

# GOLGI FRACTIONS PREPARED FROM RAT LIVER HOMOGENATES

## II. Biochemical Characterization

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### ABSTRACT

The three Golgi fractions isolated from rat liver homogenates by the procedure given in the companion paper account for 6-7% of the protein of the total microsomal fraction used as starting preparation. The lightest, most homogeneous Golgi fraction (GF<sub>1</sub>) lacks typical "microsomal" activities, e.g., glucose-6-phosphatase, NADPH-cytochrome *c*-reductase, and cytochrome P-450. The heaviest, most heterogeneous fraction (GF<sub>3</sub>) is contaminated by endoplasmic reticulum membranes to the extent of ~15% of its protein. The three fractions taken together account for nearly all the UDP-galactose:*N*-acetylglucosamine galactosyltransferase of the parent microsomal fraction, and for ~70% of the activity of the original homogenate. Omission of the ethanol treatment of the animals reduces the recovery by half. The transferase activity is associated with the membranes of the Golgi elements, not with their content. Galactose is transferred not only to *N*-acetylglucosamine but also to an unidentified lipid-soluble component.

### INTRODUCTION<sup>1</sup>

In the preceding paper (2), we have described the isolation of a series of three Golgi fractions from rat liver homogenates. The procedure relies on the overloading of Golgi elements with very low density lipoprotein (VLDL)<sup>2</sup> particles which provide an enclosed morphological marker and change the density of Golgi-derived vesicles enough to allow their separation from vesicles derived from the endoplasmic reticulum. The

overloading is produced *in vivo* by ethanol treatment.

In this paper we present the data obtained so far on the chemistry and enzymology of these fractions.

### MATERIALS AND METHODS

#### *General*

The preparation of the animals, the fractionation of liver homogenates, the isolation of Golgi fractions, and their subfractionation into membranes and content have been described in reference 2.

**COMMON GOLGI FRACTION:** For the preparation of a common Golgi fraction, the total microsome pellet was resuspended in 0.25 M sucrose and the concentration of sucrose was subsequently brought up to 1.3 M (by adding 2.0 M sucrose). 20-ml aliquots of

<sup>1</sup> A partial, preliminary report of these findings was presented at the 10th Annual Meeting of the American Society for Cell Biology (1).

<sup>2</sup> *Abbreviations used in this paper:* AcPase, Acid phosphatase; AMPase, 5'-nucleotidase; cyt., cytochrome; ER, endoplasmic reticulum; G6Pase, glucose-6-phosphatase, PLP, phospholipid; VLDL, very low density lipoprotein.

this suspension were used as bottom load under a 20-ml layer of 1.15 M sucrose and a 20-ml layer of 0.25 M sucrose. Upon centrifugation in a SW 25.2 rotor for 3.5 h at 25,000 rpm (75,500  $g_{av}$ ), a heavy band formed at the 0.25 M/1.15 M sucrose interface. This band is expected to represent the sum of GF<sub>1</sub>, GF<sub>2</sub>, and GF<sub>3</sub>.

**SUBFRACTIONATION OF MICROSOMES:** For the purpose of comparison, an aliquot of the total microsomal fraction was subfractionated into membranes and content by using the procedure worked out for Golgi fractions; i.e., the microsomes were suspended in hypotonic (0.04 M) veronal buffer, pH 8.5, passed twice through a French press, brought up to 0.25 M sucrose, and centrifuged for 60 min at 105,000  $g_{av}$ . The resulting pellet and supernate were called "microsomal membranes" and "microsomal contents," respectively (see Fig. 17 in reference 2). Presumably the treatment released much of the contents of the microsomal vesicles, just as it did in the case of the Golgi fractions. The situation was not investigated in greater detail, however, so that the use of the term "membranes" and "contents" is largely arbitrary; it implies only that these fractions have been treated in the same way as the Golgi fractions.

**PREPARATION OF MITOCHONDRIA AND LY-SOSOMAL FRACTIONS:** For certain comparative enzymological assays, mitochondrial, lysosomal, and Golgi fractions were isolated from a common homogenate. Aliquots of the latter were spun for 15 min at 485  $g_{av}$  in an International centrifuge, model SBV (International Scientific Instruments Inc., Palo Alto, Calif.), to remove unbroken cells, cell debris, and nuclei, and the resulting supernate was recentrifuged for 15 min at 3,300  $g_{av}$ . The second supernate was carefully drawn off with a pipette and saved to isolate the lysosomal fraction. The pellet was gently resuspended in 0.25 M sucrose and the suspension spun for 10 min at 3,300  $g_{av}$ . The cycle was repeated once and the final pellet, resuspended in water or buffer, is referred to as the "total mitochondrial fraction."

The supernate from the first 3,300  $g$  spin was recentrifuged for 20 min at 12,500  $g_{av}$ . The ensuing supernate was discarded, the pellet resuspended (after rinsing its surface) in 0.30 M sucrose, and the suspension spun for 10 min at 9,500  $g_{av}$ . The cycle was repeated once and the final pellet, resuspended in 0.30 M sucrose, is referred to as the "total lysosomal fraction." This procedure is a modification of that of Sawant et al. (3).

### Gross Chemistry

**PROTEIN:** Protein was assayed by the method of Lowry et al. (4) with bovine plasma albumin as a standard.

**PHOSPHOLIPID:** Lipids were extracted by the method of Folch-Pi et al. and washed with 0.73% NaCl (5). Inorganic phosphate was determined (6) in the ashed, acid-hydrolyzed extracts and phospholipid amounts were estimated by multiplying lipid P by 25.

**RNA:** Fraction aliquots were precipitated and washed several times with cold 10% TCA. The precipitates were then extracted for 20 min with hot (90°C) 5% TCA, and RNA in the extract was determined by the orcinol method (7).

### Enzyme Assays

All phosphatases were determined by previously published methods, with minor modifications as indicated below. Glucose-6-phosphatase (G6Pase) was assayed by the method of Swanson (8) using 30 mM Tris-maleate buffer (pH 6.6), 5 mM MgCl<sub>2</sub>, and 4 mM glucose-6-phosphate; 5'-nucleotidase (AMPase) by the method of Heppel and Hilmoe (9) using 28 mM Tris-HCl buffer (pH 8.5), 3 mM MgCl<sub>2</sub>, and 3.5 mM adenosine-5'-phosphate; or that of Widnell and Unkeless (10) using 83 mM Tris-HCl (pH 8.5), 17 mM MgCl<sub>2</sub>, and 17 mM adenosine-5'-phosphate; and acid phosphatase (AcPase) by the method of Wattiaux and de Duve (11). Inorganic phosphate was determined in all cases by the procedure of Ames and Dubin (6).

NADH- and NADPH-cytochrome (cyt.) *c*-reductase were assayed as described by Ernster et al. (12) and Dallner et al. (13) (without rotenone in the NADH-cyt. *c*-reductase assay); cytochrome oxidase was determined by the procedure of Cooperstein and Lazarow (14); and cyt. *b*<sub>5</sub> and cyt. P-450 were measured as done by Dallner et al. (13).

UDP-galactose:*N*-acetylglucosamine galactosyl-transferase activity was determined in two different ways. In the first procedure (Babad and Hassid [15] as modified by Fleischer et al. [16]), the assay mixture contained 0.3 M sodium cacodylate (pH 6.5), 10  $\mu$ l; 0.3 M MnCl<sub>2</sub>, 10  $\mu$ l; 0.3 M  $\beta$ -mercaptoethanol, 10  $\mu$ l; 15 mM uridine diphospho-[<sup>14</sup>C]galactose, 2.43  $\times$  10<sup>6</sup> dpm/ $\mu$ mol, 10  $\mu$ l;<sup>3</sup> 0.3 M *N*-acetylglucosamine, 10  $\mu$ l; and about 50–75  $\mu$ g protein equivalent of the cell fraction to be assayed in 10  $\mu$ l. After a 20-min incubation at 37°C, the reaction was stopped by plunging the tubes in ice and adding 20  $\mu$ l of 0.3 M EDTA (pH 7.4). The mixture was passed through a 0.5  $\times$  2.0-cm column of Dowex 2X8 (200–400 mesh; Dow Chemical Co., Midland, Mich.) in the Cl<sup>-</sup> form, and the column was eluted with 3  $\times$  0.5-

<sup>3</sup> UDP-[<sup>14</sup>C]galactose (200 mCi/mmol) was evaporated to dryness (to remove ethanol) and then resuspended in a solution of UDP-[<sup>12</sup>C]galactose at the concentration and specific radioactivity indicated.

ml samples of distilled H<sub>2</sub>O directly into scintillation vials containing Bray's solution. The samples were counted on a Beckman liquid scintillation spectrometer (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.). The blank contained all the ingredients of the assay except *N*-acetylglucosamine. The difference in radioactivity between experimental samples and blank is taken to represent the amount of galactose transferred to *N*-acetylglucosamine during the reaction.

The second procedure, based on the chromatographic separation of the reaction product, *N*-acetylglucosamine, was carried out basically as described by Fleischer et al. (16). At the end of the reaction as carried out above, the assay mixture was applied as a bar 0.5-cm long at the origin on DEAE paper (Whatman no. DE81). The developing solvent was *n*-butanol:*n*-propanol:water (3:1:1, vol/vol/vol) and chromatography was continued for 48–72 h. The paper was dried and cut into 0.5 × 2-cm strips which were extracted in 0.5 ml of water and counted in Bray's solution. Standards (100–200 μg) were run and visualized by the method of Traveyan et al. (17) as modified by J. Gregory (personal communication) using sodium hyposulfide ("hypos") to reduce the background of the stain. In some cases, lipid soluble radioactive compounds (see Results) were removed from the assay mixture before paper chromatography. The extraction procedure was carried out by adding an equal volume (80 μl) of chloroform:methanol (2 vol:1 vol) to the assay mixture. After shaking, the mixture distributed into an upper aqueous phase (~107 μl) and a lower organic phase (~53 μl). An aliquot (50 μl) of the upper phase was removed with a Hamilton syringe (Hamilton Co., Reno, Nev.) and chromatographed as described above.

### Sources of Materials

The reagents used were obtained from the following sources: glucose-6-phosphate (disodium salt), *cyt. c* (type III, from horse heart), and NADPH from Sigma Chemical Company, St. Louis, Mo.; AMP (yeast) and NADH from Mann Research Laboratories, Inc., New York, and UDP-[<sup>14</sup>C]galactose from New England Nuclear, Boston, Mass.

## RESULTS

### Protein Recovery in Golgi Fractions

In the case of ethanol-treated animals, we recovered 6–7% of the protein of the parental microsomal fraction or 1.5–1.8% of that of the original liver homogenate in our set of three Golgi fractions or in our common Golgi fraction (Table IV). Since approximately one-third of the Golgi ele-

ments of the entire cell population of the liver was probably lost in the first steps of our procedure before the isolation of microsomes (see below), we can estimate that only ~2–3% of the total protein of the liver is associated with Golgi complexes. The estimate is commensurate with the small relative volume of this cell organ in hepatocytes and agrees well with recently published stereological data (18) which assess at ~3% the fractional volume of the Golgi complex in the hepatocytes of the adult rat.

In agreement with the differences recorded in centrifugal band patterns (2), ~40% less protein was recovered in Golgi fractions prepared without previous ethanol treatment of the animals (Table V).

### Characterization of GF<sub>1</sub> and GF<sub>1</sub> Membranes

GENERAL CHEMISTRY: GF<sub>1</sub> accounts for only 0.36% of the protein of the parental microsomal fraction (Table I) and probably represents no more than ~3% of all the Golgi elements present in the original homogenate. It is, however, the most homogeneous of our Golgi fractions; moreover it contains the whole spectrum of Golgi elements present in a hepatocyte although in a definitely different mix than that found *in situ* (2). On account of its homogeneity and assuming that it is relatively representative, we have used GF<sub>1</sub> in an attempt to characterize in some detail Golgi elements and Golgi membranes. Data on protein, phospholipid, and RNA content of the whole fraction and of its membranes and contents, prepared as described in (2), are given in Table I. The high phospholipid:protein ratio of GF<sub>1</sub> (0.50) is due to the VLDLs it contains: upon their removal, the ratio in GF<sub>1</sub> membranes (0.36) becomes comparable to that found in microsomal membranes after correction for ribosomal proteins (0.35).

GF<sub>1</sub> and GF<sub>1</sub> membranes gave very low, variable, and questionable<sup>4</sup> values when assayed for

<sup>4</sup> Orcinol reacts with carbohydrates under the conditions of the RNA assay to give a product which absorbs at 665 nm (absorption maximum of RNA, reference 19) as well as at 565 nm. OD<sub>565</sub> can be used as a test for contamination of RNA by sugars (20). The ratio OD<sub>665</sub>/OD<sub>565</sub> is four for purified RNA, and 3 and ~0.4 for hot TCA extracts of microsomes and GF<sub>1</sub>, respectively. The figures in Table I represent maximal values since they were calculated directly from OD<sub>665</sub> ignoring the highly probable contamination by carbohydrates.

TABLE I  
Protein, PLP, and RNA Content of Microsomal and Light Golgi Fractions (GF<sub>1</sub>)

Fractions		Protein, $\mu\text{g/g liver}^*$	PLP, $\mu\text{g/mg protein}$	RNA, $\mu\text{g/mg protein}$
Microsomal	Total <sup>‡</sup>	15,000	260 (300) <sup>§</sup>	150
	Membranes	10,700	300 (350) <sup>§</sup>	135
	Content	2,900	75	99
GF <sub>1</sub>	Total	55 (25-80)	500 (430-560)	<15 (7-30)
	Membranes	19 (7-25)	360 (310-410)	<9 (6-40)
	Content	31	600	<26

The figures are averages of results obtained in four different experiments. Ranges for GF<sub>1</sub> total and GF<sub>1</sub> membranes are given in parentheses.

\* Wet weight

<sup>‡</sup> In these experiments the recovery of microsomes was lower than usual (15 mg protein/g wet weight liver) on account of the strong centrifugal field (10,000  $g_{av}$ ) applied to remove nuclei and mitochondria. In subsequent experiments (Tables III-VII) the field was less strong (8,800  $g_{av}$ ) and the recoveries correspondingly higher (20-25 mg).

<sup>§</sup> Values in parentheses are corrected for ribosomal proteins.

RNA. It is possible that the corresponding spectrophotometric readings reflect the presence of glycoprotein sugars rather than that of RNA; hence, it is likely that GF<sub>1</sub> contains very little, if any, RNA.

ENZYMATIC ACTIVITIES: GF<sub>1</sub> was assayed for a number of enzymes to check the degree of its homogeneity and to find out whether it has a characteristic set of activities. The figures given in Table II for a series of "marker" microsomal<sup>5</sup> enzymes such as G6Pase, NADPH-cyt. *c*-reductase, and cyt. P-450 represent the smallest concen-

trations that could have been detected in GF<sub>1</sub> and its membranes given the sensitivity of the procedures and the amounts of protein used. Since the assays were negative, it follows that these enzymes are either absent from GF<sub>1</sub> and its membranes or present in concentrations lower than indicated by the tabulated figures. In view of the differences known to exist in the sensitivity of the corresponding assays, the conclusion is firmer for G6Pase and the NADPH-cyt. *c*-reductase than for cyt. P-450. These findings strongly suggest that the enzymes mentioned are absent from GF<sub>1</sub> membranes. In this respect, they are in good agreement with the lack of cytochemically detectable G6Pase activity in the Golgi elements of hepatocytes *in situ* (21-23), and they also agree with the observation that the induction of the cyt. P-450 system by barbiturates or other drugs is accompanied by a marked proliferation of the endoplasmic reticulum (ER) (24, 25) without a comparable response from the Golgi complex (unpublished observations).

The figures given in the literature (16, 26, 27) for the specific activities of these microsomal marker enzymes in Golgi fractions are higher than ours and most probably reflect the degree of contamination of these fractions by ER-derived vesicles. In our case, ER contamination, if present, should amount to less than 4% of the protein of the GF<sub>1</sub> assuming, of course, that the latter has neither the cyt. P-450 system nor G6Pase.

GF<sub>1</sub> and GF<sub>1</sub> membranes appear to have, however, the enzymes of the second microsomal

<sup>5</sup> The term microsomes (and, hence, microsomal marker enzymes) is used here in its operational sense: it refers to small particles isolated by differential centrifugation from postmitochondrial supernates as given under Materials and Methods in reference 2. The major components of the hepatic microsomal fraction are vesicles derived from the rough and smooth ER, both of which contribute the marker enzymes. In the past ER-derived vesicles have been equated to microsomes minimizing or ignoring the presence of minor components in the same fraction, i.e., Golgi-derived vesicles, plasmalemmal fragments, and other intact or fragmented subcellular components. The separation of Golgi fractions from total microsomal fractions leaves behind a large, residual, but still heterogeneous particle preparation whose components may appear as contaminants in the Golgi fractions. These contaminants will be referred to as ER vesicles when this intracellular origin can be ascertained or as contaminating residual microsomes when their derivation is uncertain.

TABLE II  
Specific Enzymic Activities\* of GF<sub>1</sub> and Other Cell Fractions

Enzyme activities	Microsomes		GF <sub>1</sub>		Plasma-lemma	Mito-chondria	Lyo-somes
	Total‡	Mem-branes	Total	Membranes			
G6Pase (3)§	1.69	3.53	<0.07	<0.13	—	—	—
NADPH-cyt. <i>c</i> -reductase (4)	0.021	0.026	<0.001	<0.005	—	—	—
Cyt. P-450 (3)	0.031	0.051	<0.005	<0.010	—	—	—
NADH-cyt. <i>c</i> -reductase (4)	0.15	0.19	0.029	0.025	—	—	—
Cyt. <i>b</i> <sub>5</sub> (3)	0.197	0.099	0.026	0.017	—	—	—
AMPase (4)	2.15	2.23	1.59	1.93	9.2	—	—
Cyt. oxidase (5)	0.08	0.17	0.025	0.095	—	5.45	—
AcPase (3)	0.66	—	1.06	—	—	—	6.90
							33.00¶

\* Specific activity units: G6Pase and AMPase,  $\mu\text{mol P}_i/20 \text{ min/mg protein}$ ; NADPH and NADH-cyt. *c*-reductases,  $\mu\text{mol NADPH (NADH) oxidized/min/mg protein}$ ; cyt. P-450,  $\text{OD}_{450} - \text{OD}_{490}/\text{mg protein}$ ; cyt. *b*<sub>5</sub>,  $\text{OD}_{424} - \text{OD}_{410}/\text{mg protein}$ .

In all experiments, the results were checked for self-consistency by assaying (a) the microsomal fraction remaining after the isolation of GF<sub>1</sub>, (b) the GF<sub>1</sub> content fraction. The recoveries were generally high (>90%); cytochromes and enzyme activities were highly concentrated in the membrane fraction except for cyt. *b*<sub>5</sub> and AMPase of which 26% and 49%, respectively, were recovered in the GF<sub>1</sub> content fraction.

‡ Specific activity figures for all microsomal enzyme activities are comparable to those found in the literature, except for G6Pase and NADH-cyt. *c*-reductase (lower in our data). Cyt. *b*<sub>5</sub> concentration is higher than in the literature.

§ Number of experiments.

|| Widnell-Unkeless (10).

¶ Recalculated from Leighton et al. (44).

electron transport chain, i.e., NADH-cyt. *c*-reductase and cyt. *b*<sub>5</sub>. This system which is probably involved in fatty acid desaturation (28) has a rather wide distribution among subcellular components since it has been detected in microsomes (29) as well as in the outer mitochondrial membrane (30) and in plasmalemmal fractions (31). The reductase is highly labile (12) and this probably explains the wide variations recorded in our data.<sup>6</sup> The cytochrome is stable; hence, its specific activity figures can be considered reliable. These figures indicate that the enzyme is present in GF<sub>1</sub> in a concentration similar to that found in the plasmalemmal fraction (31) and eight times lower than that recorded in microsomes. Cyt. *b*<sub>5</sub> is partially solubilized by our procedure for the extraction of VLDLs:  $\sim 1/4 - 1/2$  of the original amount appears in the extracted content while the rest remains associated with the membranes of

the corresponding fractions (e.g., GF<sub>1</sub> or microsomes).<sup>7</sup>

The cyt. *b*<sub>5</sub> chain appears to be indigenous to GF<sub>1</sub> membranes. Its presence cannot be explained by contamination with either residual microsomes or mitochondria. Such contamination can be ruled out on both morphological and enzymological grounds: the fraction is free of structurally recognizable mitochondrial and ER contaminants, and its specific activities for NADH-cyt. *c*-reductase and cyt. *b*<sub>5</sub> are considerably higher than those it shows for well established markers of both ER (G6Pase, NADPH-cyt. *c*-reductase) and mitochondrial (cytochrome oxidase, see below) membranes.

GF<sub>1</sub> and its membranes have AMPase activity at  $\sim 2/3$  the concentration detected in microsomes and at a considerably lower concentration ( $\sim 1/8$ ) than found in plasmalemmal fractions. This activity also appears to be indigenous to GF<sub>1</sub>

<sup>6</sup> Specific activities for GF<sub>1</sub> range from 0.009 to 0.063 generally in parallel with variations in the activity of the total microsomal fraction.

<sup>7</sup> The finding is in agreement with Dallner's observations (32) concerning the ease of extraction of cyt. *b*<sub>5</sub> from microsomal fractions.

membranes, since morphological findings definitely rule out contamination of the magnitude required to ascribe it to contaminant ER or plasmalemmal fragments. AMPase has often been considered a plasma membrane marker on account of the high specific activity it reaches in plasmalemmal fractions (31, 10, 33-35), the large proportion of activity generally recovered in microsomal fractions (36, 37) being ascribed to contamination by plasmalemmal fragments. This view was supported by histochemical observations made on fixed liver tissue which revealed activity in the plasmalemma but not in the ER (38-40). Recently, however, Widnell (41) showed that AMPase activity can be demonstrated histochemically in unfixated microsomal fractions and found that the ER enzyme is much more sensitive to fixation than the plasmalemmal counterpart thus providing a reasonable explanation for the results obtained in fixed liver. The same situation may apply to the Golgi-associated AMPase since Goldfischer et al. (21) have noted that the complex gives a negative reaction *in situ*. Recent cytochemical tests on isolated Golgi fractions show, in fact, that there is AMPase activity in morphologically identifiable Golgi elements (42).

GF<sub>1</sub> and GF<sub>1</sub> membranes have very little cytochrome oxidase activity. Their specific activity amounts to only ~0.5% of the specific activity of the mitochondrial fractions we have isolated for comparison from the same liver homogenates. This figure should be taken, however, as a maximal value for mitochondrial contamination, since mitochondrial fractions with considerably higher (~ four times) specific activities have been prepared by more refined procedures (30, 43). This low specific activity is in good agreement with the virtual absence of structurally recognizable mitochondrial contaminants in GF<sub>1</sub> and GF<sub>1</sub> membrane pellets.

Finally, the data in Table II show that GF<sub>1</sub> has acid phosphatase activity at a concentration ~30 times lower than in liver lysosomes (cf. 3, 44). The activity could be ascribed to the contamination of the fraction by lysosomes (2) but, since morphologically recognizable lysosomes do not account for more than ~1% of the mass of GF<sub>1</sub>, the possibility of a mixed distribution of the enzyme in Golgi elements as well as in lysosomes remains open. Evidence obtained on granulocytes (45-47), monocytes (48), and macrophages (49) shows that in these cells in which the production of lysosomal enzyme is a major activity, the en-

zymes in question have a mixed distribution; they appear to be routed through Golgi elements on their way to primary (45-48) or secondary (48, 49) lysosomes. The same may generally apply for all cell types in which only secondary lysosomes can be detected (50).

Taken together, the biochemical data in Table II indicate that GF<sub>1</sub> is practically free of ER and mitochondrial contaminants and only slightly contaminated by lysosomes, although part of the lysosomal activity detected could be indigenous to Golgi elements. Recent cytochemical tests carried out on isolated Golgi fractions show that this is indeed the case (42). Accordingly, the biochemical data are in good agreement with the morphological findings already presented (2) which show that mitochondria are absent from GF<sub>1</sub> and the lysosomes present only in small numbers; the data are also compatible with the absence of ER contaminants. The same morphological observations (2) indicate that contamination by plasmalemmal fragments must be very limited, if at all present. Since the fraction is morphologically homogeneous or nearly homogeneous, the biochemical data must describe the properties of the Golgi elements and Golgi membranes in GF<sub>1</sub>. They indicate that the makeup of these membranes is in some respects qualitatively (absence of G6Pase and cyt. P-450 system) and in others quantitatively (low concentrations of the cyt. *b*<sub>5</sub>, NADH-cyt. *c*-reductase, and AMPase) different from both ER membranes and plasmalemma. The differences are in fact more extensive as will be shown by the results obtained in assaying the galactosyltransferase activity of these various membranes.

#### *Partial Characterization of GF<sub>2</sub> and GF<sub>3</sub>*

ENZYMATIC ACTIVITIES: To find out whether the properties detected for GF<sub>1</sub> apply also to heavier Golgi elements, the inquiry was extended to GF<sub>2</sub> and GF<sub>3</sub>. GF<sub>1</sub> and microsomal fractions derived from the same homogenates were included to facilitate comparison, but the assays were limited to whole fractions and to fewer enzymes than in the preceding section. According to the results obtained (Table III), the activity of ER marker enzymes remains low in all Golgi fractions, although there is a marked increase in specific activity from GF<sub>1</sub> to GF<sub>3</sub> for both G6Pase and NADPH-cyt. *c*-reductase, we assume that this reflects an increase in contamination by ER-

TABLE III  
Specific Enzymic Activities of GF<sub>1</sub>, GF<sub>2</sub>, GF<sub>3</sub>, and Other Cell Fractions

Enzyme	GF <sub>1</sub>	GF <sub>2</sub>	GF <sub>3</sub>	Micr. fr.	Plasma. fr.
G6Pase	0.05	0.10	0.40	2.60	—
NADPH-cyt. <i>c</i> -reductase	0.003	0.004	0.005	0.023	—
NADH-cyt. <i>c</i> -reductase	0.04	0.09	0.04	0.28	—
Cyt. <i>b</i> <sub>5</sub>	0.03	0.03	0.05	0.07	—
AMPase	1.5	2.5	5.5	2.50	10.0*

\* From Benedetti and Emmelot (88). Specific activity units for: G6Pase and AMPase,  $\mu\text{mol P}_i$  liberated/mg protein/20 min; the reductases,  $\mu\text{mol NADPH}$  (NADH) oxidized/mg protein/min; cyt. *b*<sub>5</sub>,  $\Delta\text{OD}$  (424 – 410 nm)/mg protein.

derived vesicles which reaches 15–20% in GF<sub>3</sub> according to the specific activity figures for G6Pase and NADPH-cyt. *c*-reductase, respectively. There is less change from one Golgi fraction to another in NADH-cyt. *c*-reductase and cyt. *b*<sub>5</sub> concentration, but there is a relatively large increase in AMPase specific activity. Since the morphology of GF<sub>2</sub> and GF<sub>3</sub> rules out extensive contamination by plasmalemmal fragments, this increase probably reflects an actual higher concentration of enzyme activity in heavier Golgi elements. According to the data in Table III, all Golgi fractions appear to be distinct from both plasmalemmal and microsomal fractions. In addition, and in agreement with the morphological findings already described (2), GF<sub>1</sub> is more similar to GF<sub>1</sub> than to GF<sub>3</sub>.

UDP-GALACTOSE :*N*-ACETYLGLUCOSAMINE GALACTOSYLTRANSFERASE: Recent work by Fleischer et al. (16, 51) and Morr  t et al. (52) has shown that Golgi-rich fractions isolated from either beef (16) or rat (51, 53) liver are highly active in transferring galactose from UDP-galactose to exogenous *N*-acetylglucosamine and has suggested (16) that this galactosyltransferase is a "marker" enzyme for Golgi membranes. In their experiments, a considerable increase in transferase activity was detected in Golgi-rich fractions over that found in homogenates and microsomes, but relatively low recoveries were recorded (~10% in beef liver and ~40% in rat liver), most of the activity separating with the microsomes.

Since in the case of ethanol-treated animals we can isolate relatively clean Golgi fractions (GF<sub>1</sub> and GF<sub>2</sub>) and since we recover in our complete set of Golgi fractions more protein than in some of the references mentioned, we assayed these fractions for galactosyltransferase activity by the procedure of Hassid and Babad (15, 16) in which UDP-[<sup>14</sup>C]-galactose is separated by ion exchange chroma-

tography from its transfer and hydrolysis products and in which it is assumed that the amount of radioactivity above the blank represents galactose transferred to *N*-acetyllactosamine. The results of a representative experiment (Table IV), out of a series of six, show that 1/3 of the transferase activity of the homogenate remains in a combined "nuclear-mitochondrial" fraction, while 2/3 separate with the microsomes. Practically 100% of the latter is recovered in our set of three Golgi fractions, with a slight overrecovery in our combined Golgi fraction, and with no detectable activity left in the residual microsomal fraction. The specific activities of the various Golgi fractions range from ~55 to 100 times higher than that of the original homogenate. In the absence of previous ethanol treatment (Table V), the recovery amounts to only 40–50% of the activity of the initial microsomal fraction, in agreement with the difference in centrifugal band pattern discussed in reference 2. The specific activities are also lower: namely, only 13–55 times higher than that of the original homogenate. In both cases, the specific activity of the combined Golgi fraction is slightly higher than that of the most active Golgi subfraction, presumably because the introduction of an additional sucrose layer in the density gradient leads to a slight degree of purification.

For work on liver cell fractions, the Hassid-Babad procedure has the disadvantage of giving high blanks for certain preparations which, in addition to transferase activity, apparently have high galactosidase (54) and nucleotide pyrophosphatase (55) activities. This is especially the case for the original homogenates and total microsomal fractions used as starting preparations for the isolation of our Golgi fractions. On account of the high blanks mentioned, the reliability of our data on total activity at the start and on recovery in the

TABLE IV  
*UDP-Galactose:N-Acetylglucosamine Galactosyltransferase Activity in Cell Fractions Isolated from the Liver of Ethanol-Treated Rats*

The assay procedure used was Babad and Hassid (15), column chromatography.

Fraction	mg protein g liver*	Specific activity‡	Total activity	Recovery %
Homogenate	195.00	1.7	331	100.0
Nuclear-mitochondrial fraction	97.00	1.1	107	32.0
Microsomal fr. (initial)§	26.00	9.0	234	70.7
GF <sub>1</sub>	0.05	93	5	1.5
GF <sub>2</sub>	0.34	102	35	10.6
GF <sub>3</sub>	1.35	150	202	61.0
Microsomal fr. (residual)	20.00	nd¶	nd¶	—
GF combined	1.5	170	255	77.0

\* Wet weight.

‡ nmol galactose transferred/h/mg protein.

§ Refers to microsomal fraction before the removal of Golgi fractions by flotation.

|| Refers to microsomal fraction left after the removal of GF<sub>1</sub>, GF<sub>2</sub>, and GF<sub>3</sub>.

¶ nd, not detectable.

TABLE V  
*UDP-Galactose:N-Acetylglucosamine Galactosyltransferase Activity in Cell Fractions Isolated from the Liver of Control Rats (No Ethanol Treatment)*

Assay procedure used was Babad and Hassid (15), column chromatography.

Fraction	mg protein g liver*	Specific activity‡	Total activity	Recovery %
Homogenate	180	2.2	396.0	100.0
Microsomal fr. (initial)	25	10.0	250.0	63.1
GF <sub>1</sub>	0.015	30.0	0.45	—
GF <sub>2</sub>	0.095	110.0	10.45	2.6
GF <sub>3</sub>	0.91	100.0	91.0	23.0
GF combined	1.05	120.0	126.0	31.8

\* Wet weight.

‡ nmol galactose transferred/h/mg protein.

end can be questioned. To obviate this disadvantage and to check on the results already obtained, we used the procedure of Fleischer et al. (16) which relies on the isolation of the product of the transfer reaction (*N*-acetylglucosamine) from the assay mixture by paper chromatography. In this procedure, UDP-[<sup>14</sup>C]galactose remains at the origin and *N*-[<sup>14</sup>C]acetylglucosamine is readily separated from [<sup>14</sup>C]galactose. However, when liver cell fractions are assayed a third unidentified radioactivity peak is detected running immediately ahead of *N*-acetylglucosamine (Fig. 1 *a* and *b*).

With the new procedure, we first tried to obtain maximal transferase activity by adding a detergent

(Triton X-100, final concentration 0.6%) to the assay mixture as done by Schachter et al. (56). In the case of GF<sub>3</sub> (Fig. 1 *a* and *b*), the addition of detergent resulted in a nearly twofold increase in the amount of radioactivity which appeared in *N*-acetylglucosamine without visibility affecting the transfer of label to the unidentified peak (arrows).

The activity in homogenates and total microsomal fractions was low and remained low (two to three times the background) even in the presence of the detergent. Moreover, a relatively large amount of radioactivity was transferred to the intermediary peak between *N*-acetylglucosamine and galactose (Fig. 2 *a* and *b*).

Attempts to increase the amount of galactose



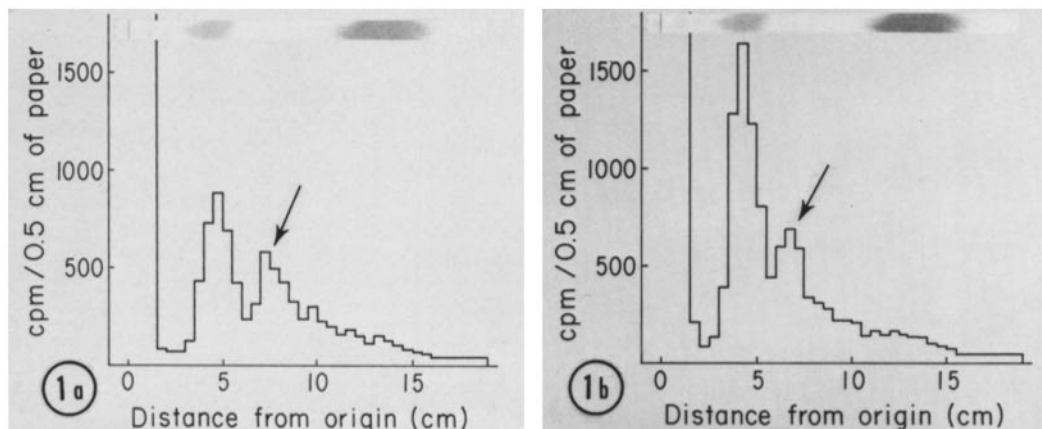


FIGURE 1 GF<sub>3</sub>. Chromatographic profiles of the products of the UDP-galactose:*N*-acetylglucosamine galactosyltransferase. (a) assay in the absence of Triton X-100:32  $\mu$ g protein, 169 nmol galactose transferred/mg protein/h; (b) assay in the presence of 0.6% Triton X-100:32  $\mu$ g protein, 292 nmol galactose transferred/mg protein/h. The arrows mark the unidentified radioactivity peak (see text). In this, and in all subsequent figures except Fig. 3 c and d, the position of the standards (left: *N*-acetyllactosamine, right: galactose) is shown on the paper strip along the upper margin of the figure.

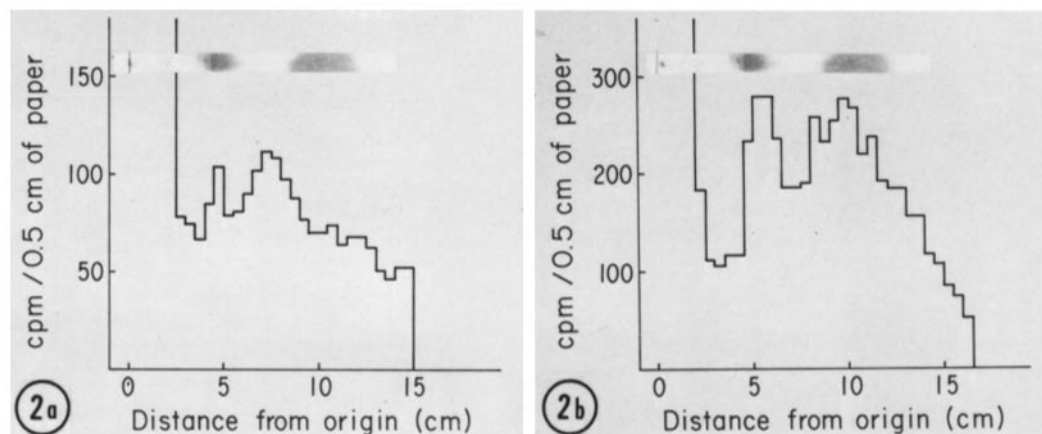


FIGURE 2 Chromatographic profiles of the products of the UDP-galactose:*N*-acetylglucosamine galactosyltransferase. Assays carried out in the presence of 0.6% Triton X-100. (a) homogenate, 75  $\mu$ g protein; (b) total microsomal fraction, 50  $\mu$ g protein.

transferred to *N*-acetyllactosamine by increasing the amount of fraction protein (enzyme) added to the assays led to extensive overlap of reaction products and inability to separate *N*-acetyllactosamine (Fig. 3 a and b) from them. The only fraction for which the reaction products appeared reasonably well separated was GF<sub>1</sub> (Fig. 4). This difficulty was finally cleared by extracting the assay mixture with a lipid solvent, i.e., chloroform:methanol, 2 vol:1 vol, at the end of the transfer reaction on the assumption that the intermediate peak is a glycolipid. The paper chromatograms of the

aqueous phases obtained in the extraction step showed that the unknown peak was effectively removed in the case of all fractions, while the mobility of *N*-acetyllactosamine and galactose was slightly increased. The results are illustrated for homogenates and total microsomes in Fig. 3 c and d and for GF<sub>3</sub> in Fig. 5 a and b. The nature of the lipid soluble compound remains to be investigated in future work.

The procedure thus modified was applied to all relevant fractions and the results (Table VI) confirmed that in the case of ethanol-treated rats,

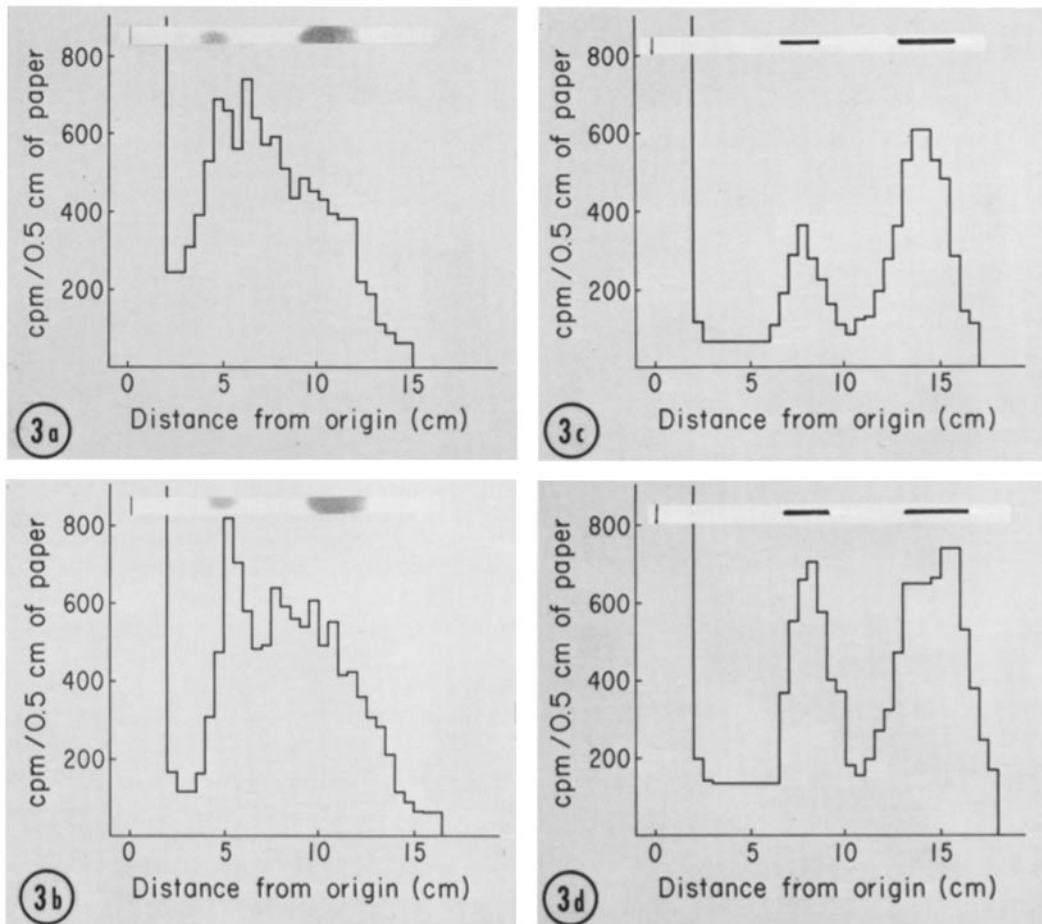


FIGURE 3 Chromatographic profiles of the products of the UDP-galactose:*N*-acetylglucosamine galactosyltransferase. Assays carried out in the presence of 0.6% Triton X-100. (a) homogenate, 375  $\mu$ g protein, whole assay mixture; (b) total microsomes, 250  $\mu$ g protein, whole assay mixture; (c) homogenate, 375  $\mu$ g protein, aqueous phase of the assay mixture after extraction with chloroform-methanol, 3.18 nmol galactose transferred/mg protein/h; (d) total microsomes, 250  $\mu$ g protein, aqueous phase of the assay mixture after extraction with chloroform-methanol, 19.2 nmol galactose transferred/mg protein/h. In Fig. 3 c and d, the positions of the standards (left: *N*-acetyllactosamine, right: galactose) is indicated by the horizontal bars along the upper margins of the graphs.

practically all the galactosyltransferase activity of the parental microsomal fraction is recovered in the set of three Golgi fractions. As in the first set of experiments, the specific activity of the Golgi fractions rises 50- to 80-fold above the activity of the original homogenates.

To localize the transferase activity in further detail, the VLDL content of Golgi elements was removed by the procedure given in reference 2 and the recovered Golgi membranes were assayed as above. The results showed that the labeled lipid-soluble product (arrow) separated with the membranes (Fig. 6), not the VLDL, and that the spe-

cific transferase activity increased twofold in the case of GF<sub>1</sub> and GF<sub>2</sub> (Table VII). No increase was detected in GF<sub>3</sub> membranes; this is in agreement with our morphological findings which show that there are relatively large amounts of removable VLDLs in GF<sub>1</sub> and GF<sub>2</sub>, but not in GF<sub>3</sub>.

The activity of Golgi membranes remains sensitive to Triton X-100 which increases it by ~25% (Table VII). This may reflect the partial reorganization of Golgi membranes into closed vesicles upon VLDL removal (2) or possibly a direct activation of the enzyme over and above the effect of

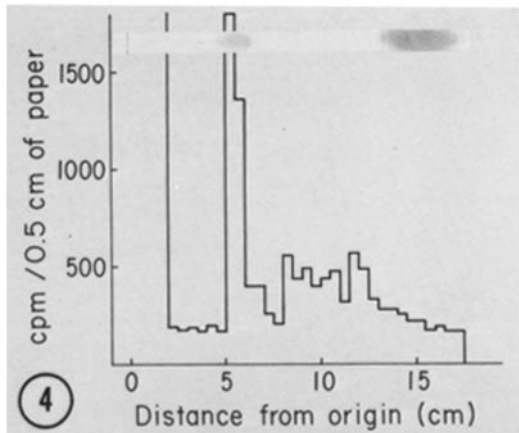


FIGURE 4 GF<sub>1</sub>. Chromatographic profile of the products of the UDP-galactose:*N*-acetylglucosamine galactosyltransferase reaction. Assay carried out in the presence of 0.6% Triton X-100 with 50 μg protein. 155 nmol galactose transferred/mg protein/h.

removal of diffusion barriers by partial membrane solubilization.

#### DISCUSSION

GF<sub>1</sub>, GF<sub>2</sub>, and GF<sub>3</sub> compare favorably in terms of purity and recovery with the Golgi fractions or Golgi-rich fractions already described in the literature.

#### *Degree of Homogeneity*

Since our morphologically cleanest fractions, GF<sub>1</sub> and GF<sub>2</sub>, have no detectable or very low G6Pase and NADPH-cyt. *c* reductase activity, we can conclude that the enzymes mentioned are either absent from, or present in very low concentrations in, the membranes and content of the Golgi elements recovered in our light and intermediate fractions. On the basis of its G6Pase activity, GF<sub>3</sub>, morphologically our least homogeneous fraction, is contaminated by ER-derived vesicles to the extent of ~15% of its total protein. Assessed on the same basis, the degree of ER contamination in the Golgi fractions or Golgi-rich fractions described in the literature varies from ~8% (16) to ~42%<sup>8</sup> (27). Cytochemical tests carried out on our Golgi fractions indicate that G6Pase reaction product is consistently absent

<sup>8</sup> This is probably a maximal value since the figure given for the G6Pase specific activity of smooth microsomes is unusually low in this reference.

from recognizable Golgi elements and present only in GF<sub>3</sub> in small vesicles of the form and size expected for smooth microsomes, i.e., vesicles derived from the smooth ER (42). Accordingly, we can conclude that Golgi elements in general, and elements of vacuolar and cisternal type in particular, lack G6Pase activity and probably NADPH-cyt. *c*-reductase and cyt. P-450. For the latter, our findings confirm data already published by Fleischer et al. (57). This conclusion is in agreement with earlier observations (21–23) on the absence of histochemically detectable G6Pase activity in the Golgi complex of hepatocytes reacted *in situ*, and on the lack of hypertrophy of the complex during the drug-induced proliferation of the hepatocytic ER. Conversely, our data confirm the view that the enzymes mentioned are truly ER membrane markers (cf. 36).

#### *Recovery*

Taken together, GF<sub>1</sub>, GF<sub>2</sub>, and GF<sub>3</sub> account for practically 100% of the galactosyltransferase activity present in our starting preparations, i.e., total microsomal fractions, which in turn account for ~70% of the activity found in the original homogenates. The recoveries reported in the literature are of ~20% (16, 51) and range from less than 10% (16) to ~40% (52, 56) with a single exception (27) in which recoveries comparable to ours (~90% of the activity of the microsomal fraction and ~70% of that of the homogenate) were obtained, but at the price of ~42% ER contamination as assessed by G6Pase activity.

Our recovery data indicate that in the liver galactosyltransferase is definitely a specific Golgi activity in confirmation of the original proposal advanced by Fleischer et al. (16) and repeated by others (27, 52), and in agreement with earlier radioautographic observations made on a variety of cell types by Neutra and Leblond (58) and by others (59).

#### *Effects of Ethanol Treatment*

Our Golgi fractions were obtained from the livers of rats acutely intoxicated with ethanol. It is known that under such conditions the lipid content of the liver increases (60) and the Golgi elements of the hepatocytes become overloaded with VLDL particles (2). It is also known that both changes are reversible and that no other structurally detectable alterations are introduced by the intoxication, with the exception of an increase in the popu-

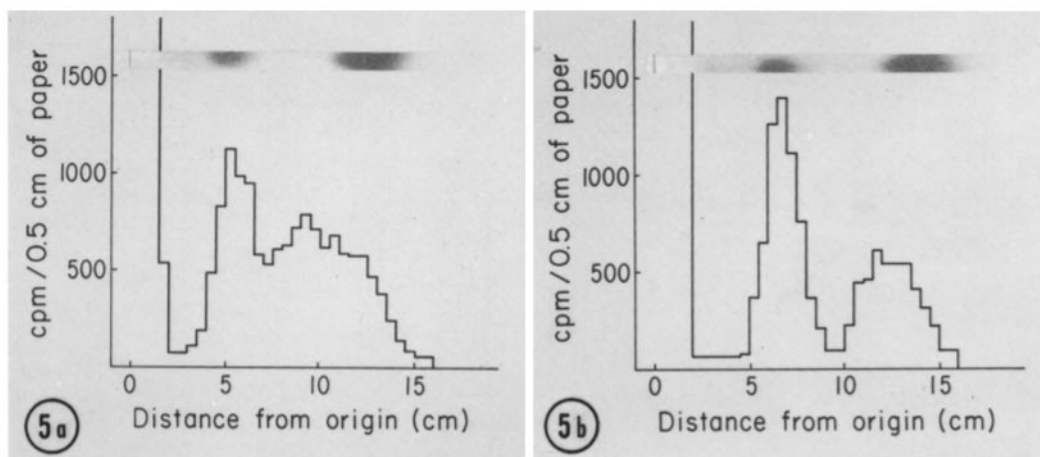


FIGURE 5 GF<sub>3</sub>. Chromatographic profiles of the products of the UDP-galactose:*N*-acetylglucosamine galactosyltransferase reaction. Assays carried out with 50  $\mu$ g protein in the presence of 0.6% Triton X-100. (a) whole assay mixture; (b) aqueous phase of the assay mixture after extraction with chloroform-methanol, 225 nmol galactose transferred/mg protein/h.

TABLE VI  
UDP-Galactose:*N*-Acetylglucosamine Galactosyltransferase Activity in Cell Fractions Isolated from the Liver of Ethanol-Treated Rats

Assay procedure used was Fleischer et al. (16) in the presence of 0.6% Triton X-100 in the incubation mixture, paper chromatography.

Fraction	mg protein g liver*	Specific activity‡	Total activity	Recovery %
Homogenate	195	3.2	624.0	100.0
Microsomal fr. (initial)	21	19	399.0	63.9
GF <sub>1</sub>	0.05	155	7.8	1.2
GF <sub>2</sub>	0.35	122	43.0	6.8
GF <sub>3</sub>	1.30	255	331.0	53.1

\* Wet weight.

‡ nmol galactose transferred/h/mg protein.

lation of large lipid droplets in the cytoplasmic matrix of the hepatocytes (60, 2). The data in Tables IV and V show that the total and specific activity of liver homogenates are comparable in normal and ethanol-treated animals, but that recoveries and specific activities are substantially lower in controls. These findings and those reported in the companion paper (2), justify our procedure since they show that the density perturbation introduced by VLDL overloading makes possible the isolation of two nearly homogeneous Golgi fractions (GF<sub>1</sub> and GF<sub>2</sub>) and allows for satisfactory recovery of Golgi elements (in GF<sub>1</sub>, GF<sub>2</sub>, and GF<sub>3</sub>) as assessed by galactosyltransferase ac-

tivity, without detectably affecting the activity of the marker enzyme used. There are, however, reports that longer or chronic treatment with ethanol increases transferase activity (61).

The literature on the effects of ethanol on the metabolism of lipids in the liver is profuse but still inconclusive concerning mechanisms leading to lipid and VLDL overloading (cf. 62, 63). It is generally assumed that the rates of synthesis of fatty acids (64) and triglycerides rise (65) as a result of an increase in the NADH:NAD ratio in the cytoplasm (66), but a decrease in the rate of discharge (67) and reduced utilization (68) may also be involved.

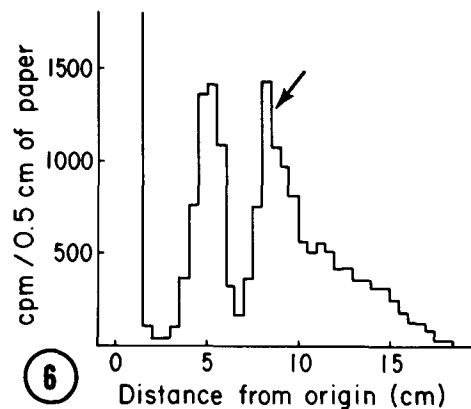


FIGURE 6 Chromatographic profile of the products of the UDP-galactose:*N*-acetylglucosamine galactosyltransferase. Assay carried out with 60  $\mu$ g protein without Triton X-100. The preparation used was a membrane subfraction isolated from GF<sub>3</sub>. Area under the *N*-acetylglucosamine peak corresponds to 164 nmol galactose transferred/mg protein/h. The arrow marks the radioactive glycolipid peak.

TABLE VII  
UDP-Galactose:*N*-Acetylglucosamine Galactosyltransferase Activity of Golgi Membranes

Fraction	Specific activity*	
	Total fr.	Membrane fr.
GF <sub>1</sub> †	93	190
GF <sub>2</sub> †	110	210
GF <sub>3</sub> †	160	170
GF combined§ + Triton X-100	—	230
GF combined§ - Triton X-100	—	172

\* nmol galactose transferred/h/mg protein.

† Assay procedure used was Hassid-Babad (15) without Triton X-100 in the incubation mixture, column chromatography.

§ Assay procedure used was Fleischer et al. (16), paper chromatography.

### Specificity of Golgi Membranes

Our data taken together with those already available in the literature indicate that the enzymic activities of Golgi membranes are definitely different from those of the membranes of the ER which in the liver have been extensively investigated by using smooth and rough microsomal frac-

tions. Hepatic Golgi membranes apparently lack enzyme activities typical for hepatic ER membranes, like G6Pase and the cyt. P-450 system. The converse applies for galactosyltransferase activity. Recent data indicate that the differences are more extensive: hepatic Golgi fractions have no detectable acyltransferase (Acyl-CoA:1,2-diacyl-*sn*-glycerol acyltransferase) and cholinephosphotransferase activity (69) and hence, lack the capacity of synthesizing phosphatidylcholine and triglycerides, which is known to be characteristic for liver microsomes (70). Conversely, there is suggestive evidence obtained by both cell fractionation (56) and autoradiography (71, 72) that other transferase activities (fucosyl and sialyl transferases) are primarily associated with Golgi membranes. The finding appears to apply for both exogenous (56) and endogenous (73) acceptors of the sugar transferred.

Data obtained on Golgi-rich fractions prepared from liver by the procedure of Morr e et al. (53) also indicate that the gross lipid composition of the Golgi fractions is different from that of both microsomal and plasmalemmal fractions (74) and is characterized by an intermediate content of sphingomyelin and cholesterol. Similar data are available for the Golgi membranes of the guinea pig pancreas isolated, in this case, as a smooth microsomal fraction (75).

It follows that Golgi membranes differ from ER membranes in both enzymic activities and lipid composition. They also appear to be different from the plasmalemma although in this case the information available is much more limited and less relevant: it pertains to whole plasmalemmal fractions when the preparation of interest should be limited to that domain of the plasmalemma with which membranes of Golgi origin interact during secretory discharge.

The data presented and discussed in this paper also indicate that the ER, Golgi, and plasma membranes share some common activities. For instance, AMPase is found in all three fractions but in different degrees of concentration, and the cyt. *b*<sub>5</sub> chain is present at least in the ER and Golgi membranes.<sup>9</sup> The true extent of the overlap (or

<sup>9</sup> Our data indicate that the whole chain (NADH-cyt. *c*-reductase and cyt. *b*<sub>5</sub>) is present in rat liver Golgi fractions, but others have reported the presence of the cyt. *b*<sub>5</sub> (57) and the absence of the reductase (51) in Golgi fractions or Golgi-rich fractions prepared from the same source. Both enzymes have been

conversely of specificity) in these membranes is, however, difficult to assess because many of their components, especially their proteins, are still unknown and because their resolution in gels is still incomplete (cf. 76) and grossly affected by the presence of the secretory proteins in the content (77 and K. Howell, unpublished observations).

A better understanding of the situation could be obtained in the future by careful isolation of the corresponding membranes (Golgi, ER) from their content and by a better electrophoretic resolution of the proteins of both subfractions. Here again our procedure has an advantage over those already published: it provides means for the separation of Golgi membranes from the content of Golgi elements. As already mentioned, the problem is different for the plasmalemmal fractions. In their case, the comparison with Golgi membranes should be limited to functionally relevant domains of the plasmalemma.

### *Functional Implications*

Although the available evidence is still fragmentary, it becomes clear that severe restrictions are imposed on the exchange of molecular components between the ER and the Golgi membranes and probably, but less certainly, between the Golgi membrane and the plasmalemma. A priori, such exchanges could take place when membranes establish continuity with one another during the intracellular transport and discharge of secretory proteins. The exact anatomy of the sites of continuity between the ER and the Golgi complex are unknown in the hepatocyte. In the pancreatic exocrine cell, it has been postulated that the transport of the secretory product involves the small vesicles at the periphery of the Golgi complex which presumably act as shuttles between the transitional elements of the rough ER and Golgi-condensing vacuoles (78). In the case of secretory granules, it is sufficiently clear that transport to the sites of discharge does not involve continuous channels and that the secretory granules discharge intermittently by fusion of their Golgi-derived membranes with the plasmalemma (79). In both cases, mechanisms seem to be at work to maintain in constant relative proportions the volumes, or surface areas, of the compartments involved in the

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detected, however, in Golgi-rich fractions prepared from beef liver (16, 57).

overall process (80). But the fate of the membranes involved in these operations, especially the fate of the secretory granule membranes after discharge, is still a matter of debate. If the continuity between ER and Golgi were permanent as postulated by Morr e et al. (81) and Claude (82), we must conclude that diffusion in the plane of the membrane for both lipids and proteins is severely restricted from one membrane to the other, although such diffusion is known to occur in other systems (83, 84) on account of the fluidity of membrane lipids. If the ER-Golgi continuity were intermittent and secretory products were moved from one compartment to the next *pari passu* with a membrane container, then we must postulate that: (a) added membrane is removed from the receiving compartment to satisfy the constancy of relative proportions under normal conditions of operation, and (b) the removal is nonrandom in character, i.e., the piece of membrane removed from the receiving compartment is exactly the piece of membrane that came from the donor compartment. Without nonrandom removal there would be complete mixing of membrane components, lipids as well as proteins, which is contrary to experimental findings at least as far as the ER and Golgi membranes are concerned.

Membrane movement and relocation have been related in the past to the transport of secretory products as well as to membrane biogenesis. The first type of relation is a reasonably well-established fact, at least in the case of the interaction between secretion granule membranes and plasmalemma. The second relation is still a postulate which proposes that the ER membrane is the precursor of the Golgi membrane which in turn is the precursor of the plasmalemma, the membrane being modified at the Golgi station before being removed to the cell surface (cf. 85). At present, there is no direct evidence for this postulate. It may well apply, but the new findings indicate that extensive and complex modifications, involving both subtraction and addition of components, must occur at least at the transition from ER to Golgi. Hence, alternative mechanisms should be considered. For instance, the membrane of each compartment may be assembled *in situ* using at least in part previously soluble components as in the case of mitochondrial (86) or chloroplast membranes (87), or the membrane container may bring forth from one compartment to the next in series not only secretory proteins but also some proteins which are selectively allowed to diffuse in the plane of the mem-

brane of the receiving compartment while the other components are retained in, and removed with, the carrier.

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