Plasma Clearance of Glycoproteins with Terminal Mannose and *N*-Acetylglucosamine by Liver Non-Parenchymal Cells

STUDIES WITH β -GLUCURONIDASE, *N*-ACETYL- β -D-GLUCOSAMINIDASE, RIBONUCLEASE B AND AGALACTO-OROSOMUCOID

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Glycoproteins having mannose and/or *N*-acetylglucosamine in the terminal non-reducing position [Stockert, Morell & Scheinberg (1976) *Biochem. Biophys. Res. Commun.* **68**, 988–993], and various lysosomal enzymes [Stahl, Schlesinger, Rodman & Doebber (1976) *Nature (London)* **264**, 86–88] are rapidly cleared from plasma by the liver after intravenous administration. A liver cell-separation technique was used to determine the cellular localization of ^{125}I -labelled β -glucuronidase, ribonuclease B, agalacto-orosomucoid and asialo-orosomucoid. On a specific radioactivity basis, all ligands except ^{125}I -labelled asialo-orosomucoid were enriched in the non-parenchymal cell fraction. Isolated cells, fixed and stained for β -glucuronidase or *N*-acetyl- β -D-glucosaminidase activity after intravenous injection of the enzymes, showed enrichment in the non-parenchymal cell fraction (probably Kupffer cells). After uptake by the non-parenchymal cells, liver lysosomal β -glucuronidase and *N*-acetyl- β -D-glucosaminidase showed degradation half-times of 2.2 and 0.4 days respectively.

It is now well established that the survival in plasma of various native and modified glycoproteins is determined by the nature of the exposed or terminal sugar residues associated with the carbohydrate chains. The concept that sugars act as determinants for specific recognition and clearance *in vivo* was originally introduced by Ashwell & Morell (1974) to account for the rapid clearance of glycoproteins with a terminal galactose. On the basis of the findings of several groups (Stockert *et al.*, 1976; Lunney & Ashwell, 1976; Baynes & Wold, 1976; Winkelhake & Nicolson, 1976) this concept can now be broadened to include the clearance of glycoproteins with terminal *N*-acetylglucosamine and mannose residues. Work from our laboratory (Stahl *et al.*, 1976*a,b,c*) and that by Achord and his co-workers (Achord *et al.*, 1977*a,b*; Sly *et al.*, 1978) has established that many highly purified lysosomal glycosidases, including human and rat β -glucuronidase, rat β -galactosidase, α -fucosidase, α -mannosidase and *N*-acetyl- β -D-glucosaminidase, are promptly cleared from plasma by a specific recognition system largely associated with liver. After uptake, the above enzymes are rapidly transported to liver lysosomes (Schlesinger *et al.*, 1976; Achord *et al.*, 1977*a*). Lysosomal enzyme clearance is mediated by receptor recognition of both *N*-acetylglucosamine and mannose residues associated with the enzymes. Important in this regard is the study

(Achord *et al.*, 1977*c*) showing that glycoproteins with terminal mannose and *N*-acetylglucosamine appear to compete for the same clearance system. Studies with alveolar macrophages have confirmed this observation and demonstrated that glucose, when conjugated to macromolecules, competes for the same receptor (Stahl *et al.*, 1978). The latter may be appropriately termed mannosyl/glucosyl recognition (Sly *et al.*, 1978), which is distinct from the galactosyl/glucosyl recognition associated with liver parenchymal cells (Ashwell & Morell, 1974).

In the present study, it is demonstrated (i) that lysosomal enzymes and glycoproteins terminating in *N*-acetylglucosamine or mannose are preferentially taken up by the non-parenchymal cells of liver and (ii) that after uptake, lysosomal glycosidases show intralysosomal survival times distinct from those characteristic of resident hepatic lysosomal enzymes.

Materials and Methods

Materials

Female Wistar rats (200g) were obtained from National Laboratory Animals, St. Louis, MO, U.S.A., or Harlan Industries, Cumberland, IN, U.S.A. Collagenase type I, glucose 6-phosphate, albumin, histochemical and chromogenic substrates were obtained from Sigma, St. Louis, MO, U.S.A. Pronase was purchased from Calbiochem, San Diego, CA, U.S.A. Calcium-free and complete

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Hanks solutions were purchased from GIBCO, Grand Island, NY, U.S.A. All other reagents were laboratory grade obtained from local sources.

Methods

Ligands. β -Glucuronidase was prepared from rat preputial gland by the method of Keller & Touster (1975) to a specific activity of greater than 2200 units/mg of protein. Rat liver lysosomal β -glucuronidase was purified by immuno-affinity chromatography as described by Owens & Stahl (1976) (specific activity 1500 units/mg). Rat liver lysosomal *N*-acetyl- β -D-glucosaminidase was purified by affinity chromatography by using *p*-aminophenyl *N*-acetyl- β -D-thioglucoaminide coupled to Sepharose as described by Doebber & Stahl (1978). A preparation with specific activity 7500 units/mg was used. All enzyme units are expressed as μ mol of substrate converted/h. Ribonuclease B was isolated by the method of Baynes & Wold (1976). Asialo- and agalacto-orosomucoid were prepared by using sequential enzymic degradation of orosomucoid with bacterial neuraminidase and β -galactosidase respectively, and given by Dr. Gilbert Ashwell, National Institutes of Health, Bethesda, MD, U.S.A. ^{198}Au -labelled colloidal gold (sp. radioactivity 1.5mCi/mg) was obtained from Mallinckrodt, St. Louis, MO, U.S.A. The ^{125}I -labelled proteins were prepared by the techniques of Bolton & Hunter (1973) as modified by Schlesinger *et al.* (1976). ^{125}I -labelled β -glucuronidase was isolated by chromatography on Sephadex G-50 and DEAE-cellulose (Schlesinger *et al.*, 1976). ^{125}I -labelled asialo-orosomucoid, agalacto-orosomucoid and ribonuclease B were prepared in 200 μ g batches and then dialysed sequentially against 1M-NaCl, 0.01M-KI and 0.005M-Tris/HCl, pH7.5. The radioactivity associated with radioactively labelled proteins was over 95% precipitable after addition of 5% (w/v) phosphotungstic acid. Specific radioactivities varied from 9 to 780 μ Ci/mg. Variations in labelling had no effect on cell localization.

Cell separation. Liver parenchymal and non-parenchymal cells were prepared by using the selective toxicity of Pronase to liver parenchymal cells previously described by Berg & Boman (1973). After injection of ligand (10–15 min), 100 U.S.P. units of heparin were injected (intravenously) and the portal vein was cannulated. The liver was blanché *in situ* with 10ml of Ca^{2+} -free Hanks balanced salt solution (Hanks & Wallace, 1949). All Hanks solutions were buffered with 20mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH7.4. The liver was arranged in a standard perfusion apparatus (Schlesinger *et al.*, 1976), where perfusion (35 ml/min) proceeded with Ca^{2+} -free Hanks solution at 37°C for 5 min without recirculation. Subsequently the liver was perfused for 10 min with 0.05% collagenase

(Sigma type I) in complete Hanks solution with recirculation. An initial cell suspension was prepared by mechanically dispersing the liver in a Petri dish followed by incubation at 37°C with shaking in 80 ml of complete Hanks solution without collagenase. Hepatocytes were isolated from half (40 ml) of the initial cell suspension by low-speed centrifugation (60g-min). Hepatocytes were washed by resuspension and sedimentation in Hanks solution three times. Non-parenchymal cells were prepared by incubation of the remaining half (40 ml) of the initial cell suspension with 0.25% Pronase for 30 min at 37°C. After Pronase digestion, parenchymal cells were nearly undetectable and non-parenchymal cells were harvested and washed three times by sedimentation at $1.2 \times 10^2 \text{g-min}$.

Assays. β -Glucuronidase was assayed with phenolphthalein glucuronide as described by Stahl & Touster (1971). *N*-Acetyl- β -D-glucosaminidase was assayed essentially as described by Stahl *et al.* (1976b), except that *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide was used as substrate. Non-specific esterase (Schiff *et al.*, 1970), β -galactosidase (Owens *et al.*, 1975), glucose 6-phosphatase (Owens *et al.*, 1975) and cathepsin D (Barrett, 1973) were measured by standard assays. The Laurell rocket test was carried out by standard methods (Laurell, 1965) by using goat anti-(rat β -glucuronidase) immunoglobulin G (Owens *et al.*, 1975; Owens & Stahl, 1976). Protein was measured by the Miller (1959) method, with bovine serum albumin from Armour Pharmaceuticals, Kankakee, IL, U.S.A., as standard.

Histochemical analysis. Non-parenchymal cells were isolated from saline (0.15M-NaCl)-injected or enzyme-injected rats as described earlier. The cells were allowed to settle on glass slides (30–60 min) and the slides were washed with cold 0.15M-NaCl. Fixation with 3% glutaraldehyde and staining were carried out at room temperature. Enzyme activity was measured with 6-bromo-2-hydroxy-3-naphthoyl-*o*-anisidine *N*-acetylglucosaminide or β -glucuronide followed by staining of the reaction product (6-bromo-2-hydroxy-3-naphthoyl-*o*-anisidine) by coupling to Fast Red Violet (Sigma) as described by Hayashi (1964). The enzyme (1500 units of *N*-acetyl- β -D-glucosaminidase or 2000 units of β -glucuronidase) was injected in 0.5 ml of 0.15M-NaCl 15 min before the rats were killed and the cells prepared.

Results

Localization of labelled ligands in isolated liver parenchymal and non-parenchymal cells

The cellular distribution of intravenously injected radioactively labelled ligand was determined by separating hepatic parenchymal cells from non-parenchymal cells, and assaying each for the appro-

Table 1. Localization of radioactively labelled ligands after intravenous injection in isolated non-parenchymal cell and parenchymal cell fractions

Anaesthetized rats (200g) were injected with ^{125}I -labelled β -glucuronidase, ^{125}I -labelled agalacto-orosomucoid, ^{125}I -labelled ribonuclease B, ^{198}Au -labelled colloidal gold and ^{125}I -labelled asialo-orosomucoid. After 10–15 min, non-parenchymal (NP) and parenchymal (P) cell fractions were prepared as described under 'Methods'. Glucose 6-phosphatase and cathepsin D were assayed in each fraction. Numbers in parentheses refer to the numbers of preparations used: the percentage recovery was computed as (total c.p.m. in all fractions and all washes/c.p.m. administered) $\times 100$ and total c.p.m. represents c.p.m. measured in an isolated-cell fraction.

Ligand	Fraction	Glucose 6-phosphatase activity (units/mg)	Cathepsin D activity (munits/mg)	Total radioactivity (c.p.m.)	c.p.m./mg	NP/P	Recovery (%)
^{125}I -labelled β -glucuronidase (3)	NP	1.6	24	35656	700	11.0	28
	P	3.0	9	8360	61		
^{125}I -labelled agalacto-orosomucoid (2)	NP	1.1	19	6732	157	3.0	39
	P	1.8	7	11246	48		
^{125}I -labelled ribonuclease B (2)	NP	0.8	21	22556	211	8.5	22
	P	1.9	5	10683	24		
^{198}Au -labelled colloidal gold (1)	NP	1.0	17	16812	170	6.0	36
	P	2.4	5	8478	28		
^{125}I -labelled asialo-orosomucoid (3)	NP	1.2	23	8876	62	0.7	45
	P	2.4	7	25088	90		

priate radioactive isotope. Anaesthetized rats were injected with approx. 5×10^5 c.p.m. of ^{125}I -labelled ligand, 1.7–3.4 μg of β -glucuronidase, 25–30 μg of agalacto-orosomucoid, 10 μg of ribonuclease B, 40 μg of asialo-orosomucoid or ^{198}Au -labelled colloidal gold in 0.5–1.0 ml of 0.15 M-NaCl. Nephrectomized rats (4,12) were used for experiments involving ribonuclease B. At 10–15 min after injection of labelled ligand, 100 units of heparin were injected intravenously, the portal vein was cannulated and the liver was prepared for cell isolation by perfusion as described under 'Methods'. After the initial collagenase perfusion to prepare a single-cell suspension, a portion was digested with Pronase, which selectively destroys the parenchymal cells. The results from the cell-separation experiments are summarized in Table 1. The data are expressed as specific radioactivity (total c.p.m. recovered/mg of cell protein) in the cell fractions. The ratio of the specific radioactivities in the non-parenchymal (NP) and parenchymal (P) cell fractions, the NP/P ratio, was used to compare the relative retention of the labelled glycoproteins by the two cell fractions. The resulting enrichment of the two cell classes was verified in part by measuring the NP/P ratios of the endogenous glucose 6-phosphatase and cathepsin D in the resulting fractions. Glucose 6-phosphatase was enriched 1.8-fold in the parenchymal cell fraction, and cathepsin D was enriched 2–3-fold in the non-parenchymal cell fraction. Other markers include ^{198}Au -labelled colloidal gold, which is selectively taken up by non-parenchymal (Kupffer) cells, and asialo-orosomucoid, which is assimilated by parenchymal cells (Ashwell & Morell, 1974). A

NP/P ratio of 6.0 was observed for ^{198}Au -labelled colloidal gold, reflecting selective uptake by non-parenchymal cells (probably Kupffer cells). Moreover, most of the ^{198}Au was recovered in the non-parenchymal fraction. The parenchymal cell marker ^{125}I -labelled asialo-orosomucoid was recovered largely in the parenchymal fraction (74%) with a NP/P ratio of 0.7. The experimental ligands β -glucuronidase and ribonuclease B were highly enriched in the non-parenchymal cell fraction with NP/P ratios of 11.0 and 8.5 respectively. Moreover, total radioactivity recovered in the two cell fractions indicates that most of the label was present in the non-parenchymal cell fraction. The cellular distribution of ^{125}I -labelled agalacto-orosomucoid was considerably more complex. As shown by comparing specific radioactivities, agalacto-orosomucoid was enriched in the non-parenchymal cell fraction, but significantly more of the total radioactivity recovered was found in the parenchymal cell fraction.

Histochemical localization of β -glucuronidase and *N*-acetyl- β -D-glucosaminidase in non-parenchymal cells

To confirm the cell-separation results, a histochemical test for β -glucuronidase and *N*-acetyl- β -D-glucosaminidase was applied to isolated cells prepared from the liver of rats previously injected (10 min) with a large dose of highly purified rat preputial gland β -glucuronidase, rat liver lysosomal *N*-acetyl- β -D-glucosaminidase or 0.15 M-NaCl. In place of the Pronase digestion, a non-parenchymal cell-enriched

Fig. 1. Osmotically sensitive β -glucuronidase after a large intravenous dose

Experimental rats were injected with 3800 units of rat preputial-gland β -glucuronidase and control rats received only 0.15M-NaCl. At selected times, rats were killed and livers were homogenized in 0.005M-Tris/HCl, pH 7.5 (5 ml/g of liver), with three bursts in a Polytron homogenizer (Brinkman, Westbury, NY, U.S.A.) at setting 5 for 10, 10 and 5 s respectively. The homogenate was centrifuged in a Beckman Spinco centrifuge at high speed ($3 \times 10^6 g$ -min). The pellets were resuspended in the same buffer (5 ml/g), rehomogenized and recentrifuged. The supernatants were pooled and the final pellets were suspended in 8 vol. of buffer containing 0.2% Triton. β -Glucuronidase (a and b) and β -galactosidase (c and d) activities were assayed in both the supernatants (a and c) and pellets (b and d) for experimental (○) and control (●) animals.

fraction was prepared by centrifuging the initial cell suspension, as described under 'Methods', at 60g-min to remove most of the parenchymal cells. The remaining unsedimented cells, a mixture of non-parenchymal and parenchymal cells, were then allowed to sediment at 1g and attach to glass slides. The attached cells were fixed and stained for β -glucuronidase or *N*-acetyl- β -D-glucosaminidase activity. Staining was allowed to proceed for 90 min, and slides containing cells from the enzyme-injected liver showed an intense granular staining reaction limited to the non-parenchymal cells. Parenchymal cells from control livers remained unstained at 150 min, whereas control non-parenchymal cells showed only a slight stain, probably reflecting endogenous enzyme activity. Most of the cells that adhered to glass were probably Kupffer cells, since they form rosettes when incubated for 30 min with mouse erythrocytes coated with

immunoglobulin G. In a control experiment, the attached non-parenchymal cells failed to form rosettes with uncoated erythrocytes. Similar results have been observed for cultured Kupffer cells and macrophages (Reynolds *et al.*, 1975; Munthe-Kaas *et al.*, 1975).

Survival of lysosomal glycosidases after uptake by liver lysosomes

Previous studies have shown that most of the injected enzyme is recovered in the liver (Schlesinger *et al.*, 1976). To measure the intralysosomal turnover of exogenously administered β -glucuronidase and *N*-acetyl- β -D-glucosaminidase, the purified enzymes were administered intravenously to the anaesthetized rat as described previously (Stahl *et al.*, 1976c). Animals were killed at intervals after recovery from surgery. Three different enzyme preparations were used: A, rat liver lysosomal β -glucuronidase; B, rat preputial-gland β -glucuronidase; C, rat liver lysosomal *N*-acetyl- β -D-glucosaminidase. In all the experiments, animals were starved overnight before injection and overnight before death. Three experimental protocols were used for recovery and estimating the injected enzyme in hepatic tissue. Total recovery of injected enzyme (experimental minus control) was always 70–90% at 1 day after injection. Previous work in this laboratory has shown that at short times after injection (1–2 h) injected enzyme is quantitatively recovered in the liver (Schlesinger *et al.*, 1976). (A) Liver lysosomal β -glucuronidase (1000 units; 1500 units/mg) or 0.15M-NaCl was injected on day zero. Livers were fractionated (Stahl & Touster, 1971) into mitochondrial/lysosomal, microsomal and supernatant fractions. β -Galactosidase or *N*-acetyl- β -D-glucosaminidase and esterase were used as markers for lysosomes and microsomal fractions respectively. Specific activities of these markers in the subcellular fractions were the same when control and experimental animals were compared. Most of the injected β -glucuronidase activity was recovered in the mitochondrial/lysosomal fraction, which resulted in a 2.5-fold increase in enzymic activity/mg of protein over that in the mitochondrial/lysosomal fraction from control rats 24 h after injection. A semi-logarithmic plot of specific activity in the mitochondrial/lysosomal fraction (experimental minus control) against time yielded a straight line, and a half-time for enzyme decay of 2.2 days was estimated graphically. (B) Rat preputial-gland β -glucuronidase (3800 units; 2375 units/mg) or 0.15M-NaCl was injected as described earlier except that livers, taken from starved control and experimental rats, were homogenized in hypo-osmotic buffer and fractionated as described in Fig. 1. Two fractions were obtained, a supernatant fraction that contained the contents of lysosomes disrupted by hypo-osmotic shock and

Fig. 2. Time-dependence of β -glucuronidase activity in the livers of injected rats

After a large intravenous dose of rat preputial-gland β -glucuronidase (3800 units) the osmotically extractable activity was prepared as described in Fig. 1. β -Glucuronidase specific activity (units/mg in experimental minus units/mg in control animals) is plotted on a logarithmic scale against the day after injection the animal was killed. There were two animals each in experimental and control groups. The half time for the loss of injected enzyme activity was 2.2 days as determined from the slope of this semi-logarithmic plot.

a crude pellet. Fractions were assayed for β -galactosidase, β -glucuronidase and esterase. The results are summarized in Fig. 1, where specific activities of β -glucuronidase and β -galactosidase in the supernatant and pellet fractions are plotted against time in days. β -Galactosidase and esterase (not shown) activities in both supernatant and pellet fractions were constant with respect to time, whereas that of β -glucuronidase in the supernatant fraction was increased in experimental animals. Most of the β -galactosidase activity was solubilized by the homogenization in hypo-osmotic medium and is

reflected in the higher specific activity of the supernatant fraction. The relatively high activity of β -glucuronidase in the pellet probably reflects endogenous microsomal β -glucuronidase activity, which may account for as much as 40% of liver β -glucuronidase activity (Owens & Stahl, 1976). A semi-logarithmic plot of supernatant β -glucuronidase specific activity (experimental minus control) (Fig. 2) allowed an estimation of the half-time of enzyme decay of 2.2 days. (C) Rat liver lysosomal *N*-acetyl- β -D-glucosaminidase (2000 units; 8600 units/mg) was injected into anaesthetized rats, and the same protocol was used as with preputial-gland β -glucuronidase (i.e. B), except that before centrifugation the homogenate was made 0.15M with respect to NaCl because *N*-acetyl- β -D-glucosaminidase remains with the membrane pellet under conditions of low ionic strength. The half-time of decay of activity of *N*-acetyl- β -D-glucosaminidase in the supernatant fraction was estimated graphically as 0.42 day (results not shown).

An independent measure of β -glucuronidase degradation was made by quantifying the β -glucuronidase antigen in liver fractions from β -glucuronidase-injected animals by using the Laurell rocket test (Laurell, 1965) and goat anti-(rat β -glucuronidase) immunoglobulin G (Owens & Stahl, 1976). These experiments (results not shown) demonstrated a loss of β -glucuronidase antigenic activity from liver with the same half-time as that estimated by following enzymic activity.

Discussion

There are at least two sugar-specific recognition systems operating in mammals for the clearance of glycoproteins from plasma. Ashwell & Morell (1974) have characterized a liver hepatocyte recognition system that mediates clearance of glycoproteins with terminal galactose. Krantz *et al.* (1976) have shown that the 'Ashwell' receptor also recognizes a glucose analogue covalently bound to bovine serum albumin. Consequently, this system might be referred to as a galactosyl/glucosyl-recognition system. A second recognition system has been described that mediates the clearance of glycoproteins with terminal *N*-acetylglucosamine and mannose. This system recognizes most lysosomal glycosidases, which are glycoproteins presumably having mannose or *N*-acetylglucosamine in the terminal position. An important contribution to the understanding of this system was the study by Achord *et al.* (1977c) showing cross-competition between glycoproteins with terminal mannose and *N*-acetylglucosamine for clearance *in vivo*. These findings have been corroborated by studies *in vitro* with alveolar macrophages, where it has been shown that glycoproteins or glycoconjugates with terminal glucose, mannose or *N*-acetylglucosamine demonstrate reciprocal competition when tested for binding

to isolated cells (Stahl *et al.*, 1978). Thus the recognition system for clearance of lysosomal enzymes might be referred to as the mannosyl/glucosyl system. The terminology used in the present paper was originally suggested by W. S. Sly (unpublished work).

The studies reported in the present paper show that the mannosyl/glucosyl-recognition system is located in liver non-parenchymal cells, in contrast with the galactosyl/glucosyl system, which is confined to the parenchymal cells (Ashwell & Morell, 1974). Four different glycoproteins, which showed preferential localization in non-parenchymal cells, were used in the present study. Ribonuclease B is a glycoprotein with terminal mannose (Plummer & Hirs, 1963), and agalacto-orosomucoid has terminal *N*-acetylglucosamine. β -Glucuronidase has considerable mannose and *N*-acetylglucosamine covalently bound to the enzyme, but the terminal sugars are unknown (Tulsiani *et al.*, 1975). Rat liver *N*-acetylglucosaminidase is a glycoprotein of unknown sugar composition. In the cell-separation experiments, ribonuclease B and β -glucuronidase showed preferential localization in the non-parenchymal cell fraction. In fact, on the basis of the NP/P ratio the localization of the last two was more specific than that observed with the Kupffer-cell marker, ^{198}Au -labelled colloidal gold. The cellular localization of agalacto-orosomucoid was considerably more complex in that a NP/P ratio of 3.0 was observed. Nevertheless, a clear distinction exists between the cellular distribution of asialo-orosomucoid and agalacto-orosomucoid.

The histochemical studies confirm the cell-separation results and show a preferential localization of β -glucuronidase and *N*-acetyl- β -D-glucosaminidase in non-parenchymal cells attached to glass. These cells were positive when tested for rosette formation with antibody-coated erythrocytes, suggesting that the uptake observed in the present study is due to liver Kupffer cells. Recent studies showing that alveolar macrophages display a mannosyl/glucosyl receptor (Stahl *et al.*, 1978) are consistent with the notion that reticulo-endothelial cells (fixed tissue macrophages) are engaged in uptake *in vivo*. However, because of the qualitative nature of the histochemical studies, we cannot rule out the possibility that liver sinusoidal endothelial cells are also engaged in glycoprotein uptake.

After hepatic uptake, β -glucuronidase (liver lysosomal and preputial) and liver lysosomal *N*-acetyl- β -D-glucosaminidase show rather short intralysosomal survival times. The half-life of infused β -glucuronidase in these experiments is similar to that observed for human placental β -glucuronidase in rat liver (2.8 days) (Achord *et al.*, 1977a). These observations are noteworthy in view of results obtained from various studies with fibroblasts (Glaser *et al.*, 1975; Warburton & Wynn, 1976) and liver (de Duve &

Wattiaux, 1966; Wang & Touster, 1975; Tsuji & Kato, 1977) (probably parenchymal cells), where lysosomal enzymes showed long half-times. The turnover rate of a given lysosomal enzyme may depend both on the enzyme and on the specific lysosomal environment in which it is operating. In particular, there is an extensive lysosomal system in Kupffer cells and other liver non-parenchymal cells (Blouin *et al.*, 1977), which may be a result of their acquisition of derelict lysosomal hydrolases that have been expressed to the extracellular space from many tissue sites and are available for clearance. This potent lysosomal system in reticulo-endothelial cells may explain the brisk turnover of newly acquired exogenous β -glucuronidase and *N*-acetyl- β -D-glucosaminidase.

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