Golgi β -Glucuronidase of Androgen-Stimulated Mouse Kidney

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Subcellular fractions were prepared from mouse kidney homogenates by differential and sucrose-gradient centrifugation. A fraction enriched in Golgi apparatus was obtained, which had considerably enriched galactosyltransferase and thiamin pyrophosphatase activities, and was morphologically typical of Golgi material. This preparation also had high β -glucuronidase activity, which increased concomitantly with microsomal β -glucuronidase activity during the specific stimulation of the enzyme in male mouse kidney after androgen administration. The degree of stimulation was much greater in the Golgi fraction. Gel-electrophoretic patterns of Golgi β -glucuronidase resembled more closely those of the enzyme located within lysosomes, but contained minor bands similar to those described previously (Swank & Paigen, 1973) as characteristic of the microsomal enzyme. It was concluded that the Golgi complex is involved in the distribution of the enzyme after its synthesis to both lysosomal and microsomal fractions.

The dual localization of some acid hydrolases in both lysosomal and microsomal fractions was demonstrated by the results of biochemical and histochemical studies. Microsomal acid phosphatase (EC 3.1.3.2) and arylsulphatase (EC 3.1.6.1) differ from the lysosomal enzymes in enzymic properties, especially with regard to their substrate specificities, as well as in their association with the cytoplasmic membranes (Ide & Fishman, 1969; Lin & Fishman, 1972; Milsom *et al.*, 1972; Sasaki & Fishman, 1973). β -Glucuronidase (EC 3.2.1.31) has been shown to be also enriched in the microsomal fractions of liver homogenates and the microsomal enzyme is associated with the intracellular membranes (Ide & Fishman, 1969; Fishman *et al.*, 1967, 1969). In liver 35% and in kidney 20–25% of the total tissue β -glucuronidase is associated with the microsomal fraction.

Paigen (1961*a,b*) demonstrated that in the mouse the same single structural gene codes for the β -glucuronidase protein in both lysosomal and microsomal fractions, suggesting a common origin of synthesis within the cell with subsequent distribution to the different cellular locations.

Investigation of the mechanism of the intracellular transport and distribution of β -glucuronidase has been aided by the specific induction of this enzyme in the male mouse kidney by administration of androgen. The increase of the enzyme activity is due to the increase of synthesis *de novo* of the enzyme

protein (Pettengill & Fishman, 1962; Frieden *et al.*, 1964; Swank *et al.*, 1973).

Kato *et al.* (1970, 1972) found that the dramatic increase in kidney β -glucuronidase activity in male mice treated with gonadotrophin occurred initially in the microsomal fraction and was followed only after a lag of about 40 h in the lysosomes, indicating a precursor-product relationship. This temporal pattern of induction has been confirmed by Ganschow (1973). It was postulated that after synthesis of this enzyme on membranes of the rough endoplasmic reticulum, β -glucuronidase entered its cisternae to be transferred via the smooth endoplasmic reticulum into primary lysosomes. However, this hypothesis was disputed by the genetic and biochemical studies of Swank & Paigen (1973), who held that the majority of the microsomal enzyme was not the precursor of the lysosomal enzyme.

The Golgi apparatus has been suggested as a site of assembly, transport and intracellular distribution of macromolecules (Novikoff *et al.*, 1971; Whaley *et al.*, 1972). It was therefore the purpose of our study to investigate whether or not the Golgi apparatus is involved in the transport and distribution of β -glucuronidase in the mouse kidney cell. The results were obtained from the study of β -glucuronidase in a Golgi-rich fraction prepared from mouse kidney. From the results of the determination of the rate of enrichment of this enzyme in the Golgi fraction during the process of androgen stimulation, there is evidence for the supposition that the Golgi apparatus is participating in the translocation of β -glucuronidase in the mouse kidney cells.

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A preliminary report of the present work has been presented (Marsh *et al.*, 1973).

Experimental

Methods

Preparation of the Golgi-rich fraction from mouse kidney. The method described by Leelavathi *et al.* (1970) was modified for the preparation of the Golgi-rich fraction from mouse kidney. In this procedure, a 15% (w/v) homogenate of the tissue was prepared by homogenizing the kidney tissue in a buffered ice-cold sucrose solution which contained 0.5 M-sucrose, 50 mM-Tris-HCl buffer, pH 6.5, and 10 mM-MgCl₂. The homogenization was performed in a loosely fitted Duall glass homogenizer (Kontes Glass Co., Vineland, N.J., U.S.A.; 0.3–0.4 mm clearance) at 1500 rev./min for 2 × 30 s. The homogenate was then centrifuged at 600g for 10 min in a Sorvall centrifuge. The sediment of unbroken cells and nuclei was discarded and the supernatant subjected to two steps of discontinuous sucrose-density-gradient centrifugation. In the first step, 10–15 ml of the low-speed supernatant was layered over 15 ml of 1.3 M-sucrose in 50 mM-Tris-HCl buffer, pH 6.5, containing 10 mM-MgCl₂, and centrifuged at 76000g for 60 min in a Spinco ultracentrifuge in the 25.1 swing-bucket rotor. The top supernatant layer was removed and the interphase layer subjected to a further density-gradient centrifugation by layering 10 ml of diluted sample (containing 3.5 ml of the interphase layer diluted to 10 ml with 0.5 M-sucrose in the same Tris-Mg²⁺ buffer) over two layers of sucrose (1.1 M and 1.3 M, in the Tris-Mg²⁺ buffer). This system was then centrifuged in the swing-bucket rotor for 60 min at 76000g and resulted in the separation of most of the particulate material in the two interphase layers. The upper interphase layer was found to be the fraction enriched in Golgi apparatus and was used for the subsequent studies.

Preparation of lysosomal and microsomal fractions from mouse kidney. Concurrent isolation of Golgi, lysosomal and microsomal fractions from the same kidney homogenates was not feasible. Previous attempts, performed almost entirely on liver preparations, to isolate Golgi fractions have demonstrated the necessity of minimum homogenization to preserve the integrity of the Golgi complex; such homogenization is inadequate for vesiculating the endoplasmic reticulum. Lin & Fishman (1972) showed that much longer periods of tissue disruption of mouse kidney tissue were essential to obtain adequate vesicle formation in the microsomal preparation.

The procedure described by Lin & Fishman (1972) was thus followed for the preparation of lysosomal and microsomal fractions; the kidney was homogenized in a tight-fitting ground-glass Duall homogenizer (0.10–0.15 mm clearance) for 3 min. Conven-

tional differential-centrifugation methods were then used for the preparation of lysosomal and microsomal fractions from the homogenate.

Assay of enzymic activity. For the assay of galactosyl-transferase activity the methods of Morré *et al.* (1969) were followed. UDP-[¹⁴C]galactose was used as substrate. The incubation mixture (total vol. 200 μl) contained: 80 μl of enzyme sample, 1.0 μmol of MgCl₂, 0.5 μmol of MnSO₄, 10 μmol of 2-mercaptoethanol, 2.0 μmol of Tris-HCl buffer (pH 7.5), 0.04 μmol of UDP-[¹⁴C]galactose (18000 d.p.m.) and 0.5 mol of *N*-acetylglucosamine (omitted from the control tubes). Incubation was carried out at 37°C for 15 min. At the end of incubation, the unchanged substrate was separated from the product of reaction by an anion-exchange column of AG1-X2 (Bio-Rad Laboratories, Richmond, Calif., U.S.A.). [¹⁴C]Galactose and ¹⁴C-labelled *N*-acetyl-lactosamine were eluted from the column with distilled water. The radioactivity was determined by liquid-scintillation counting by using Bray's (1960) scintillation fluid. Enzyme units are expressed as nmol of UDP-galactose transferred/h of incubation.

For thiamin pyrophosphatase determination, the assay mixture (1.0 ml) contained 5 μmol of thiamin pyrophosphate, 0.15 nmol of Ammediol (2-amino-2-methylpropane-1,3-diol) buffer, pH 8.6, and 5 μmol of MgCl₂. After 1 h of incubation at 37°C, the reaction was terminated by adding 1 ml of 10% (w/v) trichloroacetic acid. P_i released was then determined by the method of Lowry & Lopez (1946).

The conditions for the assay of glucose 6-phosphatase are those of Nordlie & Arion (1966); for acid phosphatase, Lin & Fishman (1972); for alkaline phosphatase, Fishman & Ghosh (1967); for β-glucuronidase, Fishman (1967); β-galactosidase, α-mannosidase and β-*N*-acetylglucosaminidase were assayed by standard methods (Levy & Conchie, 1966) by using 1 mM-*p*-nitrophenyl substrates at pH 4.0, 5.0 and 5.0 respectively.

Quantitative terms for enzyme activities reported in the present paper are μmol of P_i/h per mg of protein for glucose 6-phosphatase and thiamin pyrophosphatase; μmol of *p*-nitrophenol/h per mg of protein for α-mannosidase, β-galactosidase and β-*N*-acetylglucosaminidase; nmol of phenol/min per mg of protein for alkaline phosphatase; nmol of UDP-galactose transferred/h per mg of protein for galactosyltransferase; nmol/20 min per mg of protein for acid phosphatase; and Fishman units (μg of phenolphthalein/h) per mg of protein for β-glucuronidase.

Electron microscopy. Samples for negative staining were applied to Formvar grids and washed twice with 0.9% NaCl before being stained with aq. 2% (w/v) phosphotungstic acid (pH 6.8) containing 0.01% bovine serum albumin. Photographs were taken with a Jeolco 100B electron microscope.

The staining of β-glucuronidase in the Golgi frac-

tion was carried out by the method of Smith & Fishman (1969). The fraction was centrifuged at 105000g for 1 h to obtain a pellet which was fixed in 2.5% (v/v) glutaraldehyde (in 0.1 M-sodium phosphate buffer, pH 7.4) before staining. Naphthol AS-BI β -D-glucuronic acid (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was used as substrate and *p*-acetoxymercuric aniline diazotate was used as coupling agent.

Electrophoresis of β -glucuronidase. Electrophoretic studies of the enzyme were carried out by the method of Clarke (1964) and of Swank & Paigen (1973); 7% (w/v) polyacrylamide gels (4mm $\times$ 90mm) were prepared in 0.1 M-Tris–0.4 M-glycine buffer, pH 8.5, measured at room temperature. Samples from various subcellular fractions were extracted with 5% (v/v) Triton X-100 in 0.25 M-sucrose and 0.02 M-imidazole buffer, pH 7.4, and centrifuged at 105000g for 1 h to obtain the clear supernatants, which were then applied for electrophoresis (10–40 μ l per gel). The electrode buffer solution was 5 mM-Tris–40 mM-glycine, pH 8.1. A constant voltage of 200 V was applied and the electrophoresis was carried out in the cold-room (4°C) for 2 h with cold water in circulation around the gel tubes.

After electrophoresis, gels were preincubated in 0.2 M-sodium acetate buffer at pH 5.2 for 45 min before being stained for β -glucuronidase activity to detect the presence of the enzyme in the gel. The procedure for the staining was adapted from the histochemical method described by Hayashi *et al.* (1964), which used naphthol AS-BI glucuronide (0.25 mM) as substrate and coupled simultaneously with hexazonium pararosanilin (0.3 mM) in 0.1 M-sodium acetate buffer, pH 5.2.

Protein determinations. These were performed by the method of Lowry *et al.* (1951).

Materials

A/Jax strain (Jackson Laboratory, Bar Harbor, Maine, U.S.A.) mice, about 3 months old, were used in this study. This strain of mouse has been shown to be very responsive to androgen induction of the kidney β -glucuronidase activity (Fishman & Farmelant, 1953). Human chorionic gonadotrophin (Follutin, E. R. Squibb and Sons, New York, N.Y., U.S.A.), 2.5 i.u. in 1 ml of physiological saline (0.9% NaCl), was injected intraperitoneally. The mice were killed by decapitation and the kidneys from groups of six mice pooled, cleaned and weighed before homogenization.

UDP-[14 C]galactose was purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.) with a specific radioactivity of 250 mCi/mmol, and was diluted with the unlabelled compound. Phenolphthalein β -D-glucosiduronic acid was synthesized in our laboratory by the biosynthetic method described by Fishman (1967). *p*-Nitrophenyl β -galactoside, *p*-nitrophenyl α -mannoside and *p*-nitrophenyl β -N-acetylglucosaminide were obtained from Koch–Light Laboratories Ltd. (Colnbrook, Bucks., U.K.).

Results

Enzyme characteristics of the Golgi-rich fraction

The activities of several marker enzymes in the Golgi-rich fraction prepared from normal mouse kidney, in comparison with those in the tissue homogenate and in two other fractions obtained in the process of the preparation, are summarized in Table 1. These are results of a single experiment to verify the procedure in obtaining a Golgi-enriched fraction from mouse kidney. The specific activity of galactosyltransferase was increased sevenfold in the Golgi-rich

Table 1. Marker enzyme activities in fractions of Golgi preparation

The crude homogenate and subcellular fractions were obtained as described in the text, as are methods for enzyme activity and protein content. The specific activity of galactosyltransferase is expressed as nmol of UDP-galactose transferred/h per mg of protein, thiamin pyrophosphatase and glucose 6-phosphatase as μ mol of P_i/h per mg of protein, β -glucuronidase as μ g of phenolphthalein/h per mg of protein, acid phosphatase as nmol of phenol/20 min per mg of protein and alkaline phosphatase as nmol of phenol/min per mg of protein. Fraction (A), crude kidney homogenate; fraction (B), interphase layer of first sucrose-density-gradient centrifugation; fraction (C), upper interphase layer of second sucrose-density-gradient centrifugation (Golgi fractions); fraction (D), lower interphase layer of second sucrose-density-gradient centrifugation. Results are from a single typical experiment.

Enzymes	Fractions ...	Specific activities (units/mg of protein)			
		(A)	(B)	(C)	(D)
Galactosyltransferase		6.5	8.7	42.5	3.0
Thiamin pyrophosphatase		8.4	19.2	39.0	7.0
Glucose 6-phosphatase		1.9	2.4	3.4	1.8
β -Glucuronidase		15.4	27.2	22.6	20.0
Acid phosphatase		0.39	—	1.00	0.24
Alkaline phosphatase		12.1	22.1	23.6	32.2

fraction compared with the crude homogenate, and the ratio of the specific activities of galactosyltransferase to that of glucose 6-phosphatase was raised considerably. An increase in the specific activity of thiamin pyrophosphatase, an enzyme associated specifically with the Golgi elements according to Novikoff & Goldfischer (1961), was also noted, but to a lesser extent. This enzyme is considered less specific in cellular location than galactosyltransferase (Leelavathi *et al.*, 1970). Its use as a marker enzyme was further complicated by the fact that the Golgi fractions were found also to have a high thiamin monophosphatase activity, which therefore contributed to the apparent thiamin pyrophosphatase activity.

The Golgi fractions obtained in this study also had elevated specific activities of glucose 6-phosphatase and acid phosphatase. The latter finding agreed with results which have demonstrated histochemically and biochemically that the Golgi apparatus is one of the sites of acid phosphatase localization (Riechen & Pearce, 1965; Goldstone & Koenig, 1973). Alkaline phosphatase was also present in this fraction, which probably indicates a modest degree of contamination by the plasma membrane, in whose brush border this enzyme is essentially localized (Jacobsen *et al.*, 1967; Fishman & Lin, 1973).

Plate 1 shows the appearance of the Golgi fraction viewed under the electron microscope. The majority of the structures observed were typical of Golgi elements. In the sections stained for β -glucuronidase activity (Plate 1b), numerous vesicles of dimensions 0.1–0.4 μ m were observed which positively stained for enzyme activity.

Distribution of β -glucuronidase

Table 2 shows the distributions of β -glucuronidase and other glycosidases in mouse kidney subcellular fractions from animals not undergoing androgen treatment. Whereas β -glucuronidase activity was relatively high in both lysosomal and microsomal fractions, other glycosidase activities were more localized within the lysosomal fraction only. For galactosyltransferase, the activity in the microsomal

fraction was 2.8 times that in the crude homogenate, indicating that the homogenization procedure probably resulted in the disruption of the Golgi apparatus into particles which sedimented with this fraction. This may be of considerable relevance in evaluating microsomal β -glucuronidase in view of the great increase in Golgi β -glucuronidase specific activity after gonadotrophin treatment as described below. Golgi β -glucuronidase activities also displayed considerable latency, being increased approximately twofold in the presence of 0.1% (v/v) Triton X-100, i.e. an effect similar to that obtained with the lysosomal preparations.

Effects of gonadotrophin

Changes in the β -glucuronidase activities in the subcellular fractions (Golgi, lysosomal and microsomal) were examined for a 5-day period after a single intraperitoneal injection of gonadotrophin (2.5 i.u./mouse). Fig. 1 shows that increases in activity in the lysosomal and microsomal fractions were similar in temporal sequence to those reported by Kato *et al.* (1972), in that whereas the microsomal enzyme activity began to rise within 12 h of treatment and reached a peak value after 3 days, little change occurred in the lysosomal activity until 24 h after injection, and maximum specific activity in this fraction occurred after 4 days.

Up to 48 h after injection of gonadotrophin, the increase in the specific activity of the Golgi β -glucuronidase, relative to its initial value, was intermediate between those of lysosomal and microsomal fractions. However, at 3 days after injection, the relative specific activity of the Golgi fraction did increase to a much higher value (11-fold for Golgi fraction versus fivefold for microsomal and lysosomal fractions), and remained greatly enhanced 5 days after the treatment. This result indicates that the Golgi complex is involved in the induction of this enzyme by androgen, and might appear to contribute materially to the apparent enzyme increase observed in the microsomal fraction, if the Golgi fragments are part of the microsomal particles resulting from the

Table 2. Glycosidase activities in kidney subcellular fractions of untreated mice

The preparation of crude homogenate and subcellular fractions and methods for enzyme and protein assays are described in the text. The specific activity of β -glucuronidase is expressed as μ g of phenolphthalein/h per mg of protein and those of β -galactosidase, α -mannosidase and β -N-acetylglucosaminidase are expressed as μ mol of *p*-nitrophenol/h per mg of protein. Results are from a single experiment.

Fraction	Specific enzyme activity (units/mg of protein)			
	Crude homogenate	Lysosomal	Microsomal	Golgi
β -Glucuronidase	16.8	29.3	28.6	19.1
β -Galactosidase	0.40	0.85	0.27	0.40
α -Mannosidase	0.17	0.32	0.17	0.16
β -N-Acetylglucosaminidase	1.46	3.30	1.52	2.0

EXPLANATION OF PLATE I

Golgi fraction viewed by electron microscopy

(a) Golgi fraction negatively stained with 2% (v/v) phosphotungstic acid, pH 6.8. Magnification $\times 140000$. (b) The same fraction stained for β -glucuronidase activity by the method of Smith & Fishman (1969). Magnification $\times 40000$. Arrows point to membrane sites where β -glucuronidase is positively stained.

EXPLANATION OF PLATE 2

Electrophoretic pattern of β -glucuronidase in lysosomal (L), microsomal (M) and hormone-stimulated (Gs) and non-stimulated (Gn) Golgi fractions

Samples were extracted with 5% Triton X-100 before being applied for electrophoresis. The gels were 7% (w/v) polyacrylamide in Tris-glycine buffer (pH 8.5). The electrophoresis was carried out in Tris-glycine buffer (5–40mM, pH 8.1) for 2h at 4°C. The enzyme activity was stained by using naphthol AS-BI glucuronide (0.25mM) as substrate and coupled with hexazonium pararosanilin (0.3mM) in 0.1M-sodium acetate buffer, pH 5.2. For other details, see the text. The designation of the enzyme components (M1–M4, L and X) follows the nomenclature of Swank & Paigen (1973). The fast band, G (for Golgi), is observed in the hormone-stimulated Golgi fraction (Gs).

long homogenization process. The prolonged elevation of the relative enzyme specific activity in the Golgi fraction also suggests that a portion of Golgi β -glucuronidase is retained in the Golgi apparatus after the enzyme activity in the microsomal and lysosomal locations has decreased to normal values.

The effects of gonadotrophin on other glycosidases and acid hydrolases were also examined (Table 3). A significant increase in the specific activity of galactosyltransferase in the crude kidney homogenate occurred as a result of the hormone treatment, but

there was relatively little change in the specific activity of this enzyme in the isolated Golgi fractions. This indicates a general increase in Golgi material in renal cells after androgen stimulation, which has also been observed by electron microscopy. Among the acid hydrolases examined, β -glucuronidase is the only enzyme to respond significantly to androgen induction.

Electrophoresis of β -glucuronidase

The electrophoretic patterns of β -glucuronidase for the microsomal, lysosomal and Golgi fractions are shown in Plate 2. The overall isoenzyme patterns generally confirm those described by Swank & Paigen (1973) who reported that the mouse kidney β -glucuronidase consists of at least six electrophoretic components; four (M_1 to M_4) being of microsomal origin, one (L) of lysosomal origin and an X component, which was believed to be the precursor of the microsomal isoenzymes. All these six components were observed in our study. The Golgi fraction from non-stimulated kidney showed all the five components of lysosomal and microsomal origins, with the L component being the most predominant. The Golgi β -glucuronidase of the hormone-stimulated kidney demonstrated significant increases in L, M_1 and M_2 components, with the L form most significantly intensified. The X component was also observed in the Golgi fraction of the hormone-stimulated kidney, as well as a hitherto undescribed fast band, G.

Fig. 1. Response of β -glucuronidase in mouse kidney homogenate (○), lysosomal (●), microsomal (■) and Golgi (□) fractions to gonadotrophin injection

Relative specific activities were calculated with respect to the non-injected controls. Each subcellular preparation was made from the pooled kidneys of six mice.

Discussion

Results of this study indicate that the Golgi apparatus is participating in the translocation of β -glucuronidase in the gonadotrophin-stimulated mouse kidney and presumably in similar events in

Table 3. Specific activities of galactosyltransferase, thiamin pyrophosphatase, glucose 6-phosphatase and several acid hydrolases at various time-intervals after gonadotrophin injection

Kidney homogenate and Golgi fraction were prepared as described in the text. Six male mice were killed at each time-interval after injection of gonadotrophin (2.5 i.u./mouse). For details of enzyme and protein measurements and units of enzyme activities see the text.

Enzymes	Time after injection (h)	...	Specific activities (units/mg of protein)							
			Kidney homogenate				Golgi fraction			
			0	24	48	72	0	24	48	72
Galactosyltransferase			6.5	11.7	18.7	10.3	42.5	43.0	51.5	40.0
Thiamin pyrophosphatase			8.4	8.0	7.3	7.0	39	35	30	38
Glucose 6-phosphatase			1.9	1.7	1.8	1.9	3.4	1.8	2.9	3.2
β -Glucuronidase			15	31	86	112	23	45	212	303
α -Mannosidase			0.15	0.15	0.16	0.17	0.24	0.23	0.42	0.38
β -Galactosidase			0.35	0.35	0.35	0.39	0.70	0.45	0.55	0.68
β -N-Acetylglucosaminidase			1.44	1.52	1.54	1.54	4.3	3.2	4.8	5.3
Acid phosphatase			0.39	0.40	0.36	0.35	1.00	0.85	0.52	0.64

normal physiological conditions. However, it is not possible to decide from the available data at which step in the temporal sequence the Golgi apparatus is involved. The apparent retention of β -glucuronidase by the Golgi apparatus after androgen stimulation suggests that in addition to its previously accepted role as the location of modification of the nascent enzyme, part of the Golgi function may serve as a reservoir for the enzyme to be subsequently transported into other cellular sites, such as the primary lysosomes. Alternatively, passage of the enzyme in the Golgi tubules could be the rate-limiting process in the overall translocation. The electrophoretic pattern of β -glucuronidase, which showed a high intensity of the lysosomal component in the hormone-induced Golgi fraction, implies that a conversion of the microsomal form into the lysosomal one may be occurring in the Golgi apparatus.

Goldstone & Koenig (1973) have suggested that acid hydrolases, after being synthesized in a restricted portion of the rough endoplasmic reticulum as bound basic glycoproteins, migrate through the Golgi apparatus where acidic sugar molecules are attached by a glycosyltransferase mechanism. This converts the acid hydrolases into the 'lysosomal types'. Such a concept is consistent with our observations on the possible role of Golgi apparatus in the translocation and distribution of β -glucuronidase. This is also in agreement with earlier experimental results of Plapp & Cole (1967), which suggested that multiple forms of bovine liver β -glucuronidase resulted from differences in the carbohydrate content of the enzyme.

Swank & Paigen (1973) have shown that the microsomal components of mouse β -glucuronidase are higher polymers than the lysosomal component, which has a tetramer structure. The sixth component (X), which is also localized in microsomal fractions after androgenic stimulation, appears to be similar to the lysosomal component in molecular weight but not in charge. The four microsomal components can be converted into component X *in vitro* by heating or by trypsin digestion in urea. However, the L and X components are not affected by these treatments. Swank & Paigen (1973) suggested that component X may be the precursor of a normal microsomal component, which is then converted into other microsomal forms by modification of the polypeptide chains and polymerization. The lysosomal form, according to this hypothesis, is derived directly from the monomer and not from the transformation of the microsomal forms.

The interpretation of the molecular nature of the various isoenzyme forms in a mammalian tissue observed by Swank & Paigen (1973) is different from that suggested earlier by reports of Plapp & Cole (1966, 1967). From the results of chromatography, ultracentrifugation, amino acid analysis and studies of enzymic properties on purified bovine liver, Plapp

& Cole (1966, 1967) suggested that the multiple forms of β -glucuronidase resulted from their differences in carbohydrate content, not in their molecular weights.

Since the results of our experiment suggested that Golgi apparatus is involved in the translocation of β -glucuronidase, it is possible that this organelle may also be involved in the transformation of the enzyme into various isoenzyme forms, particularly if the idea of differences in carbohydrate content contributing to the formation of multiple enzyme forms is to be accepted. This is consistent with the findings of Goldstone & Koenig (1973) that Golgi apparatus is involved in the attachment of acidic carbohydrate molecules to the protein moieties of acid hydrolases.

It is clear that the Golgi organelle should now be included as one of the cellular components to be investigated for the elucidation of the pathway of the intracellular translocation and the mechanism of the interconversion of different isoenzyme forms of β -glucuronidase. The direction of the transport and distribution of β -glucuronidase from its location of synthesis must remain speculative. Conclusive evidence in this respect cannot be drawn from the electrophoretic enzyme patterns derived from Golgi and microsomal fractions unless these are demonstrably not cross-contaminated. Thus the M_3 and M_4 enzyme forms, which Swank & Paigen (1973) believe to be exclusively microsomal, appeared in our preparations (see Plate 2) to be associated more with the normal and hormone-stimulated Golgi preparations. It is also possible that a portion of the enzyme which appears in the Golgi fraction may be subsequently transferred into cellular structures other than the lysosomes. At a high degree of androgen stimulation, some of the nascent enzymes synthesized may, for example, remain as part of the endoplasmic-reticulum membrane and eventually be incorporated into the plasma membrane, possibly by a process such as 'membrane flow' hypothesized by Morré *et al.* (1971). The status of androgen stimulation in this case would be a factor in determining the distribution of β -glucuronidase transported by different routes.

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