- Cartier, P. & Picard, J. (1955c). *Bull. Soc. Chim. biol., Paris*, **37**, 1169.
- Dawson, J. & Jones, E. A. (1957). *Int. Congr. clin. Chem., Stockholm*, p. 86.
- Dawson, J., Weidmann, S. M. & Jones, H. G. (1957). *Biochem. J.* **66**, 116.
- Engfeldt, B. & Zetterström, R. (1954). *Endocrinology*, **54**, 506.
- Laskin, D. M. & Engel, M. B. (1956). *Arch. Path.* **62**, 296.
- Neuman, W. F. & Neuman, M. W. (1953). *Chem. Rev.* **53**, 1.
- Neuman, W. F. & Neuman, M. W. (1957). *Amer. J. Med.* **22**, 133.
- Neuman, W. F. & Neuman, M. W. (1958). In *The Chemical Dynamics of Bone Mineral*, ch. 7. Chicago: University Press.
- Rasmussen, H. (1957). *J. biol. Chem.* **229**, 781.
- Rogers, H. J., Weidmann, S. M. & Jones, H. G. (1953). *Biochem. J.* **54**, 37.
- Sobel, A. E., Burger, M. & Deane, B. C. (1957). *Int. Congr. clin. Chem., Stockholm*, p. 129.
- Weidmann, S. M. (1956). *Biochem. J.* **62**, 593.
- Weidmann, S. M. & Rogers, H. J. (1958). *Biochem. J.* **69**, 338.
- Whitehead, R. G. & Weidmann, S. M. (1957). *Nature, Lond.*, **180**, 1196.

Mammalian Glycosidases

DISTRIBUTION IN THE BODY

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The enzyme β -glucuronidase is universally distributed in mammalian tissues, and has been extensively studied from many aspects (see Levvy, 1956; Levvy & Marsh, 1959). Investigations on the changes in activity of the enzyme *in vivo* in response to extrinsic agents have been complicated by genetic factors which can lead to considerable variations in activity and response within a species. It has been concluded, however, that the enzyme is under endocrine control, though its function in the body remains obscure.

It was considered that the mammalian cell might contain other glycosidases behaving similarly to β -glucuronidase, and to obtain some preliminary information on this point various tissues have been screened for glycosidase activity with a number of chromogenic substrates. With those enzymes which appeared most active in all the tissues examined, namely α -mannosidase, β -galactosidase and β -*N*-acetylglucosaminidase, a study has been undertaken of their distribution in the mouse and the rat. For purposes of comparison, β -glucuronidase activities have also been measured, and extended to a number of hitherto unexamined tissues.

Parts of this work have been described in preliminary communications (Conchie, Findlay & Levvy, 1956, 1957; Conchie, 1957). Comparative studies of β -glucuronidase, α -mannosidase and β -*N*-acetylglucosaminidase in cancer tissues have already been published (Conchie & Levvy, 1957a), as has the distribution of glycosidases in semen, sperm and male accessory secretions of many mammalian species (Conchie & Mann, 1957).

A study of the changes *in vivo* of α -mannosidase, β -galactosidase, β -*N*-acetylglucosaminidase and

β -glucuronidase in response to hormones and other extrinsic agents has been made by Conchie & Findlay (1958).

EXPERIMENTAL

Materials

Phenolphthalein β -D-glucuronide was prepared biosynthetically by the method of Talalay, Fishman & Huggins (1946).

o-Nitrophenyl β -D-glucoside, prepared as described for the galactoside (Seidman & Link, 1950), had m.p. 152°, $[\alpha]_D^{16} - 107^\circ$ in water (c, 1.2); Montgomery, Richtmyer & Hudson (1942) give the same constants.

Phenyl α -D-galactoside, prepared as described for the glucoside (Montgomery *et al.* 1942), had m.p. 145°, $[\alpha]_D^{16} + 215^\circ$ in water (c, 1.4); Helferich & Appel (1932) give m.p. 146°, $[\alpha]_D + 217^\circ$.

p-Nitrophenyl α -D-mannoside, prepared as by Jermyn (1955), had m.p. 180–181° and $[\alpha]_D^{19} + 145^\circ$ in water (c, 0.2).

The following compounds were also prepared: *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide (Findlay, Levvy & Marsh, 1958); phenyl *N*-acetyl- α -D-glucosaminide and phenyl *N*-acetyl- β -D-glucosaminide (Conchie & Levvy, 1957b); *o*-nitrophenyl β -D-galactoside (Seidman & Link, 1950); *p*-nitrophenyl α -D-glucoside and phenyl β -D-xyloside (Montgomery *et al.* 1942); phenyl β -D-mannoside (Helferich & Winkler, 1933); D-mannono-1:4-lactone (Nelson & Cretcher, 1930).

2-Acetamido-2-deoxy-D-gluconolactone (*N*-acetylglucosaminolactone) was prepared by oxidation of *N*-acetylglucosamine with HgO as described by Findlay *et al.* (1958).

D-Galactono-1:4-lactone was obtained from L. Light and Co. Ltd.

Saccharate solutions, prepared from potassium hydrogen saccharate (British Drug Houses Ltd.), were boiled before use for 30 min. to cause one-third conversion into saccharo-1:4-lactone (Levy, 1952).

The white mice used were from our Institute colony (Tyler's Original: Mill Hill Colony). The C3H strain was obtained from the Institute of Physiology, Glasgow University.

Enzyme preparations

After dissection, tissues were gently pressed between pieces of hardened filter paper before recording the wet weight. All tissues to be assayed were homogenized in water with a glass homogenizer (Jencons Ltd.), and the final volume of enzyme preparation arranged to give a suitable liberation of aglycone in the enzyme assay (30–40 μ g. of phenolphthalein, 60–120 μ g. of *p*-nitrophenol, 80–140 μ g. of *o*-nitrophenol and 30–50 μ g. of phenol).

Enzyme assays

β -Glucuronidase. Liberation of phenolphthalein from 0.625 mM-phenolphthalein β -glucuronide in 0.05 M-acetic acid–NaOH buffer, pH 5.2, was measured as by Levvy (1952).

α -Mannosidase. For some of the earlier assays, liberation of *p*-nitrophenol from 2 mM-*p*-nitrophenyl α -mannoside in 0.2 M- Na_2HPO_4 –0.1 M-citric acid buffer, pH 4.6 (McIlvaine, 1921), was measured as by Conchie (1954) for the hydrolysis of *o*-nitrophenyl β -glucoside. Later, the following modification was adopted. To 3 ml. of buffer, pH 4.6, was added 0.5 ml. of 16 mM-*p*-nitrophenyl α -mannoside solution (giving a final concentration in the incubation mixture of 2 mM), and 0.5 ml. of enzyme preparation. The mixture was incubated for 1 hr. at 37°, and the reaction stopped by the addition of 4 ml. of 0.4 M-glycine–NaOH buffer, pH 10.8. After centrifuging at 1500 *g* for 20 min., the liberated *p*-nitrophenol was measured on the Spekker photoelectric absorptiometer with Ilford no. 601 violet filters (peak transmission 430 m μ).

β -Galactosidase. Liberation of *o*-nitrophenol from 2.5 mM-*o*-nitrophenyl β -galactoside in 0.2 M- Na_2HPO_4 –0.1 M-citric acid buffer, pH 3.6 (McIlvaine, 1921), was measured as described in the above modification for α -mannosidase assays.

β -N-Acetylglucosaminidase. (a) Liberation of *p*-nitrophenol from 5 mM-*p*-nitrophenyl *N*-acetyl- β -glucosaminide in 0.05 M-citric acid–NaOH buffer, pH 4.4, was measured as described by Findlay *et al.* (1958).

(b) Liberation of phenol from 10 mM-phenyl *N*-acetyl- β -glucosaminide in 0.05 M-citric acid–NaOH buffer, pH 4.3,

was measured by the procedure of Kerr, Graham & Levvy (1948), using the Spekker absorptiometer with Ilford no. 608 red filters (peak transmission 680 m μ). The conditions of assay were those described by Pugh, Leaback & Walker (1957). The liberation of *N*-acetylglucosamine (measured as by Aminoff, Morgan & Watkins, 1952) and phenol took place in equimolecular proportions, confirming the previous observations of Pugh *et al.* (1957). Roseman (1957) has observed that animal tissues do not hydrolyse *N*-acetylglucosamine.

Lactase. Liberation of glucose from lactose was measured specifically by the glucose oxidase method of Huggett & Nixon (1957).

Other glycosidases. α - and β -Glucosidase, α -galactosidase, β -mannosidase, β -xylosidase and α -*N*-acetylglucosaminidase activities were measured according to the procedures described by Conchie & Levvy (1957b).

RESULTS

Screening of tissues

We screened a selection of tissues for glycosidase activities, using as substrates all the chromogenic glycosides at our disposal. These were *p*-nitrophenyl α -glucoside, *o*-nitrophenyl β -glucoside, phenyl α -galactoside, *o*-nitrophenyl β -galactoside, *p*-nitrophenyl α -mannoside, phenyl β -mannoside, phenyl β -xyloside, phenyl *N*-acetyl- α -glucosaminide, *p*-nitrophenyl *N*-acetyl- β -glucosaminide, phenyl *N*-acetyl- β -glucosaminide and phenolphthalein β -glucuronide. Tissues screened were mouse liver, spontaneous mammary tumours of C3H mice, rat pancreas, rat epididymis, human kidney and a human Grawitz tumour. Mean enzyme activities found in these tissues are shown in Table 1. Semen and the male accessory gland secretions of various animals have already been investigated for glycosidase activities (Conchie & Mann, 1957).

In all tissues examined the enzymes displaying the greatest activities were α -mannosidase, β -galactosidase and β -*N*-acetylglucosaminidase. β -Glucuronidase showed moderately high activities.

Table 1. *Glycosidase activities in some mammalian tissues*

For methods of assay see text. Results are expressed as μ g. of *o*- or *p*-nitrophenol (N), phenol (P) or phenolphthalein (PP) liberated/g. of tissue (wet weight) in 1 hr.

Enzyme	Mouse liver	Spontaneous mammary tumour (C3H mice)	Rat pancreas	Rat epididymis	Human kidney	Grawitz tumour
α -Glucosidase (N)	260	630	420	990	770	210
β -Glucosidase (N)	770	130	60	430	840	80
α -Galactosidase (P)	Nil	80	50	340	60	Nil
β -Galactosidase (N)	1 480	2 430	1 150	47 300	1 930	180
α -Mannosidase (N)	2 600	3 080	2 650	75 310	1 110	350
β -Mannosidase (P)	Nil	40	60	230	—	—
β -Xylosidase (P)	Nil	Nil	Trace ?	Nil	Nil	Nil
α - <i>N</i> -Acetylglucosaminidase (P)	Nil	40	Trace ?	Trace ?	Nil	Nil
β - <i>N</i> -Acetylglucosaminidase (P)	7 310	3 910	870	42 900	22 040	4 300
β - <i>N</i> -Acetylglucosaminidase (N)	24 110	15 110	4 830	290 920	—	—
β -Glucuronidase (PP)	2 600	690	860	5 290	1 790	640

Spontaneous mammary tumours from C3H mice, which have already been shown to be rich in α -mannosidase, β -N-acetylglucosaminidase and β -glucuronidase (Conchie & Levvy, 1957a), had in addition high β -galactosidase and moderately high α -glucosidase activities. The specimen of a Grawitz tumour showed lower activities than did uninvolved human kidney, but β -galactosidase and α -glucosidase were still present. Although we have not investigated as many types of tumour for β -galactosidase and α -glucosidase as for α -mannosidase, β -glucuronidase and β -N-acetylglucosaminidase, it seems probable that activities of the former would parallel those of the latter group.

Sheep liver was not screened, but is rich in both β -glucuronidase (7210 units/g.) and α -mannosidase (2640 units/g.).

α -Mannosidase, β -galactosidase, β -N-acetylglucosaminidase and β -glucuronidase in the mouse and the rat

In view of the high activity of α -mannosidase, β -galactosidase and β -N-acetylglucosaminidase in almost all the tissues examined, and their extremely high activity in epididymis, further investigations were restricted to these enzymes. β -Glucuronidase assays were also done, partly for purposes of comparison and partly to examine tissues not previously studied. Tables 2-5 show mean values for the distribution of the four enzymes in adult and infant tissues of the male and female mouse and rat. Infants are defined as animals between 2 and 14 days old. In many experiments, tissues from one animal were assayed for several enzymes. The

Table 2. α -Mannosidase in the tissues of the mouse and the rat

For method of assay see text. Results are expressed as μ g. of *p*-nitrophenol liberated/g. of tissue (wet weight) in 1 hr. M, male; F, female; i, infant.

	Mouse				Rat			
	M	F	iM	iF	M	F	iM	iF
Liver	2 660	1 980	3 660	3 010	1 710	1 990	4 620	4 550
Spleen	1 510	970	1 320		670	810	1 570	2 080
Kidney	4 010	2 760	12 300	9 410	3 770	3 100	10 220	9 270
Intestine	1 110	1 060	—	—	1 000	1 020	—	—
Adrenals	—	Nil	—	—	650	350	—	—
Pancreas	—	—	—	—	2 650	—	—	—
Heart	170	120	—	—	120	Nil	—	—
Lung	380	320	—	—	460	440	—	—
Muscle (leg)	90	—	—	—	Nil	Nil	—	—
Brain	320	230	—	—	160	180	—	—
Eye	—	—	—	—	80	—	—	—
Blood	—	—	—	—	10	20	—	—
Preputials	1 490	1 310	—	2 070	810	1 420	590	1 230
Prostate	320	—	—	—	2 540	—	2 100	—
Testis	1 690	—	430	—	610	—	920	—
Seminal vesicle	2 890	—	—	—	610	—	2 620	—
Epididymis	16 340	—	1 240	—	75 390	—	2 570	—
Epididymal sperm	—	—	—	—	7 730	—	—	—
Ovary	—	Nil	—	1 820	—	950	—	620
Uterus	—	1 690	—	2 120	—	1 000	—	1 510
Vagina	—	1 390	—	1 130	—	160	—	810

Table 3. β -Galactosidase in the tissues of the mouse and the rat

For method of assay see text. Results are expressed as μ g. of *o*-nitrophenol liberated/g. of tissue (wet weight) in 1 hr. M, male; F, female; i, infant.

	Mouse				Rat			
	M	F	iM	iF	M	F	iM	iF
Liver	1 500	1 420	2 460	2 010	4 710	4 840	5 400	6 240
Spleen	7 650	2 610	1 350	1 000	9 810	6 550	7 100	6 270
Kidney	4 510	3 660	5 570	4 930	11 400	11 100	16 090	15 760
Pancreas	—	—	2 170	810	1 150	1 040	2 420	2 590
Brain	580	660	—	—	—	—	—	—
Preputials	1 190	—	—	—	2 910	1 530	—	—
Testis	2 890	—	—	—	1 960	—	—	—
Seminal vesicle	2 080	—	—	—	710	—	—	—
Epididymis	6 000	—	1 080	—	47 300	—	5 100	—
Ovary	—	2 510	—	2 640	—	7 500	—	—
Uterus	—	3 190	—	3 350	—	4 110	—	3 010
Vagina	—	1 100	—	—	—	2 040	—	—

Table 4. β -N-Acetylglucosaminidase in the tissues of the mouse and the rat

For methods of assay see text. Results are expressed in (a) μ g. of phenol, (b) μ g. of *p*-nitrophenol liberated/g. of tissue (wet weight) in 1 hr. M, male; F, female; i, infant.

(a) Phenyl *N*-acetyl- β -glucosaminide as substrate

	Rat			
	M	F	iM	iF
Liver	5 650	8 660	6 100	5 400
Spleen	3 150	4 200	—	4 300
Kidney	12 400	14 380	10 000	13 400
Intestine	1 850	2 450	—	4 100
Adrenals	1 600	1 900	—	1 400
Pancreas	872	—	—	—
Heart	950	400	—	900
Lung	2 000	2 600	—	2 100
Muscle (leg)	Nil	300	—	900
Brain	2 100	—	—	—
Preputials	1 100	3 330	1 500	1 100
Prostate	1 500	—	—	—
Testis	3 500	—	2 150	—
Seminal vesicle	900	—	—	—
Epididymis	42 900	—	3 300	—
Epididymal sperm	1 440	—	—	—
Ovary	—	3 630	—	1 930
Uterus	—	3 600	—	2 400
Vagina	—	1 200	—	1 900

(b) *p*-Nitrophenyl *N*-acetyl- β -glucosaminide as substrate

	Mouse				Rat			
	M	F	iM	iF	M	F	iM	iF
Liver	23 620	41 090	62 610	68 830	44 610	59 840	46 970	49 810
Kidney	83 640	50 990	52 880	51 250	126 650	109 970	91 770	98 960
Pancreas	—	—	—	—	4 830	—	—	—
Preputials	20 800	18 450	11 250	19 000	—	11 000	—	—
Testis	15 850	—	13 880	—	17 590	—	15 670	—
Epididymis	207 920	—	15 820	—	290 920	—	9 570	—
Ovary	—	30 600	—	21 740	—	26 110	—	12 410
Uterus	—	31 620	—	17 570	—	15 530	—	17 170

Table 5. β -Glucuronidase in the tissues of the mouse and the rat

For method of assay see text. Results are expressed in μ g. of phenolphthalein liberated/g. of tissue (wet weight) in 1 hr. M, male; F, female; i, infant.

	Mouse				Rat			
	M	F	iM	iF	M	F	iM	iF
Liver	2 600	2 700	4 210	4 840	19 340	13 350	19 280	20 520
Kidney	2 250	1 010	1 780	2 270	3 470	3 230	6 180	6 020
Pancreas	—	—	—	—	860	—	—	—
Brain	110	100	—	—	—	—	—	—
Eye	540	—	—	—	—	90	—	—
Preputials	850	600	—	—	119 760	2 298 000	205 750	710 600
Prostate	Nil	—	—	—	2 200	—	2 330	—
Testis	460	—	—	—	390	—	1 260	—
Seminal vesicle	870	—	—	—	90	—	800	—
Epididymis	4 030	—	1 750	—	5 290	—	3 120	—
Ovary	—	1 670	—	1 940	—	8 480	—	4 200
Uterus	—	2 570	—	1 700	—	11 020	—	3 230
Vagina	—	970	—	—	—	8 900	—	3 230

number of animals examined varied from 3 to 30. Some β -N-acetylglucosaminidase activities in the rat are given with both the phenyl and *p*-nitrophenyl glycosides as substrates: typical values for mouse tissues with the phenyl glycoside as substrate are female liver 7780 units/g., uterus 7090 units/g.

All four enzymes were widely distributed, being present in almost every tissue examined. In adult animals of both species the epididymis was the richest source of them all, subject to two exceptions. In the male mouse, β -galactosidase values for spleen tended to be higher than those for

epididymis, though spleen values vary considerably. In rats of all ages, the female preputial gland was the richest source of β -glucuronidase, as already observed by Beyler & Szego (1954); even in males the analogous organ was by far the richest source of this enzyme in these animals, and liver was the second richest tissue in β -glucuronidase activity.

Infant figures were generally similar to adult values, though in some cases they were appreciably higher. Uterus levels were often somewhat lower than those for adults. Infant epididymis activities were very much lower than the adult values, showing the greatest differences in those cases where the adult levels were particularly high.

Genetic factors

The C3H strain of mice is one with a genetically-determined low β -glucuronidase activity (Law, Morrow & Greenspan, 1952). Table 6 shows the distribution of β -glucuronidase in some tissues of adult animals and figures for infant liver. The low level of glucuronidase activity in the liver and kidney of adults, and the sex difference in the kidney values, agree with observations already made by Morrow, Carroll & Greenspan (1951). All the other tissues examined also had values much lower than those for normal strains. The increase in infant-liver values already observed with mice of normal strains (Levy, 1956) was even more marked in the C3H mice.

Water homogenates of the livers of adult C3H

Table 6. β -Glucuronidase in C3H mice

For conditions of assay see text. Results are expressed in μ g. of phenolphthalein liberated/g. of tissue (wet weight) in 1 hr. M, male; F, female; i, infant.

	M	F	iM	iF
Liver	260	420	2460	2510
Kidney	900	260	—	—
Brain	10	30	—	—
Preputials	230	30	—	—
Epididymis	1070	—	—	—
Uterus	—	710	—	—

mice, when fractionated with 0.1M-acetic acid-NaOH buffer, pH 5.2, as by Kerr & Levy (1951), displayed all, or almost all, of their β -glucuronidase activity in the supernatant, in contrast with liver homogenates of normal strains, which showed only 60% of their activity in the supernatant, as already observed by these authors. Liver homogenates of infant C3H mice, on the other hand, behaved in a manner similar to those of other strains, showing about 80% of their total activity in the supernatant.

Tissues of C3H mice assayed for α -mannosidase, β -galactosidase and β -N-acetylglucosaminidase activities had the same levels as those obtained from other strains. In the course of our investigations on α -mannosidase in our own laboratory strain, however, it seemed possible that there might be some genetic factor controlling this enzyme. Liver values have been obtained varying from about 600 to 4000 units/g. Generally, an animal with a low α -mannosidase activity in liver had low activities in other tissues examined. This variation was also noted during the investigation of mixed strains from other Laboratories. It also occurred, though to a less extent, with the other enzymes studied. Although this variation in enzyme activities has not been further investigated, it has been taken into account when planning the experiments to obtain mean values.

Enzyme differentiation

The similarity of distribution of the four enzymes throughout mammalian tissues raises the question of their differentiation. Fortunately, the question of the identity or non-identity of any or all of the enzymes can be settled at once by the use of the selective inhibitors saccharo-1:4-lactone (Levy, 1952), mannono-1:4-lactone and galactono-1:4-lactone (Conchie & Levy, 1957b), and N-acetylglucosaminonolactone (Findlay *et al.* 1958). Table 7 shows the results obtained when each enzyme is tested with all four specific inhibitors in suitable concentrations. No cross-inhibitory effects whatever were obtained, indicating that all four enzymes were quite distinct.

Table 7. Effect of lactones on glycosidases from rat epididymis

For methods of assay see text. Results are expressed as percentages of controls.

	Inhibition (%)			
	α -Mannosidase	β -Galactosidase	β -N-Acetylglucosaminidase	β -Glucuronidase
Mannonolactone, 10 mm	71	0	0	0
Galactonolactone, 10 mm	0	73	0	0
N-Acetylglucosaminonolactone, 0.1 mm	0	0	98	0
Boiled saccharate,* 0.25 mm	0	0	0	93

* One-third of anion present as saccharo-1:4-lactone, the inhibitory principle.

In view of the existence of lactase in mammalian tissues (Heilskov, 1951), the possibility of the identity of β -galactosidase and lactase was considered. When used as competing substrates 10 mM-lactose caused 11% inhibition of the hydrolysis of 2.5 mM-*o*-nitrophenyl β -galactoside and 10 mM-methyl β -galactoside caused 26% inhibition. When preparations of rat liver and rat epididymis were each assayed for lactase activity by using 10 mM-lactose as substrate the ratio liver:epididymis activity was 1:11. Assayed with nitrophenyl β -galactoside as substrate, the ratio was 1:25. The enzyme would thus appear to have considerable lactase activity.

DISCUSSION

The most notable feature of this investigation was the very wide distribution of the glycosidases α -mannosidase, β -galactosidase and β -*N*-acetylglucosaminidase. All appear to be almost universally distributed in the tissues of the mouse and the rat, and certainly occur in human tissues and in the secretions of the male genital tract in many mammalian species (Conchie & Mann, 1957). The presence of a widely distributed α -mannosidase in mammalian tissues was quite unexpected. This relatively rare enzyme has been noted in various moulds (Hérissey, 1921; Hockenhull, Ashton, Fantes & Whitehead, 1954), lucerne seed (Hill, 1934), in the liver of the snail *Helix laeda* Gould (Nagaoka, 1949) and in the common limpet, *Patella vulgata* (Conchie, Levvy & Marsh, 1956). Although β -galactosidase has already been observed in tissues of various mammalian species (Nishizawa, 1951; Cohen, Tsou, Rutenburg & Seligman, 1952), and may be a lactase, its high activity in the genital tract could scarcely have been foreseen. β -*N*-Acetylglucosaminidase has long been known to occur in mammalian tissues (Watanabe, 1936*a*), and the properties of some partially purified extracts have been investigated (Watanabe, 1936*b*; East, Madinaveitia & Todd, 1941; Pugh *et al.* 1957), but once again the pattern of distribution in the male genital tract was surprising.

In the rat, the outstanding source of these three enzymes was the epididymis, a tissue not previously examined for glycosidase activity. Assays done separately on the caput epididymis and cauda epididymis generally showed slightly greater activity in the cauda than in the caput, though exceptions did occur. β -Glucuronidase activity in the rat epididymis was moderately high, but by far the richest source of this enzyme in the rat, indeed the richest known source, was the female preputial gland, as already observed by Beyler & Szego (1954). Even in the male rat this gland was the most active source, though it had only about 5% of the activity of the corresponding female

gland. In the mouse, the epididymis was the richest source of β -glucuronidase as well as of α -mannosidase, β -*N*-acetylglucosaminidase and, with the possible exception of the spleen, β -galactosidase.

From a few preliminary experiments on α -mannosidase and β -*N*-acetylglucosaminidase in rat-epididymal sperm, it appeared that most of the activity of the two enzymes in epididymis lay in the epididymal tissue and secretion. After washing with calcium-free Ringer buffer (Mann, 1945) there remained in the epididymal sperm a relatively low α -mannosidase activity and very little β -*N*-acetylglucosaminidase activity. This latter observation has been confirmed by Dr P. G. Walker (personal communication). Using a histochemical technique for the localization of β -*N*-acetylglucosaminidase (Pugh & Walker, 1958) he observed that all the β -*N*-acetylglucosaminidase activity in rat epididymis was concentrated in the epithelium. Sperm showed little, if any, activity. The very high β -*N*-acetylglucosaminidase activity in the epididymis of these animals and in the epididymal secretions of many mammalian species (Conchie & Mann, 1957) suggests that this tissue, rather than the commonly used testis, might be worthy of examination as a possible source of hyaluronidase, since these two enzymes are often found in association.

In most tissues, infant values for all the enzymes were equal to, or higher than, the adult ones. In the epididymis, however, activities in both the mouse and the rat were much lower in infancy. The activity is apparently associated with the sexual development of the animals, and full adult enzyme levels in both species are not reached till about 12–14 weeks of age (Conchie & Findlay, 1958). Among the sex organs, the uterus in the female is probably the best analogue of the epididymis in the male. All the enzymes studied showed moderate activities in the uterus of both the mouse and the rat.

The genetically-linked factor producing low β -glucuronidase activities in all tissues of C3H mice did not extend to the other enzymes tested. The increase in liver β -glucuronidase activity found in normal strains of infant mice was greatly exaggerated in the C3H strain. While there was no correlation between the low β -glucuronidase activity in adult C3H mice and the other enzyme activities, the latter did show considerable variation in the livers of our own laboratory strain of mice and of other strains. The variation was most marked for α -mannosidase, and it is possible that, by selective breeding, 'high' and 'low' strains of mice with respect to this enzyme might be obtained.

As with β -glucuronidase, the function of these other glycosidases in mammalian tissues is by no

means clear. Since their distribution is similar to that of β -glucuronidase, it might be expected that this group of enzymes should all have similar functions. Like β -glucuronidase, the other glycosidases investigated are under sex-hormone control (Conchie & Findlay, 1958), although there is no question of the corresponding sugars' forming steroid conjugates.

β -Glucuronidase is a constituent of the crude hyaluronidase complex, acting on some of the oligosaccharides produced during the breakdown of hyaluronic acid and chondroitin (Hahn, 1947; Meyer, Linker & Rapport, 1951; Linker, Meyer & Weissmann, 1955). It is possible that its main role—apart from hydrolysis of steroid glucuronides—lies in the field of mucoid catabolism.

β -N-Acetylglucosaminidase also acts on degradation products from hyaluronic acid, and its action may be expected to extend to chondroitin, since it appears to hydrolyse β -N-acetylgalactosaminides (Heyworth, Boroah & Leaback, 1957; Findlay *et al.* 1958). Since β -N-acetylglucosamine is accompanied by mannose and galactose in the sugar components of many mucoproteins, all the glycosidases investigated above may have a role in mucoid catabolism. A function in intermediary carbohydrate metabolism in seminal secretions cannot, however, be excluded.

A notable feature of these mammalian enzymes is the existence of powerful and highly specific inhibitors for each of them in the form of the aldono-lactone of configuration corresponding to the sugar component of the glycoside hydrolysed. Such inhibitors offer possibilities for the control of physiological and pathological processes with which the enzymes are connected. Saccharo-1:4-lactone, the first of these inhibitors to be discovered (Levy, 1952), has been employed to prevent hydrolysis in the bladder of glucuronic acid conjugates of carcinogens by urinary β -glucuronidase (Boyland & Williams, 1956). It might also be employed to relieve metabolic disorders resulting from an inadequacy in glucuronide synthesis, e.g. in certain forms of jaundice (Brown & Zuelzer, 1958; Lathe & Walker, 1957; Axelrod, Schmid & Hammaker, 1957), in an analogous fashion to the well-known use of eserine in conditions resulting from a failure of, or abnormal demand upon, acetylcholine synthesis, i.e. by slowing down the degradation of the synthetic product by the corresponding hydrolytic enzyme. In the same way, one may foresee possible uses of the inhibitors for α -mannosidase, β -galactosidase and β -N-acetylglucosaminidase.

SUMMARY

1. Like β -glucuronidase, three other glycosidases— α -mannosidase, β -galactosidase and β -N-

acetylglucosaminidase—have been found to be widely distributed throughout mammalian tissues. Tables of their distribution in the mouse and the rat are given.

2. The adult epididymis in both the mouse and the rat is a particularly rich source of the enzymes. Infant-epididymis values were much lower than adult ones.

3. The low-glucuronidase C3H strain of mice showed no differences from other strains in its other glycosidase activities. The increase in β -glucuronidase activity of infant-mouse liver compared with adult liver observed with all strains was greatly exaggerated in the C3H strain.

4. All four enzymes can be differentiated by use of the appropriate selective inhibitors.

5. In view of their similarity in distribution, a common function for this group of enzymes is suggested.

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REFERENCES

- Aminoff, D., Morgan, W. T. J. & Watkins, W. M. (1952). *Biochem. J.* **51**, 379.
 Axelrod, J., Schmid, R. & Hammaker, L. (1957). *Nature, Lond.*, **180**, 1426.
 Beyler, A. L. & Szego, C. M. (1954). *Endocrinology*, **54**, 334.
 Boyland, E. & Williams, D. C. (1956). *Biochem. J.* **64**, 578.
 Brown, A. K. & Zuelzer, W. W. (1958). *J. clin. Invest.* **37**, 332.
 Cohen, R. B., Tsou, K.-C., Rutenburg, S. H. & Seligman, A. M. (1952). *J. biol. Chem.* **195**, 239.
 Conchie, J. (1954). *Biochem. J.* **58**, 552.
 Conchie, J. (1957). *J. Physiol.* **139**, 20 P.
 Conchie, J. & Findlay, J. (1958). *J. Endocrin.* (in the Press).
 Conchie, J., Findlay, J. & Levy, G. A. (1956). *Nature, Lond.*, **178**, 1469.
 Conchie, J., Findlay, J. & Levy, G. A. (1957). *Biochem. J.* **65**, 18 P.
 Conchie, J. & Levy, G. A. (1957a). *Brit. J. Cancer*, **11**, 487.
 Conchie, J. & Levy, G. A. (1957b). *Biochem. J.* **65**, 389.
 Conchie, J., Levy, G. A. & Marsh, C. A. (1956). *Biochem. J.* **62**, 24 P.
 Conchie, J. & Mann, T. (1957). *Nature, Lond.*, **179**, 1190.
 East, M. E., Madinaveitia, J. & Todd, A. R. (1941). *Biochem. J.* **35**, 872.
 Findlay, J., Levy, G. A. & Marsh, C. A. (1958). *Biochem. J.* **69**, 467.
 Hahn, L. (1947). *Biochem. Z.* **318**, 138.
 Heilskov, N. S. Chr. (1951). *Acta physiol. scand.* **24**, 84.
 Helferich, B. & Appel, H. (1932). *Hoppe-Seyl Z.* **205**, 231.
 Helferich, B. & Winkler, S. (1933). *Ber. dtsh. chem. Ges.* **66**, 1556.
 Hérissé, H. (1921). *C.R. Acad. Sci., Paris*, **172**, 766.
 Heyworth, R., Boroah, J. & Leaback, D. H. (1957). *Biochem. J.* **67**, 21 P.

- Hill, K. (1934). *Ber. sächs. Ges. (Akad.) Wiss.* **86**, 115. Quoted in *Chem. Abstr.* (1934). **28**, 5843.
- Hockenhull, D. J. D., Ashton, G. C., Fantes, K. H. & Whitehead, B. K. (1954). *Biochem. J.* **57**, 93.
- Huggett, A. St G. & Nixon, D. A. (1957). *Lancet*, **273**, 368.
- Jermyn, M. A. (1955). *Aust. J. Chem.* **8**, 403.
- Kerr, L. M. H., Graham, A. F. & Levvy, G. A. (1948). *Biochem. J.* **42**, 191.
- Kerr, L. M. H. & Levvy, G. A. (1951). *Biochem. J.* **48**, 209.
- Lathe, G. H. & Walker, M. (1957). *Biochem. J.* **67**, 9P.
- Law, L. W., Morrow, A. G. & Greenspan, E. M. (1952). *J. nat. Cancer Inst.* **12**, 909.
- Levy, G. A. (1952). *Biochem. J.* **52**, 464.
- Levy, G. A. (1956). *Vitam. & Horm.* **14**, 267.
- Levy, G. A. & Marsh, C. A. (1959). *Advanc. Carbohydr. Chem.* (in the Press).
- Linker, A., Meyer, K. & Weissmann, B. (1955). *J. biol. Chem.* **213**, 237.
- McIlvaine, T. C. (1921). *J. biol. Chem.* **49**, 183.
- Mann, T. (1945). *Biochem. J.* **39**, 451.
- Meyer, K., Linker, A. & Rapport, M. M. (1951). *J. biol. Chem.* **192**, 275.
- Montgomery, E. M., Richtmyer, N. K. & Hudson, C. S. (1942). *J. Amer. chem. Soc.* **64**, 690.
- Morrow, A. G., Carroll, D. M. & Greenspan, E. M. (1951). *J. nat. Cancer Inst.* **11**, 663.
- Nagaoka, T. (1949). *Tohoku J. exp. Med.* **51**, 131.
- Nelson, W. L. & Cretcher, L. H. (1930). *J. Amer. chem. Soc.* **52**, 403.
- Nishizawa, K. (1951). *J. Fac. Textiles Sericult. Shinshu Univ. Ser. C1*, 1. Quoted in *Chem. Abstr.* (1953). **47**, 11272.
- Pugh, D., Leaback, D. H. & Walker, P. G. (1957). *Biochem. J.* **65**, 464.
- Pugh, D. & Walker, P. G. (1958). *4th Int. Congr. Biochem., Vienna*, p. 45.
- Roseman, S. (1957). *J. biol. Chem.* **226**, 115.
- Seidman, M. & Link, K. P. (1950). *J. Amer. chem. Soc.* **72**, 4324.
- Talalay, P., Fishman, W. H. & Huggins, C. (1946). *J. biol. Chem.* **166**, 757.
- Watanabe, K. (1936a). *J. Biochem., Tokyo*, **24**, 297.
- Watanabe, K. (1936b). *J. Biochem., Tokyo*, **24**, 315.

The Relationship of Opsopyrroledicarboxylic Acid to the Biosynthesis of Porphyrin

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Porphobilinogen is known to be a specific precursor of porphyrins (Bogorad & Granick, 1953; Falk, Dresel & Rimington, 1953) and, by use of radioactive porphobilinogen, it was shown by Dresel & Falk (1956b) that porphobilinogen alone is a sufficient substrate for the formation of haem in haemolysates prepared from fowl red cells.

The steps involved in the conversion of porphobilinogen into porphyrins of series III are not known; since the only derivatives of porphyrins to be used biologically are of series III and include chlorophyll, haemoglobin and other haem enzymes, the problem is of general importance. The particular difficulty in formulating a mechanism by which porphobilinogen might be converted into a por-

phyrin III arises as follows. The condensation of four molecules of porphobilinogen in the manner shown would, on cyclization, give rise to a series-I structure, which may be described briefly as (AP·AP·AP·AP). The series III structure is (AP·AP·AP·PA), the side chains being those of uroporphyrin. The state of oxidation of the tetramer need not be considered at present; only the relative positions of the β -side chains are relevant. Mechanisms proposed have included (1) condensation of porphobilinogen to give a radial trimer which might be split in two ways to give either (AP·AP) or (AP·PA) (Bogorad & Granick, 1953; Shemin, Russell & Abramsky, 1955), (2) migration of the α -side chain of porphobilinogen (Robinson, 1955; Bullock, Johnson, Markham & Shaw, 1958; D. Mauzerall, personal