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The Relationship Between Glucuronidase and Galacturonidase Activity in the Limpet and in Mammalian Tissues

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In the first systematic study of the enzyme β -glucuronidase, Masamune (1938) showed that ox-kidney preparations did not hydrolyse (-)-menthyl α -D-glucuronide, and this compound was also not hydrolysed by mouse-liver β -glucuronidase (Levvy & Marsh, 1952) and by a snail preparation rich in β -glucuronidase and in other simple glycosidases (Utusi, Huzi, Matumoto & Nagaoka, 1949). Nevertheless, although it is not so ubiquitous as the β -glucuronide residue, there is evidence for a naturally occurring α -conjugated glucuronic acid, for example in wheat straw (Bishop, 1953; Roudier, 1953). It has been inferred that uridine diphosphoglucuronic acid also has the α -configuration (Storey & Dutton, 1955; Strominger, Maxwell, Axelrod & Kalckar, 1957).

The synthesis of chromogenic α -glucuronides (see Appendix) made a search for an enzyme hydrolysing these compounds feasible, and a rich source was found in the visceral hump of the common limpet (*Patella vulgata*), which was already known to contain β -glucuronidase (Dodgson, Lewis & Spencer, 1953) as well as many other simple glycosidases (Conchie & Levvy, 1957). α -Glucuronidase activity was also detected elsewhere, but not in mammalian tissues.

During this investigation it was observed that limpet preparations hydrolysed β -D-galacturonides and, to a lesser extent, α -D-galacturonides. Hydrolysis of both types of β -glucuronide was inhibited by saccharo-1:4-lactone and less powerfully by boiled mucate solution, suggesting that a single enzyme was responsible for both reactions. Since these inhibitors act similarly on mammalian β -glucuronidase (Levvy, 1952; see also Conchie & Levvy, 1957), it was considered possible that this enzyme should also hydrolyse β -galacturonides, and this has been verified. Previous evidence from experiments with competing substrates had sug-

gested that β -galacturonides are unaffected by β -glucuronidase (Levvy & Marsh, 1952), although the enzyme was known to be inhibited by D-galacturonic acid as well as by D-glucuronic acid (Levvy, 1952).

The method of synthesis employed for aromatic glucuronides and galacturonides (see Appendix) was extended to the preparation of phenyl α - and β -N-acetylglucosaminuronides. These are compounds of particular interest in that the sugar moiety is said to occur in bacterial polysaccharides (Park, 1951; Webster, Clark & Freeman, 1954) and is closely related to one of the structures proposed for neuraminic acid and sialic acid (Klenk, Faillard, Weygand & Schöne, 1956), although this structure is open to doubt (Cornforth, Daines & Gottschalk, 1957). Neither glucosaminuronide was, however, hydrolysed by the appropriate glucuronidase or glucosaminidase.

EXPERIMENTAL

Materials

Phenolphthalein glucuronide (Talalay, Fishman & Huggins, 1946) and veratroyl glucuronide (Sammons & Williams, 1946) were biosynthetic. The synthesis of other aromatic glucuronides employed and of borneol α -D-glucuronide are described in the Appendix (Marsh & Levvy, 1958); aliphatic glucuronides were prepared as described by Marsh (1952). D-Mannuronic and D-mannuronic acid were prepared from methyl α -D-mannuronide. Sources of other compounds used are given in a recent paper (Conchie & Levvy, 1957).

Two types of solution of the inhibitory saccharo-1:4-lactone were employed: (a) the freshly dissolved pure lactone; (b) aqueous mM-potassium hydrogen saccharate maintained at 100° for 30 min. to convert one-third of the anion into saccharo-1:4-lactone as described by Levvy (1952). Where the latter was employed it is referred to as 'boiled saccharate' in the text. Similarly, 'boiled mucate'

solutions were prepared by heating aqueous solutions of mucic acid, pH 2, for 2 hr. at 100° (Levy, 1952); the resulting equilibrium mixture contains the inhibitory galactosaccharolactone, which has never been isolated.

Enzyme preparations

Limpet. The preparations were made by the method previously described (Levy, Hay & Marsh, 1957). For estimations of reducing sugar the stock enzyme preparation (2.5 ml./g. of tissue) was dialysed in Visking sausage casing against distilled water at 0° for 12 hr. If dialysis was prolonged or done at room temperature, the sac ruptured owing to the strong 'carboxymethylcellulase' activity of the preparation (Festenstien, 1957, personal communication). A second fractionation between 20 and 80% saturation with $(\text{NH}_4)_2\text{SO}_4$ was done and the enzyme dissolved in the minimal volume of water (about 1 ml./g. of original tissue).

Mammalian. Mouse- and rat-liver preparations were made as described by Levy (1952). Most of the experiments were, however, done with the enzyme from female rat preputial (clitoral) gland, the richest known source of β -glucuronidase (Beyler & Szego, 1951), prepared as follows. Both freshly dissected glands (weighing together about 100 mg.) from an adult rat were suspended in 0.1 M-acetic acid-NaOH buffer, pH 5.2, with the glass homogenizer (Jencons Ltd.). After incubation at 37° for 1 hr. the homogenate was centrifuged at 1500 g for 15 min., and the supernatant was fractionated with $(\text{NH}_4)_2\text{SO}_4$. The precipitate obtained between 20 and 80% saturation was dissolved in 5 ml. of water/100 mg. of tissue, and diluted as required. With phenyl β -glucuronide as substrate, this preparation was not activated by deoxyribonucleic acid (cf. Bernfeld, Bernfeld, Nisselbaum & Fishman, 1954) at the optimum pH.

Enzyme assay

Liberation of phenol. The hydrolysis of phenyl glucuronides was followed by the procedure of Kerr, Graham & Levy (1948), colour intensity being measured on the Spekker absorptiometer with Ilford no. 608 red filter (wavelength of peak transmission, 680 m μ). Incubation of enzyme and substrate was for 1 hr. at 37° in 0.125 M-acetic acid-NaOH buffer, pH 5.2, the substrate concentration being 10 mM unless otherwise stated. In inhibition experiments, correction was made for interference by D-glucuronic acid and D-mannuronic acid added in relatively high concentration (Karunairatnam & Levy, 1949); D-galacturonic acid did not interfere.

Liberation of o- and p-nitrophenol. The procedure of Conchie (1954) was adopted. p-Nitrophenyl α -glucuronide was unstable at alkaline pH, especially so in the presence of tissue preparations or trichloroacetic acid filtrates (cf. Dodgson & Spencer, 1957). With this substrate therefore NaOH and glycine buffer were added to individual tubes immediately before reading the colour intensity for free nitrophenol.

Liberation of phenolphthalein. See Levy (1952).

Liberation of reducing sugar. See Levy & Marsh (1952).

RESULTS

α -Glucuronidase

Occurrence. Appreciable α -glucuronidase activity was observed only in the limpet, although trace

Table 1. Inhibition of limpet α -glucuronidase by D-sugar acids and sugar-acid lactones at pH 3.5

Substrate: mM-phenyl α -glucuronide; inhibitor concentration: 5 mM unless otherwise stated.

Inhibitor	Inhibition (%)
Glucuronic acid	68
Glucurone	9
Galacturonic acid	28
Mannuronic acid	43
Mannurone	0
Saccharo-1:4-lactone (mM concn.)	0
Mucate (after 2 hr. at pH 2 and 100°)	0
Glucono-1:5-lactone	7
Glucono-1:4-lactone	3
Galactono-1:4-lactone	8
Mannono-1:4-lactone	0
Xylono-1:4-lactone	22
Arabono-1:4-lactone	4

activities were detected elsewhere. With mM-p-nitrophenyl α -glucuronide as substrate, no activity was detected in any tissue from adult or infant rats of either sex, with the exception of the large intestine, where it appeared to be associated with the digesta rather than the organ itself. In particular, no enzyme activity was detectable in a crude preparation of female rat preputial gland in a final concentration in the incubation with substrate of 0.5% moist weight; β -glucuronidase assays in this tissue are customarily done at levels of 0.005% for the phenol and 0.00025% for the phenolphthalein method. α -Glucuronidase was also absent from human cancer tissue.

The enzyme was detected in the sediment obtained by centrifuging at high speed (10 000 g for 30 min.) the rumen liquor from a sheep fed on hay, and in the wood-rotting fungi *Polystictus versicolor* and *Coniophora cerebella*, but not in the cellulolytic organism *Sporocytophaga myxococcoides*. A commercial fungal pectinase preparation (L. Light and Co. Ltd.) possessed slight α -glucuronidase activity, hydrolysis of mM-phenyl α -glucuronide being about one-tenth that of mM-phenyl α -galacturonide.

Inhibitors and competing substrates. A number of sugar acids and sugar-acid lactones, some of them known to be specific inhibitors for other glycosidases (Conchie & Levy, 1957), were tested as inhibitors of limpet α -glucuronidase acting on mM-phenyl α -D-glucuronide in 0.125 M-acetic acid-NaOH buffer, pH 3.5. The results are shown in Table 1. Saccharo-1:4-lactone and boiled mucate solution, which are powerful inhibitors of mammalian and limpet β -glucuronidase (Levy, 1952; Levy *et al.* 1957), had no effect on α -glucuronidase, suggesting that it is a different enzyme. α -Glucuronidase resembled β -glucuronidase in that it was inhibited by D-glucuronic acid and, to a lesser

extent, D-galacturonic acid, but differed from β -glucuronidase in that it was inhibited by D-mannuronic acid and xylonolactone.

Table 2 shows the effect of other glucuronides on the hydrolysis of phenyl α -glucuronide by a limpet preparation under the same conditions. The enzyme preparation in this instance had previously been treated at pH 2.75 at room temperature to inactivate β -glucuronidase (see below) and thus reduce interference by glucuronic acid liberated from β -glucuronides. Liberation of phenol was depressed by non-chromogenic α -glucuronides, but not by β -glucuronides. Inhibition by β -glucuronic acid 1-phosphate may have been due to free glucuronic acid resulting from phosphatase activity.

Liberation of reducing sugars. Limpet preparations were found to liberate reducing sugar from (-)-menthyl α -D-glucuronide, borneol α -D-glucuronide and methyl α - and β -D-galacturonides. Hydrolysis of the β -galacturonide was presumably due to the β -glucuronidase in the preparation (see below) since it was almost completely inhibited by MM-saccharo-1:4-lactone or boiled 5 mM-mucate solution, in contrast with hydrolysis of the α -galacturonide, which was unaffected. The limpet preparation caused no hydrolysis of methyl α -D-mannuronide.

Identification of free glucuronic acid in the enzyme hydrolysate. Chromatographic identification of free glucuronic acid liberated from phenyl α -glucuronide by a limpet preparation was carried out as described for the hydrolysis of phenolphthalein β -glucuronide by a similar enzyme preparation (Levy *et al.* 1957). Hydrolysis was, however, continued for 16 hr. The only spots obtained on the chromatogram were those for glucurone and glucuronic acid.

Comparison of the different glycuronidases in the limpet

pH-Activity curves. The appropriate phenyl glycuronides were employed as substrates in a final concentration of 10 mM in 0.125M-acetic acid-NaOH buffer. Fig. 1 (a) shows the effect of

Table 2. Effect of other α - and β -glucuronides on the release of phenol from MM-phenyl α -glucuronide by limpet α -glucuronidase

Concentration of competing substrate: 5 mM; the enzyme was treated with acid.

Competing substrate	Inhibition (%)
(-)-Menthyl α -glucuronide	55
(-)-Menthyl β -glucuronide	6
Borneol α -glucuronide	41
Veratroyl β -glucuronide	9
α -Glucuronic acid 1-phosphate	55
β -Glucuronic acid 1-phosphate	22

Fig. 1. Effect of varying the pH on the α - and β -glucuronidase and galacturonidase activity of limpet preparations acting on the appropriate phenyl glycuronides in a concentration of 10 mM. A single enzyme preparation was employed throughout, and the optimum glucuronidase activity is taken as 100. (a), α -Glucuronidases; (b), β -glucuronidases; O, glucuronidase; ●, galacturonidase. The enzyme preparation was diluted tenfold in the β -series.

pH on the α -glucuronidase and α -galacturonidase activity of limpet preparations. Optimum α -glucuronidase activity seemed to be at pH 3, but investigation at lower pH was limited by the insolubility of the substrate. The optimum for α -galacturonidase activity was considerably higher at pH 4.1. At pH 3, the α -glucuronidase activity of the preparation employed in the experiments illustrated in Fig. 1 was 1900 μ g. of phenol liberated in 1 hr. by 1 g. of tissue. The ratio of α -glucuronidase to α -galacturonidase activity was 3.5 at pH 3, 1.5 at pH 4.1 and 1.3 at pH 4.5.

As shown in Fig. 1 (b), the optimum for limpet β -glucuronidase activity was at pH 3.6. β -Galacturonidase activity displayed a broader optimum around pH 3.4. At pH 3.6, the ratio of β -glucuronidase to β -galacturonidase activity was 1.4, and for this particular preparation the ratio of β - to α -glucuronidase activity was 10. An optimum at pH 3.8 was previously reported for limpet β -glucuronidase when phenolphthalein β -glucuronide was employed as substrate (Levy *et al.* 1957).

pH-Stability curves. The inactivation of limpet β -glucuronidase at extremes of the pH range has been described in a previous paper in which phenolphthalein β -glucuronide was employed as substrate (Levy *et al.* 1957) and the results are in good agreement with those reported here.

The comparative stabilities of limpet α - and β -glucuronidase and galacturonidase are shown in Fig. 2. Samples of a single enzyme preparation (20 ml./g. of tissue) were adjusted to the required pH at 0° with HCl or NaOH, incubated at 37° for 1 hr., cooled to 0° and brought back to the original pH (5.4). After suitable dilution the samples were assayed for the four glycuronidase activities, 10 mm-phenyl glycuronide in 0.125M-acetic acid-NaOH buffer, pH 4.5, being used, and compared with an untreated sample of the enzyme that had been stored at 0°.

There was little difference in pH stability between the two α -glycuronidases on the one hand and the two β -glycuronidases on the other. There was, however, a marked difference between the enzymes of the α -series and those of the β -series in that the former were stable between lower pH limits, confirming the view that the α - and β -enzymes are distinct. To obtain α -glycuronidase free from β -glycuronidase, it was found convenient to allow the limpet preparation to stand at room temperature for 5 min. at pH 2.75; 85% of the α -glycuronidase activity was thereby retained, but less than 0.5% of the β -glycuronidase activity.

Relative heat stability. An unbuffered limpet preparation (50 ml./g. of tissue) lost 60% of its α -glucuronidase, but only 15% of its β -glucuronidase activity after 5 min. at 60°. This effect was dependent on the protein and salt concentrations

of the preparation. In a concentrated, unbuffered preparation (2.5 ml./g. of tissue) there was only 7.5% loss of α -glucuronidase activity after 30 min. at 60°; repetition of this treatment after dialysis of the same preparation caused an almost complete loss of α -glucuronidase activity.

Heat treatment distinguished between α -glucuronidase and α -galacturonidase activity in limpet preparations (50 ml./g. of tissue), as shown in Fig. 3.

Fig. 2. Effect of heating for 1 hr. at 37° on limpet glycuronidase activities in preparations adjusted to varying pH with HCl or NaOH at 0°. ○, α -Glucuronidase; ●, α -galacturonidase; □, β -glucuronidase; ■, β -galacturonidase. Residual activity of each enzyme is expressed as a percentage of the initial value.

Fig. 3. Effect of heating to 60° for varying periods on the α -glucuronidase (○) and α -galacturonidase (●) activity of limpet preparations. Initial α -glucuronidase activity is taken as 100%.

After varying periods at 60°, assays were done with mm-phenyl glycuronide in 0.125M-acetic acid-NaOH buffer, pH 4.1. The ratio of α -glucuronidase to α -galacturonidase activity fell from an initial figure of 2.9 to unity after 15 min., owing to the greater percentage loss in the α -glucuronidase activity. (It should be noted that the substrate concentration here was only one-tenth of that employed in the experiments in Fig. 1.)

Table 3 shows the inhibitory effects of glucuronic acid and galacturonic acid on a similar preparation before and after heat treatment; inhibition by galacturonic acid of both α -glucuronidase activities was increased by heat treatment, whereas the effect of glucuronic acid remained unchanged.

These experiments suggest that there may be two α -glucuronidases of differing heat stability in the limpet. Brooks & Reid (1955) found that the oligosaccharidase component in a pectinase preparation was stable at 60° for 20 min.

Effect of varying the substrate concentration in the presence and the absence of inhibitors. The phenyl glycuronides were employed as substrates and the concentration was varied from 0.1 to 15 mM. In no instance was inhibition by excess of substrate observed and all inhibitors acted competitively.

Table 4 shows mean values for K_m and K_i . With the α -glucuronidases, these values were calculated by the method of Lineweaver & Burk, 1934 (see also Haldane, 1957), but the results obtained with the β -glucuronidases did not give a linear plot by this method of calculation, and values for K_m and K_i were derived from the direct plot of activity against substrate concentration.

The value for K_i for boiled saccharate or mucate solution was sufficiently close with both β -glucuronidases to support the view that these were a single enzyme. With the α -glucuronidases, K_i for glucuronic acid showed little change on passing from one substrate to the other, but the figure for galacturonic acid altered consistently by more than was expected for a single enzyme.

Values for K_i for boiled saccharate and mucate acting on limpet β -glucuronidase are in good agreement with those previously reported (Levy *et al.* 1957) with phenolphthalein β -glucuronide as substrate, namely 1.05×10^{-7} for saccharo-1:4-lactone and 6.7×10^{-5} for boiled mucate solution.

o-Nitrophenyl β -galacturonide was readily hydrolysed by limpet preparations and hydrolysis was powerfully inhibited by boiled saccharate and mucate solutions.

Table 3. *Effect of heat treatment (20 min. at 60°) on the susceptibility of α -glucuronidase and α -galacturonidase activity in a limpet preparation (50 ml./g. of tissue) to inhibition by glucuronic acid and galacturonic acid*

Substrate: mm-phenyl glycuronide; assays were done at pH 4.1.

Enzyme	Inhibitor	Inhibition (%)	
		Before heating	After heating
α -Glucuronidase	2 mM-Glucuronic acid	57	55
	10 mM-Galacturonic acid	33	50
α -Galacturonidase	mm-Glucuronic acid	65	67
	mm-Galacturonic acid	38	49

Table 4. *Dissociation constants for the complexes formed by limpet glycuronidases with substrate (K_m) and inhibitors (K_i)*

The phenyl glycuronide was employed as substrate for each enzyme, and measurements were made at pH 4.1 in the α -series and pH 3.6 in the β -series. Saccharate and mucate solutions were boiled before use, to effect lactonization (see Experimental).

Enzyme	K_m (mM)	K_i	
		Saccharate (μ M)*	Mucate (μ M)*
β -Glucuronidase	0.40	0.32	6.9
β -Galacturonidase	2.3	0.26	5.4
		Glucuronate (mM)	Galacturonate (mM)
α -Glucuronidase	0.39	0.54	4.7
α -Galacturonidase	1.8	0.60	1.0

* Apparent values; inhibition was due to lactone formed in solution.

β-Galacturonidase activity of mammalian β-glucuronidase

pH-Activity curves. With the appropriate phenyl β-glycuronide as substrate, preparations of female rat preputial gland displayed an optimum for β-glucuronidase activity at pH 4·5 and for β-galacturonidase activity at pH 3·9 (Fig. 4). Typical β-glucuronidase and β-galacturonidase activities exhibited at the optima were 600 000 and 250 000 μg. of phenol liberated in 1 hr. by 1 g. of tissue respectively. The ratio of β-glucuronidase to β-galacturonidase activity was 1·8 at pH 3·9 and

Fig. 4. Effect of varying the pH on the β-glucuronidase and β-galacturonidase activity of a single preparation of female rat preputial gland. Substrate: 10 mm-phenyl glycuronide in 0·125M-acetic acid-NaOH buffer. Optimum β-glucuronidase activity is taken as 100; O, glucuronidase; ●, galacturonidase.

3·1 at pH 4·5; identical ratios were obtained with different rat preputial-gland preparations, and the same ratio at pH 4·5 was obtained with a dialysed rat-liver preparation. It is unlikely that the freak level of β-glucuronidase activity in female rat preputial gland (about 100 times the value in rat liver) would be reflected in a different glycuronidase activity unless both were due to the same enzyme.

Effect of varying the substrate concentration in the presence and the absence of inhibitors. The effect of varying phenyl β-glucuronide and phenyl β-galacturonide concentration between 0·1 and 15 mm at the pH optima for both substrates was studied with a female rat preputial-gland preparation, and K_i was determined for glucuronate, galacturonate, boiled saccharate and boiled mucate, all of which acted competitively. Table 5 shows mean values for K_m and K_i , calculated by the method of Lineweaver & Burk (1934). Figures for K_i were in each instance so similar with both substrates as to suggest that we are dealing here with a single enzyme.

Competing substrates. In previous work (Levy & Marsh, 1952) it was found that the hydrolysis of 0·63 mm-phenolphthalein β-glucuronide by a mouse-liver preparation at pH 5·2 was inhibited to the extent of 16 % in the presence of 10 mm-phenyl β-glucuronide, but was unaffected by 10 mm-methyl β-galacturonide; it was concluded that β-glucuronidase has no action on β-galacturonides. As can be seen from Table 6, β-galacturonides can act as competing substrates for mammalian β-glucuronidase when the experiments are carried out at a lower pH. This would presumably tend to favour β-galacturonidase activity (Fig. 4).

Action of limpet and mammalian preparations on N-acetylglucosaminuronides

No hydrolysis of phenyl α- or β-N-acetylglucosaminuronide was observed after incubation with concentrated enzyme preparations from the limpet or from the female rat preputial gland. With the limpet, these experiments exclude hydrolysis not

Table 5. Dissociation constants for the complexes formed by mammalian β-glycuronidase with phenyl β-glucuronide and phenyl β-galacturonide (K_m) and with various competitive inhibitors (K_i)

The enzyme was prepared from female rat preputial gland, and saccharate and mucate solutions were boiled before use (see Experimental).

pH of assay	Enzyme	K_m (mm)	K_i			
			Saccharate (μM)*	Mucate (μM)*	Glucuronate (mm)	Galacturonate (mm)
4·5	β-Glucuronidase	1·4	0·31	22	1·5	4·3
	β-Galacturonidase	11	0·47	27	1·0	4·0
3·9	β-Glucuronidase	0·59	0·36	16	—	—
	β-Galacturonidase	5·2	0·36	18	—	—

* See footnote to Table 4.

Table 6. *Effect of other β -glucuronides in a concentration of 10 mM on the release of phenolphthalein from 0.63 mM-phenolphthalein β -glucuronide by mammalian preparations*

Source of enzyme	pH of assay	Competing substrate	Inhibition (%)
Female rat preputial gland	4.5	Phenyl β -glucuronide	60
		Phenyl β -galacturonide	23
Mouse liver	4.5	Phenyl β -glucuronide	25
		Phenyl β -galacturonide	13
Mouse liver	5.2	Phenyl β -glucuronide	21
		Phenyl β -galacturonide	0

only by β -glucuronidase but also by α -glucuronidase and by α - and β -*N*-acetylglucosaminidase (Conchie & Levvy, 1957). There was again no hydrolysis of the β -*N*-acetylglucosaminuronide when tested with a concentrated β -*N*-acetylglucosaminidase preparation from rat epididymis (Conchie, Findlay & Levvy, 1956).

DISCUSSION

The first point that emerges from this work is that both mammalian and limpet β -glucuronidase also hydrolyse β -galacturonides, although it is once more emphasized that this enzyme does not attack α -glucuronides. It is noteworthy, however, that under all comparable conditions the affinity and the rate of hydrolysis were greater with β -glucuronides than with β -galacturonides; this was also true of the α -glucuronidase and α -galacturonidase activities of the limpet preparation, although there may have been more than one α -glucuronidase present.

The postulated specificity of glycosidase inhibition by the aldonolactone corresponding to the substrate in configuration (Conchie & Levvy, 1957) is borne out by the present experiments, which provide an explanation for the previously puzzling inhibition of β -glucuronidase by boiled mucate (galactosaccharolactone) solutions. It can be seen that the inhibition of β -glucuronidase (or β -galacturonidase) by saccharo-1:4-lactone and boiled mucate solution is a highly specific and selective phenomenon so far as other simple glycosidases and lactones are concerned. Neither the α -glucuronidase nor the α -galacturonidase activity of the limpet was affected by either of these compounds. Failure of the appropriate lactone to cause inhibition in the α -glycosidase series has been noted with α -galactosidase (Conchie & Levvy, 1957) and with α -*N*-acetylglucosaminidase (Marsh & Levvy, 1957), although α -glucosidase and α -mannosidase, as well as all the β -glycosidases studied, are powerfully inhibited.

β -Glucuronidase is extensively employed as a reagent for the hydrolysis of urinary-steroid conjugates. The fact that this enzyme will also hydrolyse β -galacturonides raises the question whether

aglycones released by its action may have been conjugated with galacturonic acid. All those urinary glucuronides whose structure has been confirmed by synthesis or by identification of the uronic acid are, however, glucuronides. The distribution of α -glucuronidase is consistent with the possibility that this enzyme is purely a digestive one, acting on plant material, and that it is distinct from the liver glucuronyl transferase that acts on uridine diphosphoglucuronic acid.

Smith & Mills (1953) found that below pH 4.2 β -glucuronidase is inhibited by deoxyribonucleic acid. This has been confirmed for both the β -glucuronidase and β -galacturonidase activity of a limpet preparation, tested at pH 3.6. Above pH 4.2, deoxyribonucleic acid activates dilute preparations of highly purified β -glucuronidase (Smith & Mills, 1953; Bernfeld *et al.* 1954), but this did not apply to the preputial-gland preparation employed here, in the concentration required for assay with phenyl β -glucuronide as substrate.

SUMMARY

1. Preparations from the limpet (*Patella vulgata*) hydrolysed α -glucuronides of phenol, *p*-nitrophenol, (–)-menthol and borneol, as well as the α -galacturonides of phenol and methanol.

2. In addition to its known action on β -glucuronides, the limpet preparation hydrolysed the β -galacturonides of phenol, *o*-nitrophenol and methanol.

3. Hydrolysis of both types of β -glucuronide was powerfully inhibited by boiled saccharate solution and less powerfully by boiled mucate solution.

4. The affinity constants for both these inhibitors were identical no matter which type of substrate was employed, and it was concluded that a single enzyme was involved.

5. Hydrolysis of α -glucuronides and α -galacturonides was inhibited by glucuronic acid and to a lesser extent by galacturonic acid, but was unaffected by saccharate and mucate.

6. By treatment of limpet preparations at varying pH, it was possible to distinguish between

α - and β -glucuronidase, but not between glucuronidase and galacturonidase activity in either the α - or β -series.

7. Heat treatment and the inhibitory effects of galacturonic acid suggested that there was more than one α -glucuronidase present in limpet preparations.

8. Mammalian β -glucuronidase preparations were found to possess β -galacturonidase activity; the rate of hydrolysis, the affinity for the substrate, and the pH optimum were all lower with the latter.

9. Mammalian β -galacturonidase activity was inhibited by boiled saccharate, boiled mucate, glucuronate and galacturonate solutions, in that order; the affinity constants agreed with those for β -glucuronidase, and it was concluded that the enzymes were identical.

10. Phenyl α - and β -N-acetylglucosaminuronide were not hydrolysed by α - or β -glucuronidase or by N-acetylglucosaminidase.

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APPENDIX

The Synthesis of Aryl Glycosiduronic Acids

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In connexion with the study of the relationships between different glycuronidases in the limpet (*Patella vulgata*) and in mammalian tissues (Marsh & Levvy, 1958), it was necessary to synthesize chromogenic α - and β -D-glucopyranosiduronic acids (glucuronides) and galactopyranosiduronic acids (galacturonides). For purposes of comparison, it was considered desirable in quantitative experiments to keep the aglycone constant, and all four derivatives of phenol were therefore prepared. Since, however, phenyl glycosides are unsuitable for the screening of crude-tissue preparations, owing to the presence of material that interferes in

the determination of free phenol, certain nitro-phenyl glycuronides were also prepared.

Catalytic oxidation by gaseous oxygen in the presence of platinum catalyst as a method whereby aliphatic and alicyclic hexosides in neutral aqueous solution are converted into the corresponding hexosiduronic acids has been already described (Marsh, 1952); attempts to oxidize aryl glycosides by the same procedure were unsuccessful. Subsequently, however, the syntheses of the 2-naphthyl (Tsou & Seligman, 1952) and the phenyl (Tsou & Seligman, 1953) β -D-glucopyranosiduronic acids by the same general method were reported: the latter