Novikoff, A. B. (1961). In *The Cell*, vol. 2, p. 423. Ed. by Brachet, J. & Mirsky, A. E. New York: Academic Press Inc.
Roy, A. B. (1960). *Biochem. J.* **77**, 380.
Seidman, M. & Link, K. P. (1950). *J. Amer. chem. Soc.* **72**, 4324.
Sellinger, O. Z., Beaufay, H., Jacques, P., Doyen, A. & de Duve, C. (1960). *Biochem. J.* **74**, 450.
Stern, H., Allfrey, V. G., Mirsky, A. E. & Saetren, H. (1952). *J. gen. Physiol.* **35**, 559.
Stern, H. & Mirsky, A. E. (1953). *J. gen. Physiol.* **37**, 177.
Walker, P. G. (1952). *Biochem. J.* **51**, 223.
Whittaker, V. P. (1959). *Biochem. J.* **72**, 694.
Zalkin, H., Tappel, A. L., Caldwell, K. A., Shibko, S., Desai, I. D. & Holliday, T. A. (1962). *J. biol. Chem.* **237**, 2678.

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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 (Levvy & 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 (Levvy & 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→5)-lactone.

* Part 2: Levvy & 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 $(NH_4)_2SO_4$ between 20 and 80% saturation (Levvy & McAllan, 1961). As with β-D-galactosidase (Levvy *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 0·1 M (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→5)-lactones, are described by Levvy & 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*

	Galactoside		Tetra-*O*-acetate	
Reference	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

'*p*-nitrophenolate', all authors employed the method of Glaser & Wulwek (1924), or modifications of it.

Enzyme assay. The incubation mixture contained 1 ml. of 0·2 M-Na_2HPO_4–0·1 M-citric acid buffer (McIlvaine, 1921), 0·5 ml. of enzyme preparation and 2 ml. of 5 mM-substrate. The volume was made up to 4 ml. with water or inhibitor solution. The final pH with all three substrates was 4·5 for ox liver and 3·1 for rat epididymis. (Solutions of the inhibitors are reasonably stable at these pH values, which were kept constant for each tissue preparation with different substrates, to facilitate comparison of inhibitor effects.) After 1 hr. at 37°, 4 ml. of 0·4 M-glycine–NaOH buffer, pH 10·5, was added, the mixture centrifuged and the liberated nitrophenol measured on the Spekker photoelectric absorptiometer with Ilford no. 601 violet filters (peak transmission 430 mμ). The substrates were quite stable in the alkaline medium, and the usual enzyme and substrate controls were made.

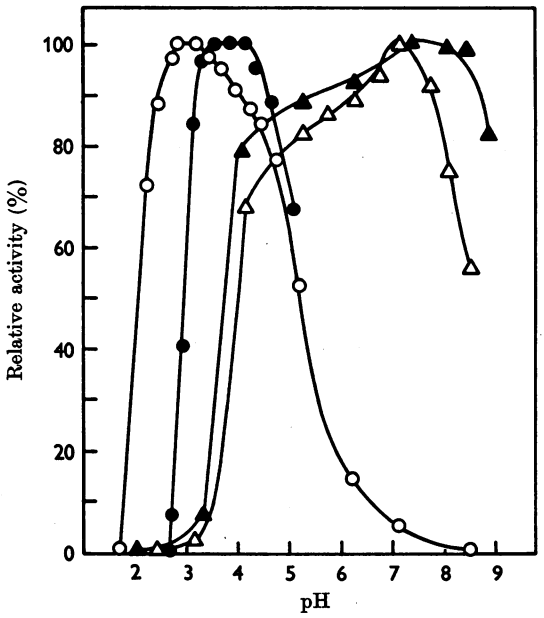

Fig. 1. Effect of pH on the rate of hydrolysis of 2·5 mM-*p*-nitrophenyl β-D-galactoside by rat epididymis (○) and ox liver (△), and of 2·5 mM-*p*-nitrophenyl β-D-fucoside by rat epididymis (●) and ox liver (▲) in phosphate–citrate buffer. At pH values below pH 2·2 HCl was added to the buffer.

RESULTS

Variation in activity with pH. Fig. 1 shows pH–activity curves for the hydrolysis of *p*-nitrophenyl β-D-fucoside and *p*-nitrophenyl β-D-galactoside by ox-liver and rat-epididymis preparations. The curves for β-D-galactosidase activity are very similar to those obtained in previous work, *o*-nitrophenyl β-D-galactoside being used as substrate (Conchie & Hay, 1959; Levvy *et al.* 1962). Results for β-D-fucosidase bore a general resemblance to those obtained for β-D-galactosidase in the earlier work in that rat liver and ox epididymis both resembled rat epididymis in displaying an optimum at pH 4, and in that changes in the composition of the buffer had little effect on pH–activity curves. Addition of sodium chloride to the rat-epididymis homogenate (see below) did not alter its pH–activity curve for β-D-fucosidase activity.

Fractionation of the preparations. There was no indication that the β-D-fucosidase and β-D-galactosidase activity in rat epididymis or ox liver could be separated by fractional precipitation with ammonium sulphate (Table 2). On centrifugal fractionation of homogenates made in 0·25 M-sucrose solution (Conchie, Hay & Levvy, 1961), more than 80 % of the β-D-fucosidase activity in ox liver was found to be soluble, whereas more than 80 % of the activity in rat liver was bound to the subcellular particles: these paralleled the partitions of β-D-galactosidase activity (cf. Levvy *et al.* 1962).

Table 2. *Fractionation of rat-epididymis and ox-liver preparations with ammonium sulphate*

The enzyme in the clear, cell-free extracts was precipitated with ammonium sulphate between the limits of saturation shown. Assays were done with *p*-nitrophenyl β-D-fucoside and *o*-nitrophenyl β-D-galactoside, and the activities towards each substrate are expressed as a percentage of the total before fractionation. In μg. of nitrophenol/g. of tissue, the 100 % values were: rat-epididymis β-D-fucosidase, 5300; rat-epididymis β-D-galactosidase, 34000; ox-liver β-D-fucosidase, 25000; ox-liver β-D-galactosidase, 36000.

Saturation with $(NH_4)_2SO_4$ (%)	Rat epididymis*		Ox liver	
	β-D-Fucosidase (%)	β-D-Galactosidase (%)	β-D-Fucosidase (%)	β-D-Galactosidase (%)
0–20	0	0	0	0
20–30	0	0	1	0
30–40	2·5	1	7	8
40–50	84	76	36	40
50–60	7	8	41	45
60–70	2·5	2	5	6
70–80	0	0	0	0

* Sodium chloride present during the extraction of the enzyme.

Variation in stability with pH. When ox liver was put through the usual preparatory procedure (see the Experimental section), the recovery of both β-D-fucosidase and β-D-galactosidase activity after ammonium sulphate fractionation was satisfactory. With rat epididymis, on the other hand, there was up to 50 % loss of β-D-fucosidase activity. This was not reflected in the β-D-galactosidase activity, measured with either *o*- or *p*-nitrophenyl β-D-galactoside. The loss in β-D-fucosidase activity was due not to incomplete extraction, but to its rapid inactivation in the homogenate by the acetic acid–sodium hydroxide buffer, pH 5·2. If the cell-free extract was prepared by merely centrifuging the homogenate at very high speed, the recovery of β-D-fucosidase was satisfactory. Adding sodium chloride or ammonium sulphate in a concentration of 0·1 M to the homogenate overcame the inactivation at pH 5·2.

The effects of these different variables are seen in the pH–stability curves (Fig. 2). Specimens were adjusted to the required pH at 0° with sodium hydroxide or hydrochloric acid, incubated for 1 hr. at 37°, cooled to 0° and brought back to the original pH (rat epididymis, 6·9; ox liver, 5·3). The activities were compared with those of an untreated control that had been kept at 0°.

In ox-liver preparations, the limits of pH stability were identical for β-D-fucosidase and β-D-galactosidase activity. For the latter, assays were done with *p*-nitrophenyl β-D-galactoside; the curve was identical with that previously obtained with *o*-nitrophenyl β-D-galactoside (Levvy *et al.* 1962). So far as these experiments are concerned, therefore, it appears that a single enzyme hydrolyses both galactosides as well as the fucoside.

Heat-inactivation. Heat-inactivation curves for ox-liver β-D-fucosidase were indistinguishable from those given for the β-D-galactosidase activity by Levvy *et al.* (1962). The fall shown in Fig. 2 for the β-D-fucosidase activity of rat-epididymis homogenates in the absence of sodium chloride at about pH 5 did not become more pronounced when the period of incubation at 37° was prolonged up to 4 hr., and elevating the temperature merely led to a simultaneous fall in β-D-fucosidase and β-D-galactosidase activity. Heating to different temperatures for 10 min. at the limit of pH stability (pH 3 or 8) gave indistinguishable curves for the inactivation of β-D-fucosidase and β-D-galactosidase in the water homogenate of rat epididymis, with a steep fall in activity above 40° at pH 8 and above 60° at pH 3.

Hydrolysis for various periods and with various amounts of enzyme. Results for the hydrolysis of *p*-nitrophenyl β-D-fucoside paralleled those described earlier for *o*-nitrophenyl β-D-galactoside (Levvy *et al.* 1962). Hydrolysis by rat epididymis

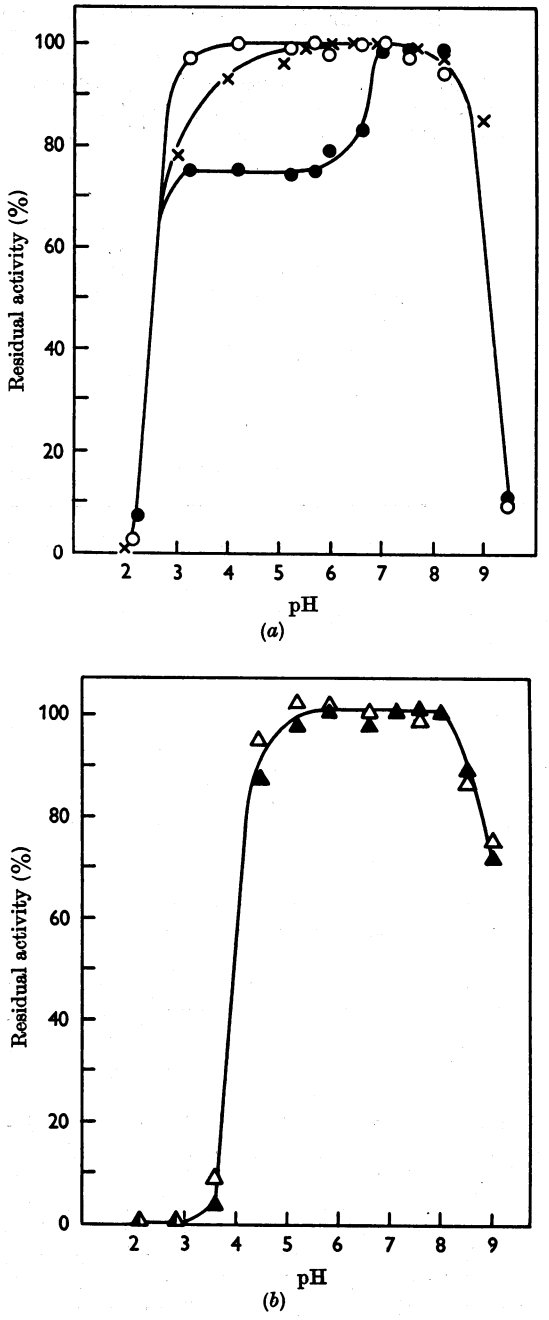

Fig. 2. Residual activity after incubation at various pH values for 1 hr. at 37° of: (*a*) rat-epididymis homogenate in water, acting on 2·5 mM-*o*-nitrophenyl β-D-galactoside (○) and on 2·5 mM-*p*-nitrophenyl β-D-fucoside (●); rat-epididymis homogenate in 0·1 M-NaCl, acting on 2·5 mM-*p*-nitrophenyl β-D-fucoside (×); (*b*) the usual fractionated preparation from ox liver, acting on 2·5 mM-*p*-nitrophenyl β-D-galactoside (△) and on 2·5 mM-*p*-nitrophenyl β-D-fucoside (▲).

Table 3. *Concentrations of galactonolactone and fuconolactone causing 50 % inhibition of the enzymes from ox liver and rat epididymis*

The experiments were done in phosphate–citrate buffer, pH 4·5 (ox liver), or pH 3·1 (rat epididymis), except for the figures quoted from Levvy *et al.* (1962), where the pH was 4·0 (ox liver) and 3·0 (rat epididymis). For activities of enzyme preparations see Tables 4 and 5.

	Concn. for 50 % inhibition (mM)			
	Ox liver		Rat epididymis	
Substrate	Galactono-lactone	Fucono-lactone	Galactono-lactone	Fucono-lactone
2·5 mM-*o*-Nitrophenyl β-D-galactoside	17*	0·09†	0·6†	No inhibition‡
1 mM-*p*-Nitrophenyl β-D-galactoside	—	0·4	—	—
1 mM-*p*-Nitrophenyl β-D-fucoside	No inhibition‡	3·7	0·05	No inhibition‡

* There was 17 % inhibition at 2·5 mM-lactone.
† Quoted from Levvy *et al.* (1962).
‡ No effect observed with 2 mM-inhibitor and 1 mM-substrate.

was linear with both time and enzyme concentration up to about 8 % hydrolysis of the substrate. With the ox-liver enzyme, the amount of fucoside hydrolysed in 1 hr. was directly proportional to the amount of enzyme preparation up to at least 10 % hydrolysis. The rate of hydrolysis, however, fell rapidly over the first hour of incubation with relatively small amounts of enzyme. This effect was not due to the products of hydrolysis, since added D-fucose did not interfere (see below) and *p*-nitrophenol caused only about 10 % inhibition at 0·1 mM. As with β-D-galactosidase, it is concluded that the enzyme is unstable in the presence of its substrate.

Effects of inhibitors. The effects of the two lactone solutions are summarized in Table 3. These results are a useful guide to the kinetic experiments described below. Inhibition always increased indefinitely with increasing inhibitor concentration, i.e. it was never 'partial'. It is evident that the relative inhibitory power of the two lactones was governed more by the source of the enzyme than by the type of substrate. Neither β-D-fucosidase nor β-D-galactosidase in rat epididymis was inhibited by 2 mM-fuconolactone in the presence of mM-substrate. Ox-liver β-D-fucosidase was unaffected by galactonolactone in a similar test. Since inhibition by the lactones increased as a power of the inhibitor concentration (cf. Levvy *et al.* 1962), such a screening test excluded all but very feeble inhibitory effects.

With ox liver from different animals, β-D-galactosidase differed slightly in its susceptibility to inhibition by galactonolactone, and all new data quoted in this and the next section were obtained with a single specimen of ox liver. Tests were done for inhibition by D-galactose and D-fucose, but the only marked effect was with the former, acting on rat-epididymis β-D-fucosidase: there was 57 % inhibition with mM-substrate and inhibitor.

Table 4. *Values obtained with rat epididymis for K_m for different substrates, and the corresponding K_i for galactonolactone*

Substrate concentrations were 0·25–7·5 mM for both galactosides and 1–10 mM for the fucoside. Typical activities of the fractionated enzyme preparation (see the Experimental section), in μg. of nitrophenol/g. of tissue, at 2·5 mM-substrate, were: 32000 with *o*-nitrophenyl β-D-galactoside, 45000 with *p*-nitrophenol β-D-galactoside and 3400 with *p*-nitrophenyl β-D-fucoside. [Since there was no apparent inhibition with fuconolactone (Table 3), K_i can be taken as infinite with this compound.]

Substrate	K_m (mM)	K_i (mM)
o-Nitrophenyl β-D-galactoside	0·38	0·064
p-Nitrophenyl β-D-galactoside	0·27	0·085
p-Nitrophenyl β-D-fucoside	66	0·038

Effect of varying the substrate concentration in the presence and absence of inhibitors. The lactones always acted competitively, and mean values for K_m, the Michaelis constant, and K_i, the inhibitor constant, are given in Tables 4 and 5. In agreement with the results in Table 3, the enzymes in rat epididymis and ox liver behaved quite differently. With rat epididymis (Table 4), the affinity in the preparation was much higher for the galactoside than for the fucoside residue, and higher still for galactonolactone: omission of sodium chloride during the preparation of the enzyme did not alter any of the constants. With ox liver (Table 5), the affinity was high for the fucoside residue and for fuconolactone, less for the galactoside residue, and there was no detectable affinity for galactonolactone.

Table 5. *Values obtained with ox liver for K_m for different substrates, and the corresponding K_i for galactonolactone and fuconolactone*

Substrate concentrations were 0·75–7·5 mM for both galactosides and 0·5–5 mM for the fucoside. Typical activities of the fractionated enzyme preparation (see the Experimental section), in μg. of nitrophenol/g. of tissue, at 2·5 mM-substrate, were: 18000 with *o*-nitrophenyl β-D-galactoside, 20000 with *p*-nitrophenyl β-D-galactoside and 12000 with *p*-nitrophenyl β-D-fucoside.

Substrate	K_m (mM)	K_i (mM) Galactono-lactone	K_i (mM) Fucono-lactone
o-Nitrophenyl β-D-galactoside	0·90	8·5	0·080
p-Nitrophenyl β-D-galactoside	1·1	20	0·29
p-Nitrophenyl β-D-fucoside	0·21	—*	0·88

* Since there was no apparent inhibition (Table 3), K_i can be taken as infinite.

Hydrolysis of *p*-nitrophenyl β-D-fucoside by rat-liver preparations at pH 3·1, gave a mean value of 28 mM for K_m. The curve relating velocity to substrate concentration fell off, without approaching enzyme saturation, up to the limit imposed by the solubility of the substrate (Fig. 3*a*). Up to the same limit, however, the corresponding curve for rat epididymis was almost linear. Saturation of the enzyme in ox liver with the fucoside was rapidly approached (Fig. 3*b*), and the Figure also gives the relative activity of the preparation towards all three substrates employed in this paper.

Hydrolysis of *p*-nitrophenyl β-D-fucoside and *p*-nitrophenyl β-D-galactoside by rat epididymis was competitively inhibited by phenyl β-D-galactoside, with K_i 1·8 and 2·0 mM respectively. These should be compared with values given by Conchie & Hay (1959) for phenyl β-D-galactoside and preparations from rat epididymis: K_i 1·9 mM in the hydrolysis of *o*-nitrophenyl β-D-galactoside and K_m 1·6 mM.

Distribution in the body. Values for the β-D-galactosidase activity of different tissues are given by Conchie *et al.* (1959) for the mouse and the rat, and by Levvy *et al.* (1962) for the ox and the pig. β-D-Fucosidase activity tended to parallel β-D-galactosidase activity in the ox and the pig, but not in the mouse and the rat. So far as rat tissues are concerned, this may be due to failure to achieve saturation of the enzyme with the β-D-fucoside (Fig. 3*a*). A limiting velocity was easily reached in the hydrolysis of *o*-nitrophenyl β-D-galactoside by rat epididymis (Conchie & Hay, 1959). With 20 mM-*p*-nitrophenyl β-D-fucoside, rat epididymis, liver and kidney had activities at pH 3·1 of 30000, 6000 and 10000 μg. of *p*-nitrophenol/g./hr. respectively. The same preparations had the following activities with the anomeric *p*-nitrophenyl α-D-fucoside (2·5 mM) as substrate, at pH 4·5: epididymis, 700; liver, nil; kidney, 1000 μg. of *p*-nitrophenol/g./hr.

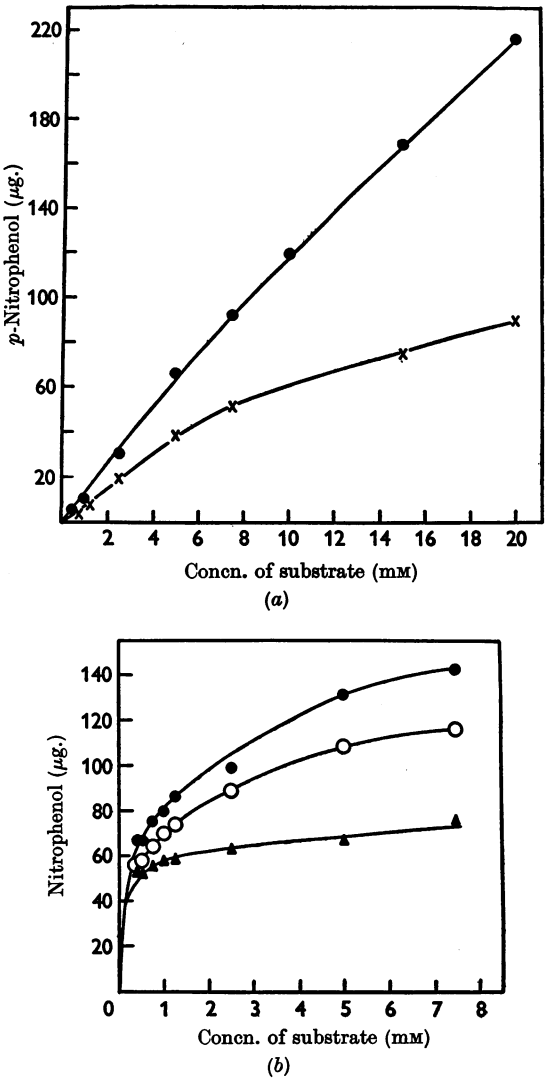

Fig. 3. Effect of varying the substrate concentration on the rate of hydrolysis of: (*a*) *p*-nitrophenyl β-D-fucoside by rat epididymis (●) and rat liver (×); (*b*) *p*-nitrophenyl β-D-galactoside (●), *o*-nitrophenyl β-D-galactoside (○) and *p*-nitrophenyl β-D-fucoside (▲) by ox liver. The ox-liver results are directly comparable.

DISCUSSION

The major question in this work is whether in the mammalian preparations the β-D-fucosidase and the β-D-galactosidase activities are due to one enzyme or two. Since the preparations from rat

epididymis and ox liver differ in their properties, it is best to consider them separately at first.

In rat epididymis, the chief evidence for the two-enzyme theory was the preferential loss of up to 50% of the β-D-fucosidase activity that occurred when the preparation was incubated at 37° for 1 hr. at slightly acid pH. This effect could not, however, be accentuated by increasing the acidity, the temperature or the period of incubation, within the limits of stability of the β-D-galactosidase activity, and it could be overcome by increasing the salt concentration. As an alternative to the two-enzyme theory, it is possible that the preparation contained a single enzyme and an unstable activator, specific for the β-D-fucosidase activity. Dissociable cofactors for hydrolytic enzymes, with different actions towards different types of substrate, are not unknown (Levvy, McAllan & Marsh, 1958; Webb & Morrow, 1959).

The kinetic experiments also were equivocal with rat epididymis. Values of K_i for galactonolactone were almost indistinguishable with *o*- or *p*-nitrophenyl β-D-galactoside as substrate, whereas there was a small, but real, fall in K_i when *p*-nitrophenyl β-D-fucoside was made the substrate. On the other hand, indistinguishable values of K_i were obtained for phenyl β-D-galactoside acting as a competitive inhibitor in the hydrolysis of *p*-nitrophenyl β-D-fucoside or β-D-galactoside. Also in favour of the single-enzyme theory was the failure of fuconolactone to inhibit either type of enzyme activity. In the limpet (Levvy & McAllan, 1963), where the β-D-fucosidase and the β-D-galactosidase activities were easily separated, each enzyme was inhibited by both fuconolactone and galactonolactone, but inhibition was much greater when the substrate and the lactone were derived from the same sugar.

With the ox-liver preparation, all the evidence was in favour of the single-enzyme theory until the inhibition experiments were reached. Fuconolactone inhibited the hydrolysis of *p*-nitrophenyl β-D-fucoside and β-D-galactoside. The value of K_i was greater with the fucoside as substrate, but the difference was smaller than that observed when *o*- and *p*-nitrophenyl β-D-galactoside were compared as substrates. The difference between the two galactosides extended to the K_i values they afforded for inhibition by galactonolactone, but this lactone had no perceptible effect on the hydrolysis of *p*-nitrophenyl β-D-fucoside.

The view that we are dealing with a single enzyme in ox liver is supported by the observation of Wallenfels & Fischer (1960) that after 2000-fold purification a calf-intestine-β-D-galactosidase preparation had considerable β-D-fucosidase activity. With purified enzyme preparations, even crystalline ones, there is always the suspicion that on the one hand two enzymes may have remained associated throughout all the stages of purification, and that on the other hand a dissociable cofactor that alters the specificity of a single enzyme may have been lost. For this reason, inhibitor constants have sometimes been relied upon to decide questions as to the unity of different types of enzyme activity. As in the present instance, however, this approach can give rise to new difficulties. In the present state of knowledge, there is probably no final answer to this kind of problem, except where the two enzyme activities can be completely separated without alteration in their properties.

It was considered that any explanation of the anomalous K_i values for ox liver must account as readily for the difference between *o*- and *p*-nitrophenyl β-D-galactoside as for that between *p*-nitrophenyl β-D-galactoside and β-D-fucoside, and that this was not impossible in terms of a single enzyme. It also seemed possible that the results for rat epididymis could be similarly explained on the basis of a single enzyme, if it were assumed that the preparation contained an unstable activator, specific for the β-D-fucosidase activity.

The affinity of an enzyme for its substrate is usually taken to be the reciprocal of K_m, but in fact it is the reciprocal of K_s, the substrate constant. It is seldom that K_s can be measured, but K_i is readily measured, and in competitive inhibition it is analogous to K_s.

Many glycosidases are competitively inhibited by the aldonolactones corresponding to the substrates in configuration, usually in accordance with the substrate specificity. On the usual enzyme theory, one would expect that experimental values for K_i for a given inhibitor and enzyme would be the same with different substrates. This ignores a factor that is implicit in the acceptance of differing affinities for different substrates. Over and above those groups in the active centre that confer activity and specificity on an enzyme, there may be adventitious subsidiary groups near by that can lead to loose combination or steric hindrance with unessential substituents in the substrate molecule. Such subsidiary groups may be different in enzymes, from different sources, that catalyse the same reaction. With an individual preparation, an increase in the affinity of the enzyme for a substrate resulting from such a secondary reaction would betray itself in an increased K_i for a given competitive inhibitor.

It is probable that β-D-glucuronidase hydrolyses β-D-galacturonides and that β-*N*-acetyl-D-glucosaminidase hydrolyses *N*-acetyl-β-D-galactosaminides, i.e. that neither enzyme is specific for the configuration at C-4 in the sugar residue. Nevertheless, the use of *o*- or *p*-nitrophenol as the aglycone led in some cases to anomalous results in otherwise consistent series of figures for K_i in the inhibition of either of these enzymes by both the

appropriate aldonolactones, and these anomalies were explained along the lines suggested above (Levvy *et al.* 1958; Findlay, Levvy & Marsh, 1958). The same argument can apply when the aglycone remains constant and the sugar residue alters (Walker, Woollen & Heyworth, 1961): even though the hydroxyl group at C-4 in the sugar residue is unimportant in considering the specificity of β-*N*-acetyl-D-glucosaminidase, its configuration may influence combination at a subsidiary group in the enzyme from one source, although not necessarily from others. (A similar argument was put forward by Conchie & Levvy, 1957, to explain the relationship between β-xylosidase and β-glucosidase in the limpet.)

Applying the argument that the affinity of a substrate can alter the apparent affinity of an inhibitor for an enzyme (and vice versa) to the present work, and assuming that we are dealing with a single enzyme in each tissue, it would appear from the values of K_i for galactonolactone in Table 4 that *o*- and *p*-nitrophenyl β-D-galactoside had similar affinities for the enzyme in rat epididymis. The smaller K_i in the presence of the fucoside, however, suggests that this substrate had a lower affinity than the galactosides for the enzyme: this may perhaps be reflected in the grossly different K_m. With ox liver (Table 5), the K_i values for galactonolactone and fuconolactone were greater, in approximately the same proportion, in the presence of *p*-nitrophenyl β-D-galactoside as substrate than the corresponding values with *o*-nitrophenyl β-D-galactoside. In other words, it would appear that *p*-nitrophenyl β-D-galactoside had a higher affinity for this enzyme than had *o*-nitrophenyl β-D-galactoside. In the same way, it can be deduced that the fucoside had a higher affinity than the galactosides for the enzyme, and this conclusion is confirmed by the experimental values for fuconolactone, as compared with galactonolactone.

Although the ox-liver enzyme, on this theory, has a higher affinity for the fucose residue, whether in the form of the lactone or the glycoside, than for the galactose residue, this has no physiological implication. The affinity of an enzyme is often higher for an 'unnatural' than for a 'natural' substrate. D-Fucose has not yet been found in animal tissues.

SUMMARY

1. The enzyme that hydrolyses *p*-nitrophenyl β-D-fucoside has been studied in ox liver and rat epididymis.

2. The enzyme from the two sources differed markedly in its properties.

3. In both types of preparation, the β-D-fucosidase and β-D-galactosidase activities were not easily separated.

4. β-D-Fucosidase activity in rat epididymis was inhibited by galactonolactone, but not by fuconolactone, whereas in ox liver the enzyme activity was inhibited by fuconolactone, but not by galactonolactone.

5. β-D-Galactosidase activity in rat epididymis was inhibited by galactonolactone, but not by fuconolactone, whereas in ox liver this enzyme activity was powerfully inhibited by fuconolactone and more feebly by galactonolactone.

6. The relationship of the two types of enzyme activity in each preparation is discussed.

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REFERENCES

Aizawa, K. (1939). *Enzymologia*, **6**, 321.
Beiser, S. M., Burke, G. C. & Tanenbaum, S. W. (1960). *J. molec. Biol.* **2**, 125.
Conchie, J., Findlay, J. & Levvy, G. A. (1959). *Biochem. J.* **71**, 318.
Conchie, J. & Hay, A. J. (1959). *Biochem. J.* **73**, 327.
Conchie, J., Hay, A. J. & Levvy, G. A. (1961). *Biochem. J.* **79**, 324.
Conchie, J. & Levvy, G. A. (1957). *Biochem. J.* **65**, 389.
Findlay, J., Levvy, G. A. & Marsh, C. A. (1958). *Biochem. J.* **69**, 467.
Glaser, E. & Wulwek, W. (1924). *Biochem. Z.* **145**, 514.
Goebel, W. F. & Avery, O. T. (1929). *J. exp. Med.* **50**, 525.
Heyworth, R. & Walker, P. G. (1962). *Biochem. J.* **83**, 331.
Leaback, D. H. (1960). *J. chem. Soc.* p. 3166.
Levvy, G. A. & McAllan, A. (1961). *Biochem. J.* **80**, 435.
Levvy, G. A. & McAllan, A. (1963). *Biochem. J.* **87**, 206.
Levvy, G. A., McAllan, A. & Hay, A. J. (1962). *Biochem. J.* **82**, 225.
Levvy, G. A., McAllan, A. & Marsh, C. A. (1958). *Biochem. J.* **69**, 22.
McIlvaine, T. C. (1921). *J. biol. Chem.* **49**, 183.
Snyder, J. A. & Link, K. P. (1952). *J. Amer. chem. Soc.* **74**, 1883.
Snyder, J. A. & Link, K. P. (1953). *J. Amer. chem. Soc.* **75**, 1758.
Walker, P. G., Woollen, J. W. & Heyworth, R. (1961). *Biochem. J.* **79**, 288.
Wallenfels, K. & Fischer, J. (1960). *Hoppe-Seyl. Z.* **321**, 223.
Webb, E. C. & Morrow, P. F. W. (1959). *Biochem. J.* **73**, 7.