DISTRIBUTION OF TERMINAL GLYCOSYLTRANSFERASES IN HEPATIC GOLGI FRACTIONS

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ABSTRACT

The distribution of the three glycosyltransferases synthesizing the terminal trisaccharide sialic acid → D-galactose → *N*-acetylglucosamine present in many glycoproteins was determined in Golgi fractions prepared from rat liver homogenates by a modification of the procedure of Ehrenreich et al. (1973, *J. Cell Biol.* **70**:671–684). The enzymes were assayed with asialofetuin, ovomucoid, and Smith-degraded ovomucoid as sugar acceptors. Careful adjustment of the pH of all sucrose solutions to 7.0 ± 0.1 prevented enzyme inactivation, and allowed quantitative recoveries at every isolation step. The three morphologically and functionally different Golgi fractions GF_1, GF_2, and GF_3 showed (in that order) decreasing specific activities of all three enzymes, but the relative amounts and relative specific activities of the three transferases in any given fraction were nearly identical. Two marginal fractions, one extra heavy (collected on the gradient below GF_3) and the other extra light (isolated by flotation from the postmicrosomal supernate) were found to contain recognizable Golgi elements. An enrichment of any transferase over the two others was not detected in either preparation.

A partial release of content from a combined GF_{1+2} was achieved by treatment with the nonionic detergent Triton X-100. Low Triton/phospholipid ratios (<2 mg/mg) led to lysis of the vesicles and cisternae and loss of very low density lipoprotein particles (ascertained by electron microscopy), but failed to separate the transferases from each other; the three enzymes sedimented together with a population of empty vesicles to a density of ~1.08 g/ml.

KEY WORDS rat liver Golgi fractions · subfractionation · detergents (Triton X-100) · sialyltransferase · galactosyltransferase · *n*-acetylglucosaminyltransferase

The Golgi complex plays a major (perhaps exclusive) role in the terminal glycosylation of glycoproteins (32, 46), in addition to its involvement in the intracellular transport, concentration, and partial proteolytic processing of secretory proteins (27, 41). The evidence is firm for exportable glycoproteins, but still in need of final validation for nonexportable species. Secretory glycoproteins—like other exportable proteins—pass sequentially through the compartments of the secretory pathway (i.e., endoplasmic reticulum [ER], Golgi complex, secretion granules), and are glycosylated while in transit (43, 44, 56) before being discharged. The most common glycoprotein oligosaccharide—found in plasma proteins, egg white proteins, immunoglobulins, as well as the few membrane glycoproteins so far examined (14, 54)—is

a branched chain that contains *N*-acetylglucosamine *N*-glycosidically linked to an asparaginyl residue (30). Its proximal segment, or common core, consists of chitobiose, several mannosyl residues, and (initially) a few glucosyl residues (25, 33, 45). The site of its synthesis and the mechanism of its transfer to the corresponding polypeptide chain have been investigated in some detail in the cells of the mammalian liver and avian oviduct (34, 43). The chain is synthesized in the ER as a dolichol pyrophosphate-oligosaccharide precursor and transferred cotranslationally as a whole unit to a nascent polypeptide (56). In contrast, the residues of the terminal trisaccharide of the chain, i.e., *N*-acetylglucosamine (GlcNAc),[1] D-galactose (Gal), and *N*-acetylneuraminic acid (NANA) are added stepwise to the common core (46, 47), after removing from the latter its glucosyl and part of its mannosyl residues (45, 53).

The glycosyltransferases that catalyze the three terminal steps have been localized to the Golgi complex by autoradiography (32) and to Golgi (or Golgi-rich) fractions by biochemical assays (4, 17, 46, 47).

As discussed in reference 15, the Golgi complex has functional polarity and consists of morphologically and cytochemically heterogeneous elements. The procedure of Ehrenreich et al. (12) resolves the Golgi complex in three successive fractions of which the heaviest and the lightest are enriched in cis- vs. trans-Golgi elements, respectively. Moreover, recently published data suggest that hepatic secretory proteins move from heavy to light Golgi elements before being discharged (3).

Given this context of findings and assumptions, it becomes of interest to find out whether terminal glycosyltransferases are distributed uniformly or sequentially among resolvable Golgi elements. If the second alternative were to apply, GlcNA-transferase would be concentrated in cis-Golgi elements (i.e., the heavy Golgi fraction), whereas sialyltransferase would be associated preferentially with trans-Golgi elements (i.e., the light Golgi fraction). To answer this question we have investigated the distribution of terminal glycosyltransferases among Golgi fractions isolated as in reference 12 and assayed as intact Golgi vesicles, as well as vesicles partially freed of their content.[2]

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 120–150 g (from Charles Rivers Breeding Laboratories, Inc., Wilmington, Mass., or from Sueddeutsche Versuchstierfarm, Tuttlingen, Germany) were starved overnight and given ethanol (6 g/kg body weight, in 50% wt/vol solution) via stomach tube 90 min before sacrifice.

Subcellular Fractions

Golgi fractions (light: GF_1; intermediate: GF_2; and heavy: GF_3) were isolated by the following procedure derived from references 12 and 21, and devised to reduce leakage of Golgi content (cf. reference 48) and to improve the separation of different types of Golgi elements.

After passage through a tissue press, the livers were homogenized in 0.25 M sucrose with only five strokes in a glass/Teflon homogenizer at low speed (950 rpm). The homogenate was adjusted to a concentration of 20% (wt/vol) and then centrifuged for 10 min at 10,000 g_{av} (Beckman L2-65 ultracentrifuge, type 30 rotor; Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.); this step removed unbroken cells, cell debris, nuclei, and mitochondria into a common large-particle pellet. The corresponding supernate was collected and centrifuged for 130 min at 78,500 g_{av} to obtain a total microsomal pellet, which was resuspended in 1.22 M sucrose with three strokes in the same homogenizer. After adjusting the refractive index of the suspension to 1.400 (corresponding to that of 1.38 M sucrose) by adding 2 M sucrose, an 8-ml sample was layered under a step density gradient (6 ml each of 1.22 M, 1.15 M, 0.86 M, 0.6 M, 0.25 M sucrose [Fig. 1, gradient M]), and centrifuged for 3 h at 82,500 g_{av} in a Beckman SW27 rotor. All sucrose solutions were carefully adjusted to pH 7.0 with 0.01 M Tris.

In addition to GF_1, GF_2, and GF_3, harvested as described in reference 12, the following fractions were collected: the 1.15-M and 1.22-M sucrose layers (light and heavy interlayers), the load section, and the pellet (resuspended in 0.25 M sucrose).

The postmicrosomal supernate was concentrated to one-tenth of its volume by ultrafiltration (N_2 pressure: 2 bar) on a Millipore Pellicon PTHK membrane in a stirred molecular filtration cell (Millipore AG, Zurich, Switzerland). Solid sucrose was added to the concentrate to give a final refractive index of 1.3970 (equivalent to 1.32 M sucrose), and 11 ml of this suspension was layered under a gradient of 1.15 M (5 ml), 0.86 M (11 ml), 0.25 M (5 ml), and 0.11 M sucrose (6 ml) (Fig. 1, gradient S). After centrifugation for 4 h at 82,500 g_{av} in a Beckman SW27 rotor, a thick fluffy white band (GF_0) was found spread over the whole 0.25-M sucrose layer. This band, and the layers indicated in Fig. 1, gradient S, and in Table V, were collected and assayed as separate fractions.

Detergent Treatment of Golgi Fractions and Continuous Gradient Centrifugation

All operations were performed at 0°-4°C. GF_1 and GF_2 were

[1] *Abbreviations used in this paper:* ER, endoplasmic reticulum; Gal, galactose; GlcNAc, *N*-acetylglucosamine; NANA, *N*-acetylneuraminic acid; PL, phospholipid; VLDL, very low density lipoprotein (particles).

[2] Some of these results have been published in preliminary form (7).

pooled (GF_{1+2}), and their volume and sucrose concentration were reduced by ultrafiltration as above, except that the beginning of the operation was carried out under constant volume conditions, so as to reduce osmotic stress to Golgi vesicles.[3] Preliminary experiments had shown that this procedure did not alter the morphology of Golgi elements in GF_{1+2} (not shown). By contrast, dilution of the fraction with water followed by pelleting and resuspension caused extensive damage to, and massive aggregation of, Golgi elements.

Samples of concentrated GF_{1+2} (1.7–3.2 mg protein, 3.2–4.3 mg phospholipid) were then mixed with appropriate amounts of [^{3}H]Triton X-100[4] to give Triton/phospholipid ratios ranging from 0.7 to 2.0 mg/mg. The mixtures were loaded onto linear sucrose gradients (either 0.3–1.15 M or 0.3–0.86 M on a 2-M cushion) containing 0.2 mg/ml [^{3}H]Triton, and centrifuged for 90 min to 11 h (at 96,000 g_{av} in a Beckman SW27.1 rotor). Under these conditions, visible bands formed, and enzyme activity peaks could be detected, at resolvable distances from the load. Triton X-100 was added to the gradient because in its absence Golgi elements lost almost entirely their detergent as they moved along the gradient. The concentration used (0.2 mg/ml) is slightly higher than the critical micellar concentration of Triton X-100 (which is 0.15 mg/ml at room temperature, but is expected to be higher at 4°C [19]).

After centrifugation, the load was aspirated with a blunt 2-mm syringe needle attached to a peristaltic pump (Perpex, H. J. Guldener, Lucerne, Switzerland), then the needle was lowered in 7-mm steps to collect a series of consecutive layers from the top to the bottom of the gradient. Collected samples were assayed for Triton X-100 radioactivity, in addition to protein, phospholipid, and transferase activities.

Electron Microscopy

Samples were fixed in suspension with 1% OsO_4 (final concentration) in 0.1 M cacodylate buffer (pH 7.4), at 4°C for 2 h or overnight. Pellicles were then prepared as in reference 2 and further processed as in reference 5. Sections of pellicles were examined and micrographed in a Philips 200 or 301 electron microscope.

Biochemical Determinations

GENERAL: Protein and phospholipid were assayed as in reference 8.

Glycosyltransferases

GENERAL ASSAY CONDITIONS: Since quantitative studies of enzymic activity distribution depend critically on the reliability of the assays used, we have reevaluated some of the properties of the three glycosyltransferases and tested a number of activating, inhibiting, or interfering factors. The results are given in Table I. The tabulated K_m are apparent values, determined by double-reciprocal plotting, without corrections for substrate degradation by pyrophosphatases and other possible variables; accordingly, they cannot be taken as true kinetic parameters; they were primarily used to optimize the assay procedures.

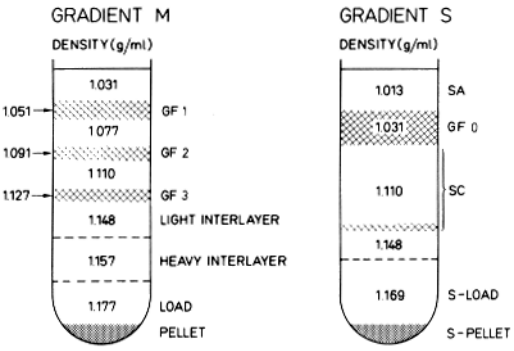

FIGURE 1 Golgi fractions were isolated from resuspended total microsomes on gradient M, and GF_0 was floated out of the postmicrosomal supernate on gradient S. The final positions of visible bands are indicated by cross-hatched areas. The densities of load sections and resulting fractions were estimated from their refractive indices and are given as equivalent densities of pure sucrose solutions.

The activation of the three transferases by Triton X-100 was investigated as in reference 8. The activation curves resembled each other closely (not shown), reaching maximal values at 2.0–2.5 mg Triton/mg phospholipid in whole homogenates as well as total microsomal fractions, and thus suggesting that activation is a membrane effect (i.e., increased permeability for macromolecular acceptors and possibly charged precursors), rather than a specific molecular response.

SPECIFIC ASSAYS: Galactosyltransferase (UDP-Gal: 2-acetamido-2-deoxy-D-glucosylglycopeptide galactosyltransferase, E.C. 2.4.1.38, with ovomucoid as acceptor) was measured as in reference 8, except that the pH of the assay was raised to 7.0, the volume was reduced to 50 μl, and 2 mM ATP was added to protect the substrate against endogenous pyrophosphatases. At the end of the incubation, the acceptor protein was recovered (by precipitation) and then dissolved in tissue solubilizer (TS 1, Koch Light Labs, Colnbrook, U. K.). The solution was neutralized with glacial acidic acid, and its radioactivity measured in a Packard B2450 Tri-Carb Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) with a xylene/Triton X-114 (3:1) scintillation cocktail containing 2,5-diphenyloxazole (PPO, 3 g/liter) and 2,2′-phenylene-bis (5-phenyloxazole) (POPOP, 0.2 g/liter).

In the assays for sialyltransferase (CMP-*N*-acetylneuraminate: D-galactosyl-glycoprotein *N*-acetylneuraminyltransferase, E.C. 2.4.99.1), and GlcNAc-transferase (UDP-2-acetamido-2-deoxy-D-glucose: glycoprotein 2-acetamido-2-deoxy-D-glucosyltransferase, E.C. 2.4.1.51), the same incubation time (30 min), volume, amount of subcellular material (0.003–2.5 mg protein), and washing procedure were used as for galactosyltransferase.

[3] To this intent, water was added dropwise from a reservoir that contained the amount needed to reduce to 0.25 M the sucrose concentration of the GF_{1+2} sample. The constant volume filtration equipment was built by Mr. C. Lehmann and Mr. K. Babl.

[4] The first batch of radioactive Triton X-100 was a gift from Dr. Alan M. Rothman, Rohm and Haas Research Laboratories, Spring House, Pa. Subsequently, [^{3}H]Triton X-100 (New England Nuclear) was diluted with nonradioactive Triton X-100 (Merck, Darmstadt, Germany) to a final sp act of 0.1 μCi/mg.

The incubation medium for sialyltransferase contained: 100 mM cacodylate buffer, pH 5.8, 40 mM β-mercaptoethanol, 0.4% Triton X-100, 1.0 mM CMP-[^{14}C]NANA (New England Nuclear, Boston, Mass., 1.68 mCi/mmol), and 17.5 mg/ml asialofetuin prepared from native fetuin (Calbiochem, San Diego, Calif.) by mild hydrolysis (in 0.05 M H_2SO_4 at 80°C for 1 h as in reference 50).

GlcNAc-transferase was assayed in the presence of 100 mM cacodylate buffer, pH 6.6, 40 mM β-mercaptoethanol, 0.4% Triton X-100, 10 mM $MnCl_2$, 2 mM ATP and 0.7 mM UDP-[^{3}H]GlcNAc, (New England Nuclear), diluted with nonradioactive UDP-GlcNAc (Sigma Chemical Co., St. Louis, Mo.) to a sp act of 5 mCi/mmol. The acceptor glycoprotein (final concentrations 25 mg/ml) was prepared from ovomucoid by Smith-degradation (periodate oxidation, $NaBH_4$ reduction, and mild hydrolysis) following Spiro's method for the removal of terminal galactose from asialofetuin (51). This procedure reduces the GlcNAc content of ovomucoid to less than half (9); it also removes some of the mannosyl residues (9)[5] thereby exposed, but enough of the latter remain for the glycoprotein to act as a GlcNAc acceptor. The preparation of Smith-degraded ovomucoid is much simpler than the enzymic or chemical removal of three terminal sugars from fetuin (6).

Untreated ovomucoid proved to be an acceptor for GlcNAc-transferase as well as for sialyltransferase, but it had in both cases a higher apparent K_m and a lower V_{max} than the acceptors specified in Table I.

CONTROLS: To assess possible secondary effects caused by glycosidases, batches of ^{14}C-resialylated fetuin, [^{3}H]galactoovomucoid, and ^{3}H-reglucosaminylated ovomucoid were purified on Sephadex G-25 and subjected to simulated assay conditions (without sugar donor) in the presence of whole homogenate samples. No significant degradation could be detected for up to 2 h. Even the relatively acid-labile sialyl residues were not affected by precipitation and washing in 0.5 M HCl containing 1% phosphotungstic acid.

RESULTS

Stability of Transferase Activities

Transferase activities in homogenates and total microsomes did not decrease during storage for 2 d on ice or for 6 wk at −20°C (Fig. 2). In Golgi fractions, however, there was a variable but generally substantial inactivation; in comparable fractions, Howell et al. (21) reported 42% inactivation of galactosyltransferase in 24 h. Preincubation with Triton X-100 for 1 d also caused variable and extensive inactivation. The addition of β-mercaptoethanol (5 mM) as sulfhydryl reagent and antioxidant to all solutions used in the fractionation procedure prevented transferase inactivation, but led to extensive aggregation, swelling, and rupture of Golgi vesicles, as observed by electron microscopy (not shown). The procedure of Ehrenreich et al. (12) uses unbuffered sucrose solutions. In our initial experiments, isolated GF_1 had a pH as low as 4.8, whereas the pH of the homogenates, total microsomes, and load sections of the gradient was ~6.8. When all sucrose solutions were carefully neutralized to pH 7.0 ± 0.1 by the addition of 0.01 M Tris, the transferases were satisfactorily stabilized in Golgi fractions, and recoveries throughout the isolation procedure ranged from 85 to 100% (Table II). This procedure also stabilized all transferase activities in fractions treated with Triton X-100 (2 mg Triton/mg phospholipid) for periods up to 11 h at 0°C.

Distribution of Terminal Glycosyltransferases in Cell Fractions Prepared by Differential Centrifugation

With the optimized procedures described above it was possible to quantitate and compare the distribution patterns of the three glycosyltransferases in hepatic cell fractions.

Table II shows that the common large-particle pellet contained >60% of the protein and phospholipid of the whole homogenate and almost half of its transferase activities. This distribution reflects the high proportion of unbroken cells and cell debris left behind by the mild homogenization procedure we used. For all three enzymes, the same fraction of the total activity (~40%) was recovered from the sucrose step gradient M. This indicates that neither transferase was enriched over the others in the total microsomal fraction.[6]

Fractions Obtained by Gradient Centrifugation and Their Morphological Survey

Subfractionation on gradient M yielded the three Golgi fractions described in reference 12 and, in addition, two fractions slightly heavier than GF_3 (light interlayer, d = 1.15 g/ml; heavy interlayer, d = 1.16 g/ml), the load section, and a small pellet (Fig. 1).

Although morphologically our Golgi fractions resembled closely those illustrated by Ehrenreich et al. (12), they are described in some detail for the following reasons: (*a*) they were collected on

[5] The acceptor properties and solubilities varied somewhat from preparation to preparation; the results reported in this paper were obtained with the best batches.

[6] Recovery for individual fractions were calculated by reference to the starting preparation (resuspended total microsomes) as well as the sum of all fractions in the gradient. The two sets of figures were comparable, but more variable (hence, less reliable) in the first alternative.

TABLE I

Properties of Hepatic Terminal Glycosyltransferases

Parameter studied	Monosaccharide transferred: NANA	Gal	GlcNAc
Monosaccharide donor	CMP-NANA	UDP-Gal	UDP-GlcNAc
Apparent K_m*	0.4 mM	0.06 mM	0.14 mM
Monosaccharide acceptor	Asialofetuin	Ovomucoid	Smith-degraded ovomucoid
Apparent K_m*	1.1 mg/ml	6 mg/ml	30 mg/ml
pH optimum	5.8	7.0	6.6
Metal ion required	none	Mn	Mn
Apparent K_m*	—	3.7 mM	2.5 mM
Inhibition by EDTA (4 mM)	0%	100%	100%
Detergent required for maximal activation: mg Triton X-100/mg phospholipid	2–2.5	2–2.5	2–2.5
Linearity of product formation with time	60 min	120 min	60 min
Product hydrolysis under assay conditions	<1%	<1%	<1%

* Determined with double reciprocal plots without corrections. The enzymes were exposed to saturating concentrations of all other reagents.

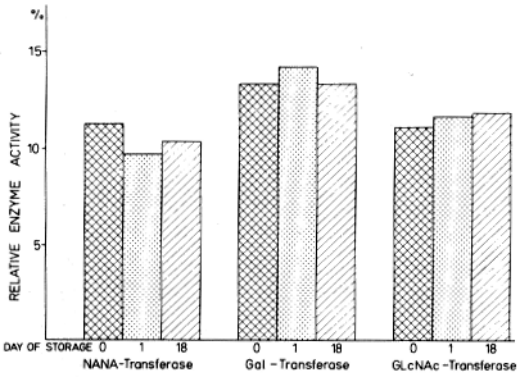

FIGURE 2 Stability of glycosyltransferase activities in stored GF_{1+2}. The transferases were assayed in homogenates immediately after the collection of the Golgi fractions (day 0), and in GF_{1+2} either at day 0, or after 24 h on ice at 0°C (day 1), or after 18 d at −20°C. The results are given as percent of the activities found in the corresponding whole homogenate. The figures are averages of two experiments.

filters (2), hence, their sampling is less open to question; (*b*) the corresponding biochemical data are not in full agreement with those reported by Bergeron et al. (4); accordingly a reexamination of the morphology of the fractions became necessary.

GF_1 consisted almost entirely of Golgi vesicles and Golgi cisternae (Fig. 3). The vesicles (Diam: 0.2–0.5 μm) were characteristically filled with clustered very low density lipoproteins (VLDLs), embedded in a dense, amorphous matrix. The cisternae appeared as dumbbell or elongated profiles (normal sections) or double-ring profiles (cross section through buckled cisternae); some of them had distended margins filled with VLDLs; a few had a festooned appearance that suggested that they derived from fenestrated cisternae. Small vesicles or tubules (Diam: 0.05–0.1 μm)—empty or containing single VLDL particles—were minority components of uncertain origin: they could have derived from either cis Golgi elements or the smooth ER.

GF_2 had the same type of components but in different proportions: cisternal profiles, with or without VLDL-filled rims, and small vesicles, empty or marked by single VLDLs, were more frequent than in GF_1 (Fig. 4).

In GF_3, "empty" structures were predominant and vesicles with more than one VLDL were rare; the majority of the components were Golgi cisternae of the types described in GF_1 and GF_2 (Fig. 5). In addition to smooth vesicles, possibly of smooth ER origin, GF_3 contained a small number of recognizable contaminants, namely rough microsomes and damaged mitochondria.

The light interlayer (not shown) underneath GF_3 resembled the latter fraction: vesicular elements containing single VLDLs were still very frequent, but empty vesicles, presumably smooth microsomes, became a major component. This fraction might be derived from smooth-surfaced elements located *in situ* at the periphery of the Golgi complex. The heavy interlayer contained a

TABLE II

*Distribution and Recovery of Glycosyltransferase Activities in Hepatic Cell Fractions**

Fraction	Protein	Phospholipid	Sialyltransferase	Galactosyltransferase	GlcNAc-transferase
Large-particle pellet	60.2 ± 4.4	63.2 ± 11.7	46.1 ± 10.7	37.9 ± 3.7	57.3 ± 10.2
Recovery from gradient M (Total microsomal fraction)	12.6 ± 1.0	23.3 ± 3.8	43.6 ± 3.3	42.6 ± 5.1	41.6 ± 4.5
Recovery from gradient S (postmicrosomal supernate fraction)	21.4 ± 2.7	1.8 ± 0.4	6.3 ± 1.2	6.3 ± 1.4	5.5 ± 0.9
Total recovery	94.2 ± 5.3	88.3 ± 12.3	96.0 ± 11.3	86.8 ± 6.5	104.4 ± 11.2

The mean absolute values per gram liver wet weight found in whole homogenates were: protein: 174 ± 14 mg; phospholipid: 39.8 ± 8.6 mg; sialyltransferase: 1,749 ± 167 nmol/30 min; galactosyltransferase: 657 ± 76 nmol/30 min; GlcNAc-transferase: 514 ± 138 nmol/30 min.

* Data in percent of whole homogenate, mean and standard deviation of four experiments.

population of small, mostly smooth-surfaced vesicles, and the load was a mixture of smooth and rough microsomes. Some contamination with VLDL-containing vesicles and dumbbell- and ring-shaped Golgi elements could still be observed in both fractions. In the pellet, rough microsomes and free polysomes were predominant, smooth vesicles were few, and recognizable Golgi structures were virtually absent.

In summary, large Golgi vesicles (i.e., predominantly trans-Golgi elements) decreased markedly from GF_1 to GF_3, concomitantly with a progressive increase in Golgi cisternae and small vesicles (i.e., predominantly cis-Golgi elements). The latter were still present in relatively large numbers in two additional fractions heavier than GF_3, i.e., the two interlayers.

Transferase Activities in Golgi Fractions

The results given in Table III show that the specific activity of the Golgi marker enzyme galactosyltransferase was 50 times higher in GF_1, and 30 times higher in GF_3, than in the whole homogenate. GlcNAc-transferase and sialyltransferase showed a similar distribution. In all three cases the specific enzyme activities decreased in the order GF_1, GF_2, GF_3, light interlayer, heavy interlayer, and were much lower in the load and the pellet than in all the other fractions.

The recoveries of the three transferases in any given fraction were almost identical. GF_1 and GF_2, the lightest and least contaminated Golgi fractions, contained 12–13% of the original homogenate activity of each transferase; GF_3 and the light interlayer accounted together for another 13%. Variations in the relative distribution of individual transferases in any single experiment were clearly smaller than variations for any transferase in separate experiments; therefore, differences in distribution patterns (if present) were below the resolution of our methods. (Table IV)

Transferase Activities in the Postmicrosomal Supernate

A small but fairly constant percentage (5–10%) of the activity of each transferase was detected in the postmicrosomal supernate which was expected to be free of all microsomal and Golgi elements (cf. reference 12). These activities could not be ascribed to truly soluble enzymes, contributed by liver cells or the blood plasma, since they were activated (2.5-fold) by Triton X-100 (2.5 mg/mg PL), and since they eluted in the void volume of a Sephadex G-200 column. Soluble glycosyltransferases should not be activated by detergent (this is the case for the transferases of the blood plasma [22–24]), and are expected to be retained by the column mentioned, if comparable in size to the soluble transferases of the colostrum (mol wt: 40,000–50,000 [42, 49, 55]). The transferase activities of the postmicrosomal supernate seemed to be bound to particles of unexpectedly low density ($\leq$1.030 g/ml) since they failed to sediment out of 0.25 M sucrose even at 105,000 *g* 12 h. When the postmicrosomal supernate was concentrated[7] and its density adjusted to that of 1.32 M sucrose, the transferase activities could be floated in a discontinuous sucrose density gradient of the type shown

[7] Millipore Pellikon PTHK membrane used for concentration has a nominal exclusion limit of 100,000 daltons.

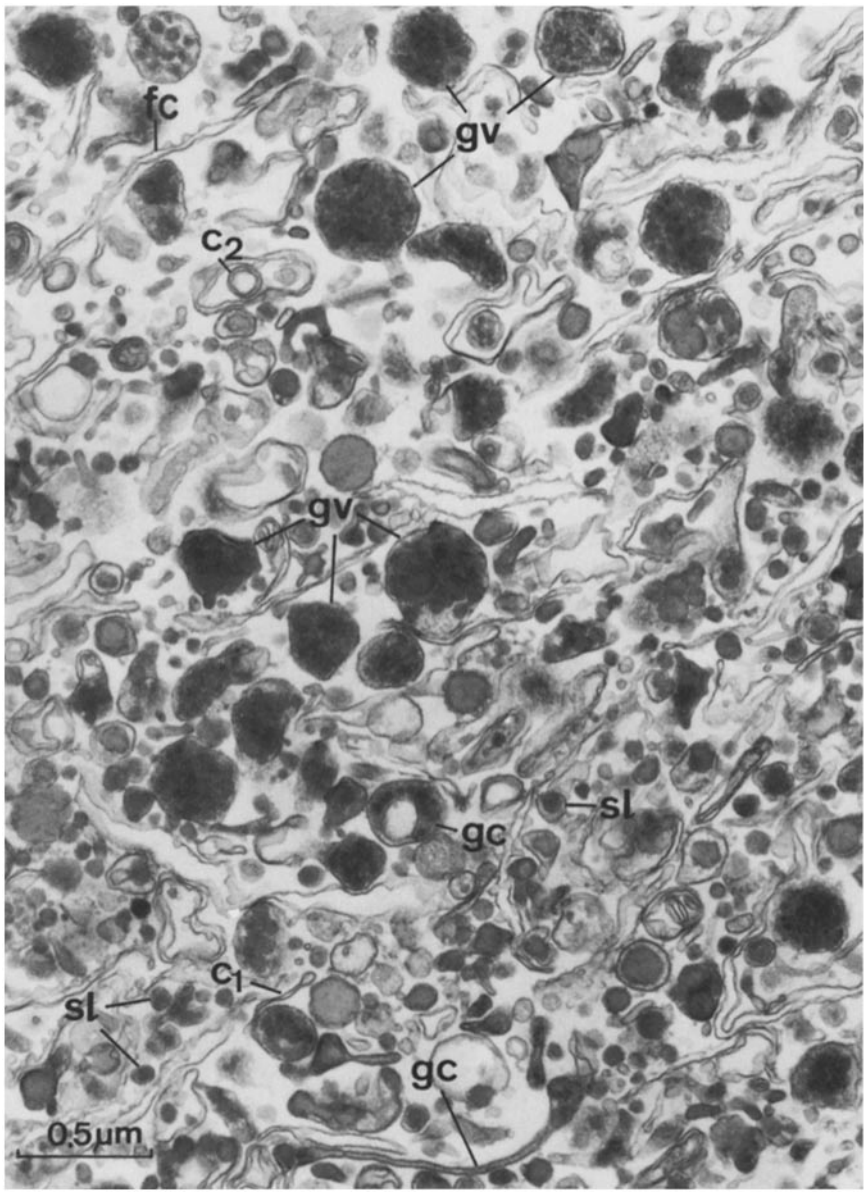

FIGURE 3 GF_1 (light Golgi fraction). Field from the middle of the pellicle, representative for the entire preparation. VLDL-filled vesicles: *gv*; cisternae with few VLDLs in their dilated rims: *gc*; small vesicles containing single VLDLs: *sl*; empty cisternae: c_1 (normal section), and c_2 (transverse section, double-ring profile); festooned cisternal profile: *fc*. × 36,000.

in Fig. 1, S. As indicated by this figure and the data in Table V, most of the activities (60%) were recovered in a thick, whitish band in a 0.25-M sucrose layer. The recovery (3–4%) and the relative sp act (6–7) were the same for each glycosyltransferase in this extra-light Golgi fraction (designated GF_0). By electron microscopy (Fig. 6), GF_0 consisted primarily of free lipid droplets of the size of VLDLs or larger (up to 0.4 µm), and contained as minority components typical Golgi cisternae and Golgi vesicles marked by clustered VLDLs. The apparent low density of these elements presumably reflects their coaggregation with free VLDL or lipid droplets.

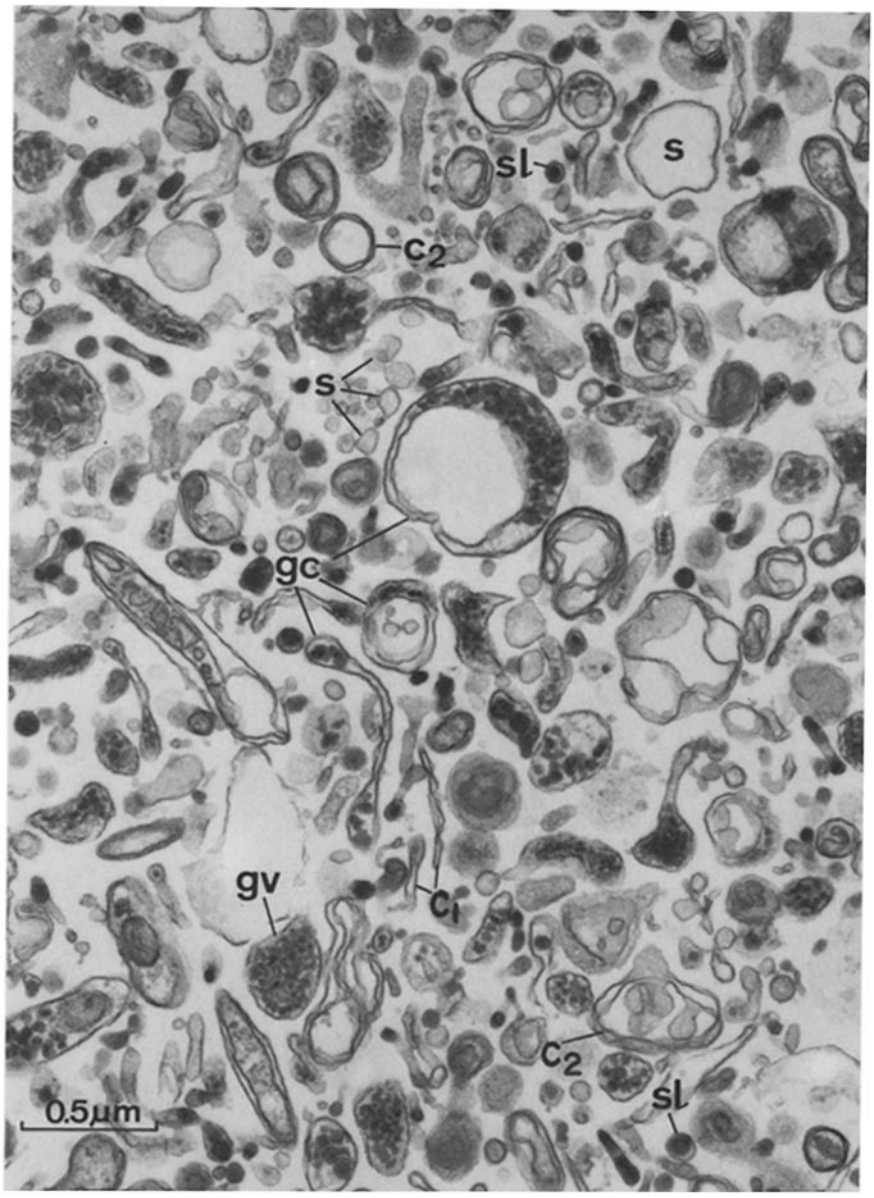

FIGURE 4 GF_2. (Intermediate Golgi fraction.) Common components for GF_2 and GF_1 are designated as in Fig. 3; empty smooth vesicles: *s*. × 36,000.

Effects of Detergent Treatment on Golgi Fractions

Since the fractionation procedures so far used failed to separate the terminal glycosyltransferases from one another, and since the main Golgi fractions studied are morphologically heterogeneous (they contain Golgi cisternae and Golgi large and small vesicles in varied ratios), we attempted to subfractionate a combined GF_{1+2} by detergent treatment. Our intent was to find out whether: (*a*) a similarly homogeneous transferase distribution applies for Golgi membranes after content removal; (*b*) differences in density, emerging after partial content removal, reveal differences in transferase distribution among different Golgi elements; or (*c*) fragmentation of Golgi vesicles by detergent separates membrane domains with dif-

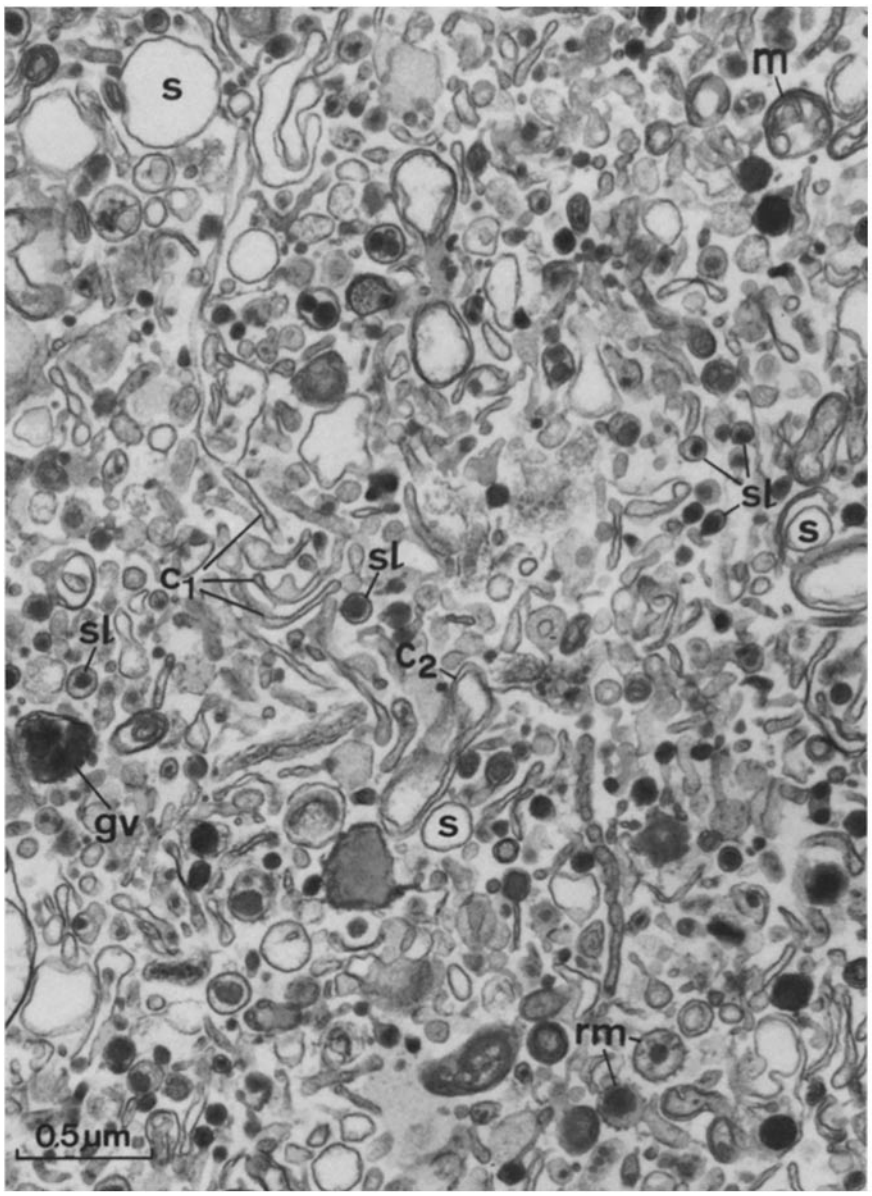

FIGURE 5 GF_3. (Heavy Golgi fraction.) Components shared in common with GF_1 and GF_2 are designated by the same letters as in Figs. 3 and 4. Morphologically recognizable contaminants include: mitochondria (*m*) and rough microsomes (*rm*). The latter have been buoyed to the density of GF_3 by the VLDL they contain. × 36,000.

ferent enzymic activities. Partial content extraction has been achieved in microsomes by deoxycholate treatment (31, 13), and the same detergent was used to separate "specialized regions" from microsomal membranes (57). For our experiments we chose the nonionic detergent Triton X-100, because, unlike deoxycholate or sodium dodecylsulfate (49), it does not inhibit the transferases (18). Triton X-100 has also been used successfully in the stepwise dissociation of Semliki Forest virus envelopes (20).

The results of these experiments showed that at a Triton X-100 concentration of 0.8 mg/mg phospholipid (R = 0.8 mg/mg) (Fig. 7 *a*), when most

of the vesicular content had already been removed, the three transferases sedimented in a common peak coincident with a visible, white band that consisted mainly of empty vesicles with discontinuous membranes. At R = 1.2 mg/mg, a common peak (coinciding with a vesicular band) was still present but ~25% of the galactosyl- and sialyltransferase activities failed to sediment. Finally at R = 2.0 mg/mg (Fig. 7*b*) when the band had practically vanished and vesicular structures were not found in the gradient, a common transferase peak was no longer present. Most of the galactosyl- and sialyltransferase activity remained in the load presumably as a result of extensive solubilization, whereas GlcNAc-transferase was found throughout the gradient, possibly on account of aggregation with other proteins (see the legend of Fig. 7 *a* and *b* for further details).

TABLE III

Relative Specific Activities of Glycosyltransferases in Fractions Resolved by Gradient M

Fraction	Sialyltransferase*	Galactosyltransferase	GlcNAc-transferase*
GF_1	42.9 ± 6.3	50.4 ± 11.5	44.7 ± 11.1
GF_2	32.1 ± 7.1	38.3 ± 10.5	33.5 ± 7.3
GF_3	23.9 ± 5.4	27.5 ± 13.1	27.8 ± 10.5
Light interlayer	11.0 ± 1.3	9.3 ± 5.5	11.1 ± 3.3
Heavy interlayer	4.2 ± 1.1	4.1 ± 1.4	4.0 ± 1.1
Load section	1.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.2
Pellet	0.3 ± 0.05	0.2 ± 0.04	0.25 ± 0.05

The specific activities of the homogenate (in nmol monosaccharide transferred/mg protein per 30 min) were: sialyltransferase: 10.1 ± 0.2; galactosyltransferase: 3.8 ± 0.6; GlcNAc-transferase: 3.0 ± 0.9.

* Relative specific activity = specific activity of the fraction/specific activity of the whole homogenate (mean and standard deviation of four experiments).

It appears, therefore, that the three terminal glycosyltransferases remain associated with Golgi membranes as long as the latter are not extensively solubilized by detergent, and that treatment with Triton X-100 does not lead to the separation of either vesicular elements or membrane domains with different transferase activity.

DISCUSSION

As shown in the original reference (12) and as confirmed by our findings, GF_1, GF_2, and GF_3 (Figs. 3–5) are enriched in structural elements derived from the trans, intermediate, and cis regions of the Golgi complex, respectively. Kinetic data, obtained in pulse-chase experiments and analyzed on a similar series of fractions, suggest that secretory proteins labeled with [^{3}H]leucine (3) progress in a general cis → trans direction (GF_3 → GF_2 → GF_1) with a peak interval of 5–7 min in between successive fractions. Similar results were reported for secretory glycoproteins labeled with [^{3}H]Gal (1); in that case, a lag was clearly resolved between radioactivity peaks passing through GF_2 and GF_1.

The evidence for a progressive cis → trans movement of secretory products through the Golgi complex, and the sequential character of terminal glycosylation are compatible with Schachter's postulate that these glycosyltransferases are serially located within the Golgi complex (46). If so, sialyltransferase would be expected to reside mostly in trans-Golgi elements, whereas galactosyltransferase and GlcNAc-transferase should be preferentially located in intermediate and cis-Golgi elements.

Our results indicate, however, that none of the enzymes is enriched over the others in any of the three main Golgi fractions. The specific activities vary significantly and consistently from one fraction to another, but the variations are always in

TABLE IV

*Distribution of Glycosyltransferase Activities among Fractions Resolved by Gradient M**

Fraction	Protein	Phospholipid	Sialyltransferase	Galactosyltransferase	GlcNAc-transferase
GF_1	0.15 ± 0.02	0.67 ± 0.23	6.6 ± 0.3	7.0 ± 1.9	6.3 ± 1.7
GF_2	0.18 ± 0.02	0.91 ± 0.28	5.6 ± 1.1	6.2 ± 0.7	5.9 ± 1.2
GF_3	0.30 ± 0.06	1.30 ± 0.24	7.1 ± 1.6	8.1 ± 3.6	8.1 ± 2.4
Light interlayer	0.51 ± 0.05	1.88 ± 0.40	5.5 ± 0.8	4.8 ± 2.9	5.6 ± 1.7
Heavy interlayer	2.19 ± 0.51	6.0 ± 0.6	8.8 ± 0.4	8.6 ± 0.6	8.3 ± 1.4
Load section	7.80 ± 0.8	10.5 ± 3.6	9.5 ± 2.4	7.5 ± 0.6	7.0 ± 2.2
Pellet	1.48 ± 0.09	2.0 ± 0.6	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1

* All values are given in percent of whole homogenate values (mean and standard deviation of four experiments). For absolute values cf. Table II.

TABLE V

*Glycosyltransferase Distribution among Fractions Resolved by Gradient S**

Fraction	Protein	Phospholipid	Sialyltransferase	Galactosyltransferase	GlcNAc-transferase
SA	0.08 ± 0.01	0.16 ± 0.03	0.29 ± 0.09	0.31 ± 0.13	0.30 ± 0.12
GF_0	0.49 ± 0.07	0.68 ± 0.24	3.60 ± 0.7	3.70 ± 1.1	3.10 ± 0.6
SC‡	0.63 ± 0.49	0.26 ± 0.10	0.58 ± 0.16	0.48 ± 0.11	0.56 ± 0.17
S-Load	19.10 ± 2.6	0.64 ± 0.26	1.60 ± 0.9	1.60 ± 0.8	1.10 ± 0.6
S-Pellet	1.13 ± 0.14	0.06 ± 0.04	0.20 ± 0.07	0.23 ± 0.11	0.40 ± 0.3

The relative specific activities of GF_0 were: 7.3 ± 0.8 for sialyltransferase, 7.6 ± 2.9 for galactosyltransferase, and 6.2 ± 0.5 for GlcNAc-transferase, representing 20- to 30-fold enrichment over the corresponding values for the postmicrosomal supernate.

* For absolute values cf. Table II. Data are given in percent of whole homogenate values (mean ± standard deviation of four experiments).

‡ SC included the 1.110 and the 1.148 density layers as well as the faint band at their interface.

parallel. GF_1 shows the highest values for all three transferases (Table III) followed by GF_2 and GF_3, the latter having about half the specific activities of GF_1. For some individual enzymes, our findings are in agreement with data already published by others. For instance, Merritt and Morré (35) have reported that galactosyltransferase is almost twice as active in secretion granules as in cisternal fractions, and Banerjee et al. (1) have observed that GF_1 is more active than any other Golgi fraction in the in vivo incorporation of sialic acid into secretory glycoproteins.

A parallel distribution of terminal glycosyltransferases was found to apply also to other fractions which contain either extra heavy (light interlayer) or extra light (GF_0) Golgi elements (Tables III–V). In both cases, the recovery figures, as well as the specific activities were similar for all three transferases.

Accordingly, it can be concluded that the procedure of Ehrenreich et al. (12) does not reveal any preferential concentration of any terminal glycosyltransferase in any Golgi or Golgi-related fraction. The results imply that the transferases are evenly distributed along the cis-trans axis of the Golgi complex, although they may be preferentially associated with some specific Golgi element. In this case, the transferase concentration would be expected to change in parallel for all three enzymes with the position (cis vs. trans) of the specific elements. The findings do not necessarily imply that all three transferases reside in the same structure, since similar results would be obtained if separate compartments, each harboring an individual transferase, were so similar in their physical properties (size, density) as to distribute in parallel during the fractionation procedure. Although unlikely, this second alternative cannot be ruled out by the evidence so far obtained.

Our data on galactosyltransferase recovery and relative specific activity in GF_1 and GF_2 are comparable to those recorded by Bergeron et al. (4), but the corresponding figures for GF_3 are quite different: both recovery and relative specific activity are considerably lower than previously reported. In addition, a substantial amount of galactosyltransferase activity was found in cell fractions ranging in density from >1.127 (GF_3) to <1.177 (residual microsomal pellet). Our morphological survey of the fractionation procedure was more extensive, and involved more satisfactory sampling than in reference 12; it revealed a higher concentration of cisternal elements in GF_1 and the presence of similar elements in relatively large number in a series of cell fractions (intermediate layers and load) which consist primarily of vesicles describable as smooth microsomes.

The reasons for this discrepancy remain to be investigated; they probably include differences in VLDL losses from Golgi vesicles during liver homogenization (as suggested by the free VLDLs recovered from the postmicrosomal supernate in G_0), differences in the collection of GF_3, as well as differences in the assay procedure (presence or absence of detergent).

Extensive heterogeneity among Golgi elements has been detected by a variety of cytochemical tests (10, 16, 38, 40), but the functional meaning of this heterogeneity is still obscure (cf. reference 15). More recently, attempts were made to isolate enzymically differentiated fragments from rehomogenized (28) or protease-treated (36, 39) Golgi fractions. Finally, Golgi elements enriched in NADPH-cytochrome P450 reductase, NADH-cytochrome b_5 reductase, and cytochrome b_5 have been isolated by an immunoadsorption procedure

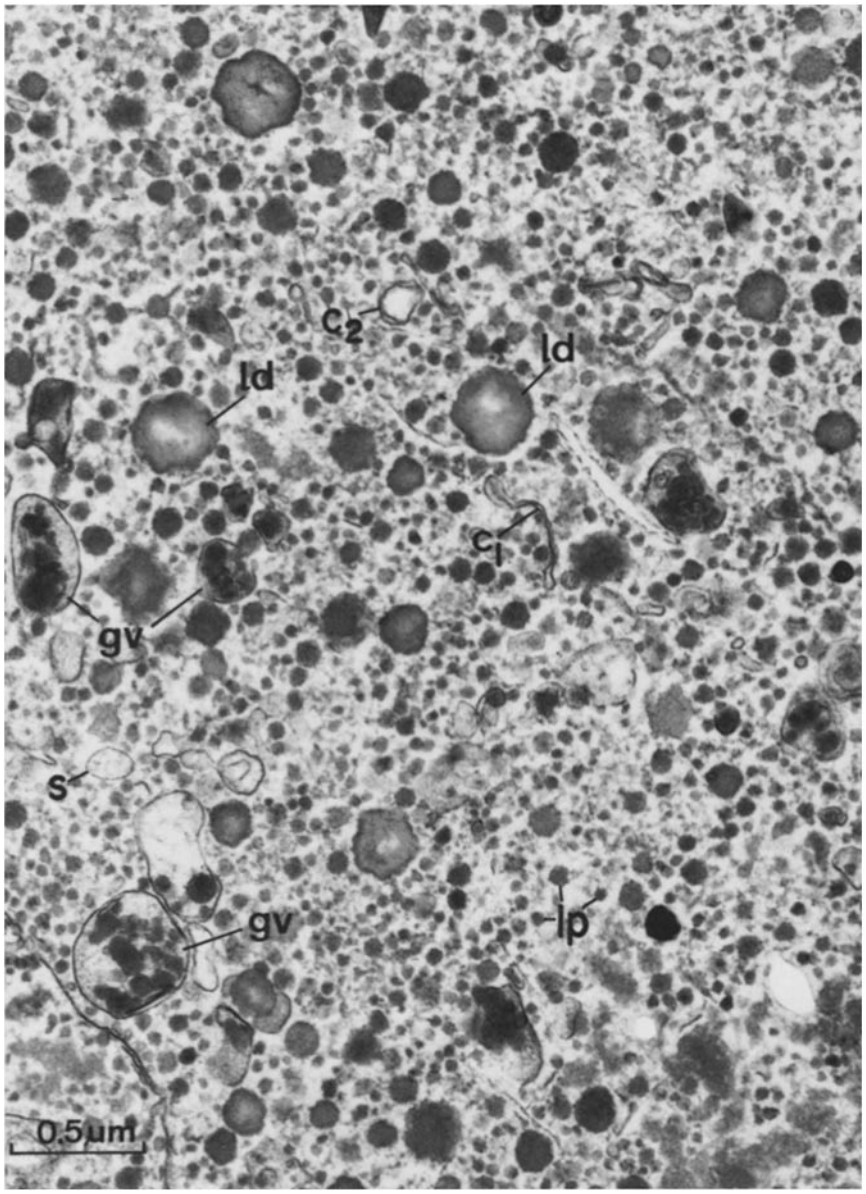

FIGURE 6 GF_0 (extra-light Golgi fraction) floated on gradient S out of the postmicrosomal supernate. Components common for all Golgi fractions (*gv*, c_1, c_2, *s*) are designated as in Figs. 3 and 4; small dense particles, presumably free VLDLs: *lp*; large lipid droplets derived from the cytoplasmic matrix: *ld*. × 36,000.

(26) from hepatic GF_{1+2}. They were found to be primarily large Golgi vesicles marked by clustered VLDL. The immunoadsorbed elements have little galactosyltransferase activity, a finding which suggests that the transferases may be located in the central part of the Golgi cisternae. This possibility deserves further investigation.

After deoxycholate treatment (57) or extensive sonication (11), hepatic microsomes have been resolved in membrane preparations with different partial spectra of microsomal enzyme activities. Since such fractionation procedures inactivate Golgi glycosyltransferases, we have used Triton X-100, a nonionic, nondenaturing detergent, to

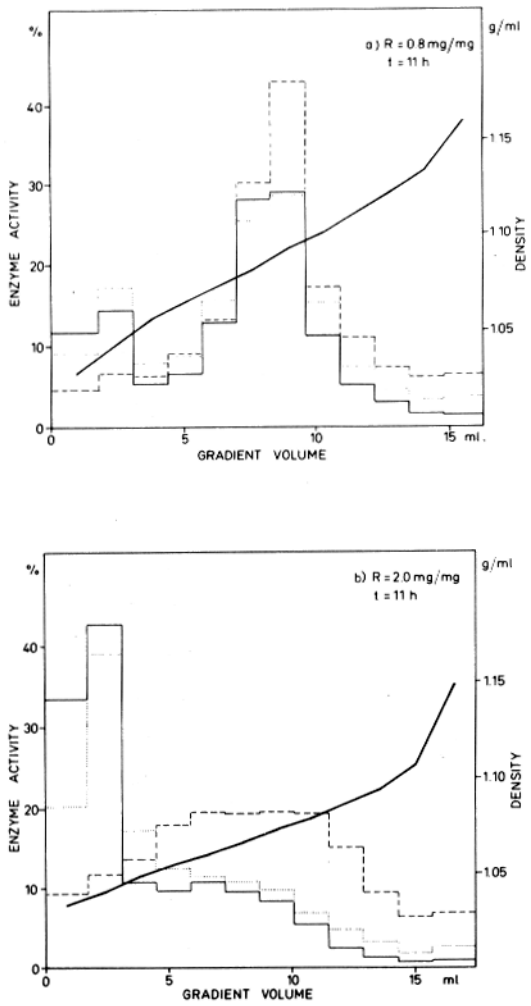

FIGURE 7 GF_{1+2} treated with Triton X-100. Distribution of transferase activities on linear sucrose gradients after detergent treatment. Pooled GF_{1+2} were mixed with Triton X-100 to give various Triton/phospholipid ratios (R), and centrifuged for 11 h at 96,000 *g*. The density of the collected fractions (oblique line) was estimated from their refractive indices. The enzyme activities per milliliter of fraction (sialyltransferase, . . .; galactosyltransferase, —; GlcNAc-transferase – – –) are given in percent of input values. A clearly visible, white band was found at $d = 1.087$ (fractions 6 and 7) in the gradient in Fig. 7*a*. At this level, the specific activities of the glycosyltransferases were twice as high as in the input. The glycosyltransferase peak coincided with a (smaller) peak of protein and phospholipid. A large fraction of the latter remained in the two fractions on top of the gradient (load). No band was visible in the gradient in Fig. 7*b*. [^{3}H]Triton X-100 radioactivity and phospholipid were distributed in parallel throughout the gradients.

find out whether membrane subfractions with different and distinct enzymatic activities can be isolated from GF_{1+2}. At low detergent/phospholipid ratios ($R = 0.8$ mg/mg to 1.2 mg/mg), Triton caused lysis of Golgi elements, i.e., loss of content with at least partial retention of membrane structures. At $R = 2.0$ mg/mg the membranes were solubilized. Except for a limited solubilization of galactosyl- and sialyltransferases, no separation of transferase activities was obtained at lytic concentrations. The results suggest that all transferases reside in the same structures or, conversely, that after lysis individual transferases are associated with different but physically similar particles. Partial separation of transferase activities was obtained only upon membrane solubilization, primarily because GlcNAc-transferase proved to be less soluble than the other two enzymes in Triton X-100 at $R = 2.0$ mg/mg.

Taken together, our results indicate that the three terminal glycosyltransferases occur throughout the Golgi complex in fairly constant proportions but at different concentrations that increase in the cis → trans direction. They can be separated from each other only after membrane breakdown. It seems more likely that they are organized in close spatial relationship to one another, rather than being individually distributed at relatively large distances along the secretory pathway, with sequential maxima in the order GlcNAc-transferase, galactosyltransferase, sialyltransferase (cf. reference 46). An organization based on close proximity would allow acceptor transfer from enzyme to enzyme without release of the intermediates into the lumen. This mechanism could explain the high precision with which the oligosaccharides of glycoproteins are synthesized, and the fact that newly synthesized plasma glycoproteins remain membrane-bound at least until the incorporation of galactosyl residues into their oligosaccharide chains (44).

It should be pointed out that our experimental protocol may not differentiate between multiple enzymes transferring the same monosaccharide to different acceptors or to different positions on the same acceptor. The existence of multiple, specialized, hepatic transferases has been reported for all three monosaccharides (29, 36, 58). Enzyme activities transferring Gal to lipids (58), or GlcNAc directly to an asparaginyl residue in ribonuclease A (29) may not be assayed with our acceptors, but the case is less clear for sialyltransferases. Fetuin

contains sialyl residues (50) in different oligosaccharides, three resembling the *O*-glycosidically linked side chains found in mucins (52); hence, asialofetuin could function as a polyspecific acceptor. Moreover, in Chinese hamster ovary cells, there are at least two different GlcNAc-transferases (37), each transferring GlcNAc to a mannose residue in a different position. The presence of a similar set of branching and elongating transferases in hepatocytes is definitely not excluded. Distribution studies should be extended to this variety of specific transferases when adequate monospecific acceptors will become available.

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REFERENCES

1. BANERJEE, D., C. P. MANNING, and C. M. REDMAN. 1976. The in vivo effect of colchicine on the addition of galactose and sialic acid to rat hepatic serum glycoproteins. *J. Biol. Chem.* **251**:3387–3892.
2. BAUDHUIN, P., P. EVRARD, and J. BERTHET. 1967. Electron microscopic examination of subcellular fractions. I. The preparation of representative samples from suspensions of particles. *J. Cell Biol.* **32**:181–191.
3. BERGERON, J. J. M., D. BORTS, and J. CRUZ. 1978. Passage of serum-destined proteins through the Golgi apparatus of rat liver. An examination of heavy and light Golgi fractions. *J. Cell Biol.* **76**:87–97.
4. BERGERON, J. J. M., J. H. EHRENREICH, P. SIEKEVITZ, and G. E. PALADE. 1973. Golgi fractions prepared from rat liver homogenates. II. Biochemical characterization. *J. Cell Biol.* **59**:73–88.
5. BOLENDER, R. O., D. PAUMGARTNER, G. LOSA, D. MUELLENER, and E. R. WEIBEL. 1978. Integrated stereological and biochemical studies on hepatocytic membranes. I. Membrane recoveries in subcellular fractions. *J. Cell Biol.* **77**:565–583.
6. BOSMAN, H. B. 1970. Glycoprotein synthesis: Purification and properties of glycoprotein N-acetylglucosaminyltransferases from guinea pig liver utilizing endogenous and exogenous acceptors. *Eur. J. Biochem.* **14**:33–40.
7. BRETZ, R., H. BRETZ, and G. E. PALADE. 1978. Distribution of glycosyltransferases in subfractions of rat liver Golgi apparatus. *J. Cell Biol.* **79** (2, Pt. 2):247*a*. (Abstr.).
8. BRETZ, R., and W. STÄUBLI. 1977. Detergent influence on rat liver galactosyltransferase activities towards different acceptors. *Eur. J. Biochem.* **77**:181–192.
9. CHATTERJEE, A. K., and R. MONTGOMERY. 1962. The carbohydrate of ovomucoid. *Arch. Biochem. Biophys.* **99**:426–432.
10. CHENG, H., and M. G. FARQUHAR. 1976. Presence of adenylate cyclase activity in Golgi and other fractions from rat liver. II. Cytochemical localization within Golgi and ER membranes. *J. Cell Biol.* **70**:671–684.
11. DALLMAN, P. R., G. DALLNER, A. BERGSTRAND, and L. ERNSTER. 1969. Heterogeneous distribution of enzymes in submicrosomal membrane fragments. *J. Cell Biol.* **41**:357–377.
12. EHRENREICH, J. H., J. J. M. BERGERON, P. SIEKEVITZ, and G. E. PALADE. 1973. Golgi fractions prepared from rat liver homogenates. I. Isolation procedure and morphological characterization. *J. Cell Biol.* **59**:45–72.
13. EHRHART, J. C., and J. CHAUVEAU. 1975. Differential solubilization of proteins, phospholipids, free and esterified cholesterol of rat liver cellular membranes by sodium deoxycholate. *Biochim. Biophys. Acta.* **375**:434–445.
14. EMERSON, W. A., and S. KORNFELD. 1976. Characterization of the oligosaccharide units of the bovine erythrocyte membrane glycoprotein. *Biochemistry.* **15**:1697–1703.
15. FARQUHAR, M. G. 1978. Traffic of products and membranes through the Golgi complex. *In* Transport of Macromolecules in Cellular Systems. S. C. Silverstein, editor. Dahlem Konferenzen, Berlin.
16. FARQUHAR, M. G., J. J. M. BERGERON, and G. E. PALADE. 1974. Cytochemistry of Golgi fractions prepared from rat liver. *J. Cell Biol.* **60**:8–25.
17. FLEISCHER, B., and S. FLEISCHER. 1970. Preparation and characterization of Golgi membranes from rat liver. *Biochim. Biophys. Acta.* **219**:301–319.
18. FLEISCHER, B., and M. SMIGEL. 1978. Solubilization and properties of galactosyltransferase and sulfotransferase activities of Golgi membranes in Triton X-100. *J. Biol. Chem.* **253**:1632–1638.
19. HELENIUS, A., and K. SIMONS. 1975. Solubilization of membranes by detergents. *Biochim. Biophys. Acta.* **415**:29–79.
20. HELENIUS, A., and H. SÖDERLUND. 1973. Stepwise dissociation of the Semliki Forest virus membrane with Triton X-100. *Biochim. Biophys. Acta.* **307**:287–300.
21. HOWELL, K. E., A. ITO, and G. E. PALADE. 1978. ER marker enzymes in Golgi fractions—what does this mean? *J. Cell Biol.* **79**:581–589.
22. HUDGIN, R. L., and H. SCHACHTER. 1971. Porcine sugar nucleotide: glycoprotein glycosyltransferases. I. Blood serum and liver sialyltransferase. *Can. J. Biochem.* **49**:829–837.
23. HUDGIN, R. L., and H. SCHACHTER. 1971. Porcine sugar nucleotide: glycoprotein glycosyltransferases. II. Blood serum and liver galactosyltransferase. *Can. J. Biochem.* **49**:838–846.
24. HUDGIN, R. L., and H. SCHACHTER. 1971. Porcine sugar nucleotide: glycoprotein glycosyltransferases. III. Blood serum and liver *N*-acetylglucosaminyltransferase. *Can. J. Biochem.* **49**:847–852.
25. HUNT, L. A., J. R. ETCHISON, and D. F. SUMMERS. 1978. Oligosaccharide chains are trimmed during synthesis of the envelope glycoprotein of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. U. S. A.* **75**:754–758.
26. ITO, A., and G. E. PALADE. 1978. Presence of NADPH-cytochrome P-450 reductase in rat liver Golgi membranes. Evidence obtained by immunoadsorption method. *J. Cell Biol.* **79**:590–597.
27. JAMIESON, J. D., and G. E. PALADE. 1977. Production of secretory proteins in animal cells. *In* International Cell Biology. B. R. Brinkley and K. R. Porter, editors. The Rockefeller University Press, New York. 308–317.
28. KATONA, E., and M. A. MOSCARELLO. 1975. The association of enzymic activities in subfractions of isolated rat liver Golgi. *Experientia (Basel).* **31**:412–413.
29. KHALKHALI, Z., F. SERAFINI-CESSI, and R. D. MARSHALL. 1977. The UDP-*N*-acetyl-glucosamine-asparagine-sequon *N*-acetyl-β-D-glucosaminyltransferase activity in preparations of rough endoplasmic reticulum from regenerating rat liver. *Biochem. J.* **164**:257–261.
30. KORNFELD, R., and S. KORNFELD. 1976. Comparative aspects of glycoprotein structure. *Annu. Rev. Biochem.* **45**:217–237.
31. KREIBICH, G., P. DEBEY, and D. D. SABATINI. 1973. Selective release of content from microsomal vesicles without membrane disassembly. I. Permeability changes induced by low detergent concentrations. *J. Cell Biol.* **58**:436–462.
32. LEBLOND, C. P., and G. BENNETT. 1977. Role of the Golgi apparatus in terminal glycosylation. *In* International Cell Biology. B. R. Brinkley and K. R. Porter, editors. The Rockefeller University Press. New York. 326–336.
33. LI, E., I. TABAS, and S. KORNFELD. 1978. Synthesis of complex-type oligosaccharides. I. Structure of the lipid-linked oligosaccharide precursor of the complex-type oligosaccharides of the vesicular stomatitis virus G. protein. *J. Biol. Chem.* **253**:7762–7770.
34. LUCAS, J. J., C. J. WAECHTER, and W. J. LENNARZ. 1975. The participation of lipid-linked oligo-saccharide in the synthesis of membrane glycoproteins. *J. Biol. Chem.* **250**:1992–2002.
35. MERRITT, W. D., and D. J. MORRÉ. 1973. A glycosyl transferase of high specific activity in secretory vesicles from isolated Golgi apparatus of rat liver. *Biochim. Biophys. Acta.* **304**:397–407.
36. MUNRO, J. R., S. NARASIMHAN, S. WETMORE, J. R. RIORDAN, and H. SCHACHTER. 1975. Intracellular localization of GDP-L-fucose: glycoprotein and CMP-sialic acid: apolipoprotein glycosyltransferases in rat and pork livers. *Arch. Biochem. Biophys.* **169**:269–277.
37. NARASIMHAN, S., P. STANLEY, D. WILLIAMS, and H. SCHACHTER. 1976.

Membrane glycoprotein synthesis in Chinese hamster ovary cells—evidence for a branching *N*-acetylglucosaminyltransferase. *Fed. Proc.* **35**:1441.

38. NOVIKOFF, P. M., and A. YAM. 1978. Sites of lipoprotein particles in normal rat hepatocytes. *J. Cell Biol.* **76**:1–11.
39. OVTRACHT, L., D. J. MORRÉ, R. D. CHEETHAM, and H. H. MOLLENHAUER. 1973. Subfractionation of Golgi apparatus from rat liver: Method and morphology. *J. Microsc. (Paris).* **18**:87–102.
40. OVTRACHT, L., and J.-P. THIERY. 1972. Mise en evidence par cytochimie ultrastructurale de compartiments physiologiquement differents dans un meme saccule golgien. *J. Microsc. (Paris).* **15**:135–170.
41. PALADE, G. E. 1975. Intracellular aspects of the process of protein secretion. *Science (Wash. D. C.).* **189**:347–358.
42. PAULSON, J. C., W. E. BERANEK, and R. L. HILL. 1977. Purification of a sialyltransferase from bovine colostrum by affinity chromatography on CDP-agarose. *J. Biol. Chem.* **252**:2356–2362.
43. PLESS, D. D., and W. J. LENNARZ. 1977. Enzymatic conversion of proteins to glycoproteins. *Proc. Natl. Acad. Sci. U. S. A.* **74**:134–138.
44. REDMAN, C. M., and M. G. CHERIAN. 1972. The secretory pathway of rat serum glycoproteins and albumin. Localization of newly formed proteins within the endoplasmic reticulum. *J. Cell Biol.* **52**:231–245.
45. ROBBINS, P. W., S. C. HUBBARD, S. J. TUREO, and D. F. WIRTH. 1977. Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins. *Cell.* **12**:893–900.
46. SCHACHTER, H. 1974. Glycosylation of glycoproteins during intracellular transport of secretory products. *In* Advances in Cytopharmacology. Vol. 2. B. Ceccarelli, F. Clementi, and J. Meldolesi, editors. Raven Press, New York, 207–218.
47. SCHACHTER, H., I. JABBAL, R. L. HUDGIN, L. PINTERIC, E. J. MCGUIRE, and S. ROSEMAN. 1970. Intracellular localization of liver sugar nucleotide glycoprotein glycosyltransferases in a Golgi-rich fraction. *J. Biol. Chem.* **245**:1090–1100.
48. SCHEELE, G. A., G. E. PALADE, and A. M. TARTAKOFF. 1978. Cell fractionation studies on the guinea pig pancreas. Redistribution of exocrine proteins during tissue homogenization. *J. Cell Biol.* **78**:110–130.
49. SMITH, C. A., and K. BREW. 1977. Isolation and characteristics of galactosyltransferase from Golgi membranes of lactating sheep mammary glands. *J. Biol. Chem.* **252**:7294–7299.
50. SPIRO, R. G. 1960. Studies on fetuin, a glycoprotein of fetal serum. I. Isolation, chemical composition, and physicochemical properties. *J. Biol. Chem.* **235**:2860–2869.
51. SPIRO, R. G. 1963. Periodate oxidation of the glycoprotein fetuin. *J. Biol. Chem.* **239**:567–573.
52. SPIRO, R. G., and V. D. BHOYROO. 1974. Structure of the O-glycosidically linked carbohydrate units of fetuin. *J. Biol. Chem.* **249**:5704–5717.
53. TABAS, I., and S. KORNFELD. 1978. Synthesis of complex type oligosaccharides. III. Identification of an α-D-mannosidase activity involved in a late stage of processing of complex-type oligosaccharides. *J. Biol. Chem.* **253**:7779–7786.
54. TOMITA, M., and V. T. MARCHESI. 1975. Amino-acid sequence and oligosaccharide attachment sites of human erythrocyte glycophorin. *Proc. Natl. Acad. Sci. U. S. A.* **72**:2964–2968.
55. TRAYER, I. P., and R. L. HILL. 1971. The purification and properties of the A protein of lactose synthetase. *J. Biol. Chem.* **246**:6666–6675.
56. WAECHTER, C. J., and W. J. LENNARZ. 1976. The role of polyprenol-linked sugars in glycoprotein synthesis. *Annu. Rev. Biochem.* **45**:95–112.
57. WINQUIST, L., and G. DALLNER. 1976. Localization of enzymes in specialized regions of the microsomal membrane. *Biochim. Biophys. Acta.* **463**:399–412.
58. ZATTA, P., D. ZAKIM, and D. A. VESSEY. 1975. The transfer of galactose from UDP-galactose to endogenous lipid acceptors in liver microsomes. *Biochim. Biophys. Acta.* **392**:361–365.