these do not apply to hydrolytic enzymes only. Such enzymes may merely more conveniently measure factors that apply equally to other enzymes in all types of subcellular particles. Without separation of the different anabolic and catabolic activities of the cell it could not exist, and 'membranes' of some type or other are an essential postulate. The term 'lysosomal', in contrast with 'latent', however, becomes meaningless unless assumptions about the function of the enzymes are

introduced. It is difficult to see what distinction can be drawn on grounds of function between 'lysosomal' enzymes and those numerous other hydrolytic enzymes that are not particle-bound or are devoid of latency. Nevertheless, however the lysosome theory is interpreted, changes in the sedimentation pattern of an enzyme under pathological conditions may still be biologically valid, as in our findings for glycosidases in tumours.

SUMMARY

1. The intracellular localization of β -galactosidase, α -mannosidase, β -*N*-acetylglucosaminidase and α -L-fucosidase in some tissues of the mouse and the rat has been studied by centrifugal fractionation of homogenates in 0.25 M-sucrose.

2. All the glycosidases were particle-bound to various degrees, the extent depending on the glycosidase and the tissue.

3. In some tissues there was considerable heterogeneity in the patterns of distribution for the enzymes.

4. The degree of latency exhibited in the particles by each enzyme varied from tissue to tissue.

5. The significance of these results with reference to the lysosome theory is discussed.

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Mammalian Fucosidases

3. β -D-FUCOSIDASE ACTIVITY AND ITS RELATION TO β -D-GALACTOSIDASE*

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During a study of mammalian tissues for α -L-fucosidase activity (Levy & McAllan, 1961), the preparations were seen to cause rapid hydrolysis of *p*-nitrophenyl β -D-fucoside, and the present paper deals with attempts to discriminate between the enzyme responsible and the extensively studied β -D-galactosidase in mammalian tissues (Conchie, Findlay & Levvy, 1959; Conchie & Hay, 1959; Levvy, McAllan & Hay, 1962). β -D-Fucosidase and β -D-galactosidase activity in the limpet, *Patella vulgata*, have been shown to be due to two different enzymes (Levy & McAllan, 1963). Levvy *et al.* (1962) have also shown that rat-epididymis β -D-galactosidase is powerfully inhibited by solutions of galactonolactone, but not of fuconolactone, whereas ox-liver β -D-galactosidase is powerfully inhibited by fuconolactone solutions, and only relatively feebly by galactonolactone solutions: in the course of this same work it was found that the potency of both inhibitors was dependent upon the proportion present in the solution in the form of the (1 $\rightarrow$ 5)-lactone.

* Part 2: Levy & McAllan (1961).

EXPERIMENTAL

Enzyme preparations. The freshly dissected tissues were homogenized in water, incubated for 1 hr. at pH 5.2 in acetate buffer, and precipitated from the clear extract with (NH₄)₂SO₄ between 20 and 80% saturation (Levy & McAllan, 1961). As with β -D-galactosidase (Levy *et al.* 1962), incubation was not really necessary for extraction of the β -D-fucosidase activity in water homogenates of rat epididymis and ox liver, but to retain complete rat-epididymis β -D-fucosidase activity it was necessary to add NaCl to the preparation at a final concentration of 0.1M (see below).

Substrates and inhibitors. The syntheses of *p*-nitrophenyl β -D-fucoside and *o*-nitrophenyl β -D-galactoside, and the preparation of solutions of fuconolactone and galactonolactone containing maximum amounts of the inhibitory (1 $\rightarrow$ 5)-lactones, are described by Levy & McAllan (1963). *p*-Nitrophenyl β -D-galactoside tetra-*o*-acetate was made by the general method of Glaser & Wulwek (1924), and deacetylated to give *p*-nitrophenyl β -D-galactoside by the procedure of Leaback (1960). Because earlier constants for these well-known compounds are not easily found in the literature, they are given in Table 1, together with our own and other recent values. Apart from Goebel & Avery (1929), who condensed the acetobromo-sugar with silver

Table 1. *Constants for p-nitrophenyl β -D-galactoside and its tetra-O-acetate*

Reference	Galactoside		Tetra-O-acetate	
	m.p.	$[\alpha]_D$ in water	m.p.	$[\alpha]_D$ in CHCl ₃
Goebel & Avery (1929)	181–182°	—	144–145°	– 8.3°
Aizawa (1939)	170	– 75°	138	—
Snyder & Link (1952, 1953)	—	– 85	—	– 9
Beiser, Burke & Tanenbaum (1960)	173–175	—	139–140	—
Heyworth & Walker (1962)	181–182	– 84	138	– 12
This paper	183–184	—	147–148	– 9