# BIOGENESIS OF MICROSOMAL MEMBRANE GLYCOPROTEINS IN RAT LIVER

## III. Release of Glycoproteins from the Golgi Fraction and their Transfer to Microsomal Membranes

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ABSTRACT

The presence in the Golgi fraction of glycoproteins destined to be incorporated into the microsomal membrane was investigated. When incubated in sucrose, washed Golgi vesicles released four major, weakly acidic glycoproteins, some of which could be incorporated into microsomal membranes by incubation. Double labeling with [$^3$H]glucosamine and [$^{14}$C]leucine demonstrated the incorporation of both protein and oligosaccharide moieties, and the main peak of radioactivity was associated with the 70,000 mol wt region after SDS-gel electrophoresis. The proteins that could be incorporated into microsomes were probably associated to a large extent with the outer surface of the Golgi membrane. Centrifugation of the proteins released from the Golgi in a KBr solution ($\rho$ = 1.24) resulted in a separation of glycoproteins, those in the top layer being most actively incorporated into microsomes. The lipoglycoproteins in the top layer that could be incorporated appeared in the 70,000 mol wt region after SDS-gel electrophoresis, as did the corresponding proteins isolated from the supernate. These results suggest that glycoproteins with completed oligosaccharide chains are released from the Golgi system to the cytosol and are subsequently transferred to microsomes as constitutive membrane components.

The central role of the Golgi system in the process by which secretory proteins and lipoproteins are transported to the blood is well established. The most thoroughly studied transport pathway is that of albumin, which is discharged from membrane-bound ribosomes in a vectorial manner, after which the completed protein is found in the lumen of the rough endoplasmic reticulum (ER)[1] (1). Albumin is transported from the rough ER to the smooth ER to the Golgi system, and finally all the newly synthesized albumin molecules appear in the blood (2). Similarly, the various types of serum lipoproteins, assembled from protein, neutral lipid, and phospholipid components in the rough and

[1] *Abbreviations used in this paper:* EDTA, ethylenediaminetetraacetic acid; ER, endoplasmic reticulum; GRP, Golgi-released protein; HDL, high density lipoproteins; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; PLP, phospholipid; SDS, sodium dodecyl sulfate; and TCA, trichloroacetic acid.



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smooth ER, are transported to the blood via the Golgi vesicles as morphologically identifiable structures (3).

The situation is more complex in the case of the serum glycoproteins. The distribution of glycosyl transferases and perhaps also of the different types of lipid intermediates suggests that the oligosaccharide moiety of these glycoproteins is completed during transport through the lumen of the ER and the Golgi system (4). The terminal sugar moieties such as galactose, fucose, and sialic acid, are transferred from the appropriate sugar nucleotides to the oligosaccharide chain exclusively in the Golgi system. This implies that a polypeptide which is synthesized on bound ribosomes and which possesses terminal galactose and sialic acid has at some time to be inside or closely associated with the Golgi system.

It was previously found that microsomes contain glycoproteins as constitutive membrane components (5). The most plausible hypothesis is that these galactose- and sialic acid-containing proteins are completed in the Golgi complex and then transferred back to the ER membranes. There is experimental evidence to indicate that after glycosylation in the Golgi complex certain glycoproteins are not secreted, but remain in the cell as intracellular components. It has been suggested that acid hydrolases (6) and β-glucuronidase (7, 8), established glycoprotein enzymes, are synthesized in the ER and Golgi system before transfer to the lysosomes. Using histochemical and autoradiographic techniques, Leblond and co-workers (9, 10) demonstrated that sugar-containing proteins from the Golgi region are not only secreted but are also used for intracellular construction of the lysosomes, dense bodies, basement and plasma membranes.

This paper describes the release of glycoproteins from the isolated Golgi complex during incubation and the purification of a released glycoprotein component by ultracentrifugation. This glycoprotein is incorporated into microsomal membranes upon incubation as a tightly attached structural component.

## MATERIALS AND METHODS

Incorporation studies, antibody treatment, SDS-gel electrophoresis, and chemical determinations were performed as described previously (11).

### *Animals*

In all experiments male albino rats with a weight of 160–180 g were used. The rats were starved for 20 h before sacrifice. When the Golgi was to be isolated, 50% ethanol (1 ml/100 g) was administered through a stomach tube 90 min before decapitation. 200 μCi [$^3$H]glucosamine was injected in the portal vein 30 min before decapitation. In appropriate cases, 60 μCi [$^{14}$C]leucine was also given simultaneously.

### *Fractionation*

Microsomes were prepared as described earlier (12). Two types of Golgi fractions were isolated, based on the observations of Ehrenreich et al. (13). In the first, total microsomes, isolated after vigorous homogenization and suspended in 1.17 M sucrose, were overlayered with layers of 1.15 M and 0.25 M sucrose. In the second type of preparation the 1.15 M sucrose was replaced by 0.86 M sucrose. In both cases centrifugation at 58,000 *g* for 3 h were employed. The isolated fractions, designated "1.15 Golgi" and "0.86 Golgi", were analyzed. In agreement with the results of Ehrenreich et al. (13), the latter was found to represent a more limited subfraction of the total Golgi fraction.

### *Incubation*

Isolated Golgi fractions were diluted with distilled water to a sucrose concentration of 0.25 M and sedimented by centrifugation at 105,000 *g* for 90 min. The pellet was resuspended in 0.15 M Tris buffer, pH 8.0; after recentrifugation, the sediment was suspended in 0.25 M sucrose (1 ml per 10 g starting weight of liver). This suspension was incubated at 37°C for 30 min, followed by centrifugation at 105,000 *g* for 90 min. The supernate, called "Golgi-released protein" (GRP), which was particle free and also devoid of nonprotein-bound radioactivity, was used in the incorporation experiments.

For the study of incorporation, the incubation medium consisted of 20 mM Tris-HCl buffer, pH 8.0, 65 mM KCl, 10 mM ethylenediaminetetraacetic acid (EDTA) and 0.4 mM CMP, Tris-water-washed total microsomes or rough microsomes, an appropriate amount of GRP, and enough sucrose to give a final concentration of 0.25 M. The total incubation volume was 3 ml. The amount of microsomal and supernatant protein used is given in the legends to the individual figures and tables. Incubation was performed at 37°C for 60 min. At the end of the incubation period, the tubes were cooled in an ice-water bath, and the microsomes were sedimented at 105,000 *g* for 60 min. The pellet was suspended in 0.15 M Tris-HCl buffer, pH 8.0, to release adsorbed proteins, and after recentrifugation the microsomal pellet was used for various analyses. All incorporation experiments were performed both with total and rough microsomes. Since both fractions gave identical results, the values for rough microsomes are given only in Table II.

### *Centrifugation in KBr Solutions*

To GRP dissolved in 0.25 M sucrose was added enough solid KBr to give a final density of 1.24 (including the density of the sucrose). 4.5 ml of this suspension



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TABLE I

*Release of Glycoprotein from the Golgi Fraction during Incubation in 0.25 M sucrose*

| Exp | Fraction | Treatment of Golgi | Protein | [$^3$H]GlN* | [$^{14}$C]Leu‡ |
|---|---|---|---|---|---|
| | | | *mg* | *cpm/mg proten* | |
| 1 | 1.15 Golgi | None | 9.8 ± 0.93 | 583,000 ± 62,000 | |
| | Released from 1.15 Golgi | Incubation | 2.4 ± 0.15 | 966,600 ± 95,000 | |
| 2 | 0.86 Golgi | None | 6.6 ± 0.70 | 247,000 ± 13,200 | 15,300 ± 2,600 |
| | Released from 0.86 Golgi | Incubation | 1.5 ± 0.09 | 360,900 ± 48,000 | 22,600 ± 1,700 |

0.86 and 1.15 Golgi were isolated and incubated in 0.25 M sucrose as described in Materials and Methods. After incubation and centrifugation both supernates were analyzed for protein and radioactivity. The values are the means ± SEM of eight determinations.

* GlN represents glucosamine.

‡ Leu represents leucine.

TABLE II

*Incorporation of Total GRP into Microsomes, Mitochondria, and Golgi Membranes*

| Fraction | [$^3$H]Glucosamine incorporated | |
|---|---|---|
| | *total cpm* | % |
| GRP | 183,000 ± 31,800 | |
| Total microsomes | 56,880 ± 4,200 | 31.1 |
| Rough microsomes | 54,230 ± 3,700 | 29.6 |
| Mitochondria | 10,490 ± 1,100 | 5.7 |
| Golgi | 15,400 ± 1,700 | 8.4 |

The various fractions (5 mg protein) were incubated with 0.3 mg GRP protein, centrifuged, washed, and the radioactivity in the pellet determined. The values are the means ± SEM of seven determinations.

containing about 2.5 mg protein was centrifuged at 405,000 *g* for 48 h in a SW 56 rotor on the Spinco-Beckman L2-65B centrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). After centrifugation, the tube contents were divided either into four aliquots of 1.1 ml each or into five aliquots (0.5 ml from the top and 1 ml for each of the four remaining aliquots). Usually, no pellet was obtained. KBr was removed by dialysis of the individual fractions against water.

## RESULTS

### *Release of Glycoproteins from Golgi Vesicles*

The possibility that glycoproteins are released from Golgi vesicles upon incubation in sucrose was studied. Two types of Golgi fractions were prepared according to the procedure described by Ehrenreich et al. (13). The preparation designated "1.15 Golgi" contained all vesicles flotated on the 1.15 M sucrose layer (Golgi 1+2+3). In the second type of preparation, designated "0.86 Golgi", the particles collected were those which flotated on the 0.86 M sucrose phase (Golgi 1 + 2). 1.15 Golgi fractions prelabeled in vivo with [$^3$H]glucosamine and washed with Tris at pH 8.0 were incubated for 30 min in 0.25 M sucrose at 37°C. After ultracentrifugation, about 25% of the total Golgi protein was found in the supernate with a specific activity twice as great as that of the pellet (Table I). A very similar result was obtained when the 0.86 Golgi was incubated in sucrose. After double labeling with [$^3$H]glucosamine and [$^{14}$C]leucine the glycoproteins released from 0.86 Golgi were also highly labeled in their protein moieties.[2]

### *Incubation of Microsomes with GRP*

The total GRP fraction, labeled in vivo with [$^3$H]glucosamine was incubated with total microsomes, resulting in a 30% incorporation of the total radioactivity into microsomes (Table II). During these incubations, EDTA and CMP were present to eliminate possible interference by CMP-sialic acid transferase (14). As further evidence for the incorporation of whole macromolecules, incubations were also performed with rough microsomes. The identical incorporation observed

[2] The GRP suspension was also extracted with phenol. The gel electrophoretic pattern of the proteins is little changed by phenol extraction. For this reason, most of the released proteins must be weakly acidic glycoproteins.



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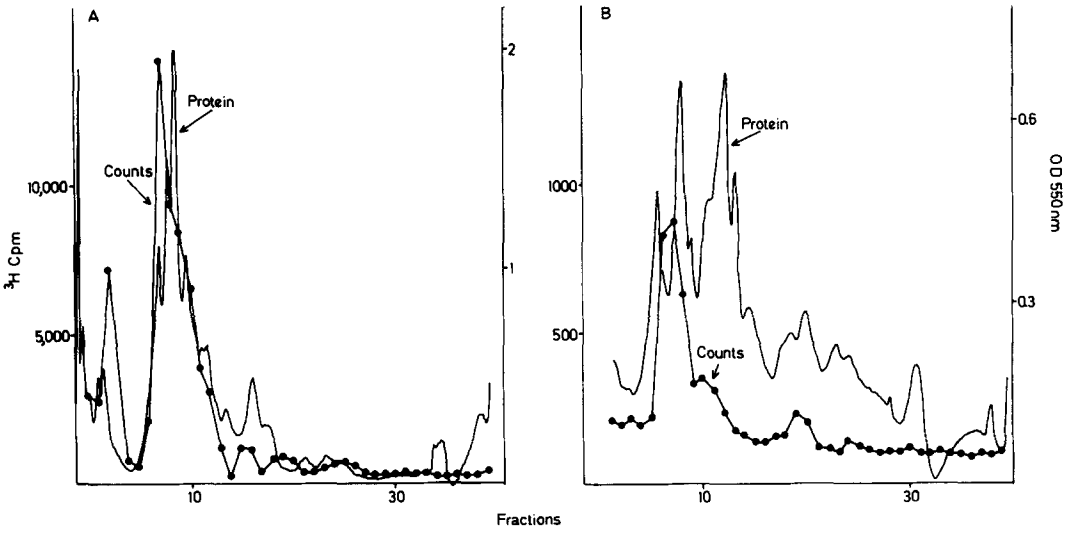


FIGURE 1 Incubation of microsomes with proteins released from Golgi vesicles. Microsomes (5 mg protein) were incubated with GRP (0.3 mg protein). GRP was prepared from Golgi membranes isolated from rats injected 30 min before decapitation with 200 $\mu$Ci [$^3$H]glucosamine. SDS-gel electrophoresis was performed as described earlier (11). (A) GRP before and (B) microsomes after incubation. $^3$H = [$^3$H]glucosamine.

provided additional evidence against the participation of sialyl transferase in this process since rough microsomes do not contain this activity. GRP proved to be incorporated into rat liver mitochondria poorly. In this case only 5.7% of the protein-bound [$^3$H]glucosamine was incorporated, and this figure is even lower when the inevitable contamination of mitochondria by microsomes is taken into account. Reincubation of the GRP with the total Golgi fraction gave incorporation which was less than one-third of that obtained with microsomes. These findings indicate that the glycoproteins are designed to be incorporated into specific membranes.

The incorporation of GRP into microsomes was shown to be dependent on time and the protein concentration of both components.[3] The sodium dodecyl sulfate (SDS)-gel electrophoretic pattern of GRP exhibits, in addition to a few minor components, four large protein components in the higher molecular weight region of the gel. All of these proteins can be labeled in vivo with [$^3$H]glucosamine (Fig. 1 A). Thus, the number of components released by the incubation is quite limited. When microsomes were analyzed by SDS-gel electrophoresis after incubation, protein with high specific activity appeared in only one region of the gel, around fraction 9 (Fig. 1 B). This electrophoretic picture suggests that a specific glycoprotein of the GRP fraction is incorporated into the microsomal membrane, seemingly without any significant change in molecular weight.

### *Antibody Treatment*

Incubation of Golgi membranes in sucrose led to the release of glycoproteins which could be incorporated into microsomes. In order to determine if the proteins released from the Golgi include serum proteins, the GRP preparation was treated with rabbit antiserum against rat serum proteins using a two-step precipitation procedure. After incubation with antiserum, about 20% of the total protein-bound glucosamine label was precipitated, indicating the presence of a moderate amount of serum glycoproteins (Table III). When the GRP remaining in the supernate after incubation with microsomes was treated with antibodies, more than 40% of the total counts were precipitated with antise-

[3] An almost linear increase of the incorporation rate was observed when microsomes were incubated with increasing amounts of GRP within certain limits (0.1–0.5 mg protein). Consequently, if the amount of the GRP fraction used was kept constant (0.3 mg) and the incubation was performed with varying amounts of microsomes (1–20 mg protein), the incorporation rate per milligram microsomal protein was decreased.



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TABLE III

*Treatment of GRP with Rabbit Antiserum against Rat Serum Proteins*

| Exp | Fraction | Treatment | [³H]GlN | | | |
|---|---|---|---|---|---|---|
| | | | Supernate | | Pellet | |
| | | | *total cpm* | % | *total cpm* | % |
| 1 | GRP before incubation with microsomes | None | 45,300 | 100 | | |
| | GRP before incubation with microsomes | Antiserum | 35,600 | 78.5 | 9,300 | 20.5 |
| 2 | GRP after incubation with microsomes | None | 14,600 | 100 | | |
| | GRP after incubation with microsomes | Antiserum | 7,040 | 48.2 | 6,560 | 44.9 |

Antiserum treatment was performed as described previously (11). The values are the means of three experiments.

TABLE IV

*Incubation of Glycoprotein from the Top Layer with Microsomes*

| Preparation | [³H]GlN | | [¹⁴C]Leu | |
|---|---|---|---|---|
| | *total cpm* | # | *total cpm* | # % |
| Top layer | 8,700 ± 480 | | 934 ± 112 | |
| Microsomes after incubation with the top layer | 5,200 ± 750 | 59.0 | 496 ± 88 | 53.0 |

Incorporation of 0.3 mg protein from the top layer obtained by KBr centrifugation with 5 mg microsomal protein was carried out as described in Materials and Methods. The values shown are means ± SEM (6).

rum. This result indicates that incorporation does not involve serum proteins but rather those components which do not react with antiserum.[4]

## *Centrifugation in KBr Solutions*

Because of its behavior, it was suspected that the protein released from the Golgi fraction by incubation was a liporotein complex. The results of various purification procedures supported this idea. The most effective of these procedures was centrifugation in a salt medium.

The GRP fraction was subfractionated in solutions of KBr as described in Materials and Methods. After incubation with microsomes, the highest incorporation was obtained with the top layer; about 60% of the total protein-bound sugar label in this layer was incorporated into microsomes after 1 h (Table IV). Simultaneous double-labeling with [¹⁴C]leucine demonstrated an almost identical degree of incorporation of labeled protein into microsomes. Thus, it appears that a glycoprotein that is especially actively incorporated into microsomes was concentrated in the top layer.

## *Gel Electrophoresis of the Top Layer*

After KBr centrifugation of the GRP, SDS-gel electrophoresis revealed two main protein peaks in the top layer; but only one of them, located around fraction 9, contained protein-bound [³H]glucosamine (Fig. 2 A). This protein in the 70,000 mol wt region also contained some [¹⁴C]leucine; however, the specific activity of this label in the 50,000 mol wt region was much higher.

Fig. 2 B demonstrates the gel electrophoretic pattern of microsomes incubated for 1 h with the top layer. The protein with the highest [³H]glucosamine and [¹⁴C]leucine label was localized in the region of fraction 9, indicating that the main glycoprotein of the top layer was incorporated into microsomes with unchanged molecular

[4] No trichoroacetic acid (TCA)-soluble radioactivity was present in the GRP fraction, i.e. there was a complete lack of small radioactive compounds.



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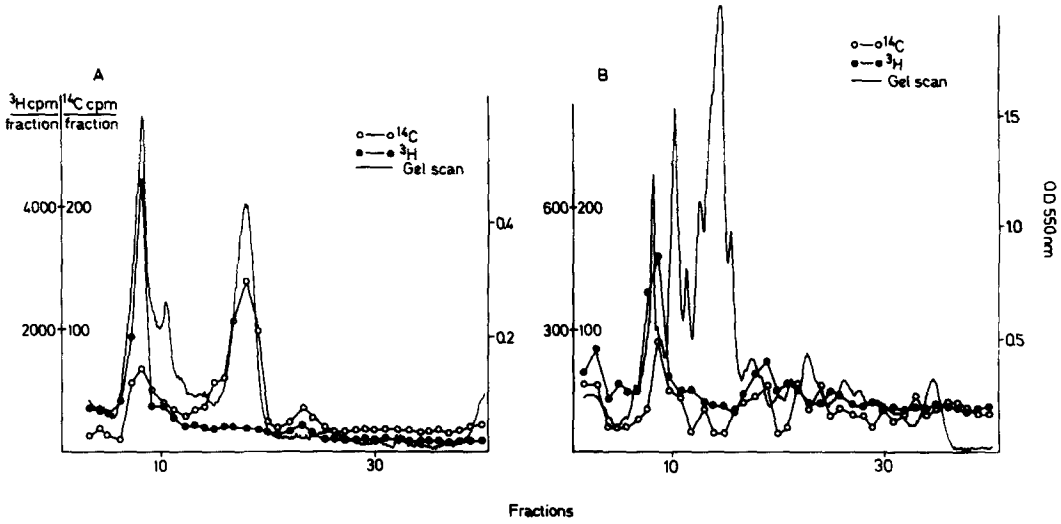


FIGURE 2 SDS-gel electrophoresis of the flotated lipoprotein and of microsomes after incubations with this lipoprotein. GRP was prepared from Golgi membranes isolated from rats injected 30 min before decapitation with 200 $\mu$Ci [$^3$H]glucosamine and 60 $\mu$Ci [$^{14}$C]leucine. The GRP was flotated in a KBr solution as described in Materials and Methods. Microsomes (5 mg protein) were incubated with GRP (0.3 mg protein) at 37°C for 60 min. (A) The top layer after centrifugation of the protein released from Golgi vesicles in KBr solutions and (B) microsomes after incubation with the proteins shown in Fig. 2 A. $^3$H = [$^3$H]glucosamine; $^{14}$C = [$^{14}$C]leucine.

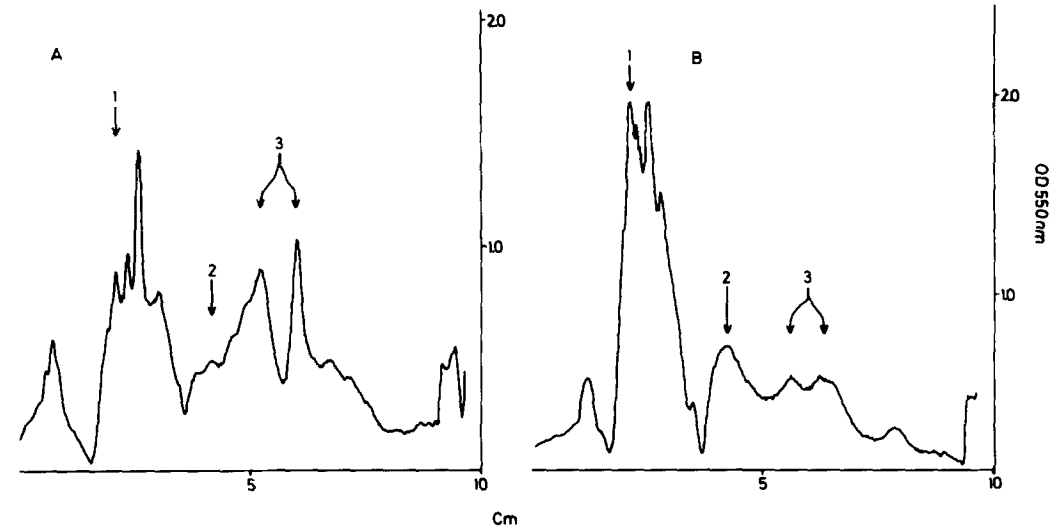


FIGURE 3 SDS-gel electrophoresis of the Golgi fraction after treatment with a French press. French press treatment was performed as described by Ehrenreich et al. (13). After treatment the fraction was centrifuged at 105,000 *g* for 2 h. The pellet was incubated to obtain GRP as described in Materials and Methods. (A) Content = the supernate after centrifugation; (B) GRP, obtained by incubation of the pelleted Golgi membranes. For explanation of numbers 1–3, see the text.

weight. There were also a number of not readily identifiable smaller peaks in the lower molecular weight regions. In a previous paper (5) isolation of a lipoprotein from liver supernate by a similar procedure was described. After incubation of this protein with microsomes, a similar heterogeneity in the distribution of label after SDS-gel electrophoresis of the microsomes was found.



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### *Treatment with a French Press*

In order to investigate the origin of the GRP, Golgi fractions prepared from rats injected with [$^3$H]glucosamine were subjected to treatment with a French press as described by Ehrenreich et al. (13). These experiments were intended to demonstrate whether the glycoproteins capable of being incorporated into microsomes that are released from the Golgi fraction during incubation are located inside the Golgi vesicles.

The gel electrophoretic pattern of the Golgi content released by treatment with a French press displayed a number of peaks throughout the gel (Fig. 3 A). The peaks labeled 1 and 2, which were not very striking, corresponded to the two peaks which were the dominating proteins in the top layer after centrifugation of GRP in KBr. The prominent double peaks of the lower mol wt region (labeled 3) were identified previously as high density lipoproteins (HDL) (15). The picture was quite different when the proteins released by incubation of Golgi membranes that had already been treated with a French press were analyzed (Fig. 3 B). Peaks 1 and 2 were now enhanced to a great extent, while the two peaks of 3 were drastically reduced in comparison with the peaks in gel A.[5] Thus, the gel electrophoretic pattern of the GRP released by incubation from membranes treated with a French press closely resembled that obtained in previous experiments involving the incubation of untreated Golgi fraction. This protein proved to be more efficient than "content" in transfer reactions.[6]

[5] Both "content" and GRP were used in incorporation experiments with microsomes and were treated with antibodies against serum protein. As could be expected from the gel patterns, the GRP was incorporated twice as effectively as the content. Rabbit antiserum against rat serum proteins precipitated about 30% of the protein-bound [$^3$H]glucosamine in the content, while the corresponding precipitation with GRP did not exceed 10%.

[6] In separate experiments the isolated Golgi fraction was incubated with CMP-sialic acid, and thereafter the membranes were subjected to a short treatment with phospholipase A in the presence of albumin, under which conditions the enzyme can attack only the outer surface of the vesicles. This enzyme treatment released 35% of the total incorporated protein-bound sialic acid, thereby indicating a surface localization of part of the newly synthesized sialoprotein. Furthermore, SDS-gel electrophoresis of these released proteins exhibited a pattern very similar to that of GRP.

## DISCUSSION

Experiments with isolated Golgi fractions revealed that upon incubation in 0.25 M sucrose glycoproteins were released. When the released proteins were incubated with microsomes, they became tightly associated with the microsomal membrane.

In these experiments extensive efforts were made to eliminate contamination with the various types of serum proteins which are synthesized in the liver and also found in the liver homogenate. Several experimental observations including gel electrophoretic patterns, extent of incorporation, and precipitation by antibodies to rat serum proteins demonstrate that both the GRP and the supernatant lipoprotein are different from serum lipoproteins.

As described in a preceding paper (5), the particle-free supernate of perfused liver contains several glycoproteins, partly in lipoprotein form, and some of these can be incorporated into microsomes. How can such a macromolecule be localized in the cytosol, which does not contain glycosyl transferases? The experiments described in this study throw some light on this question. Golgi membranes seem to possess the capacity to house lipoglycoproteins which may be released into the cytoplasm and under appropriate conditions incorporated into the ER.

The biogenesis of microsomal and Golgi membranes is, at least in part, supposed to involve a process generally described as membrane flow (16). There are undoubtedly strong experimental data supporting such a flow for many membranous macromolecules. In the newborn rat there is clear structural and biochemical maturation of the rough ER before development of the smooth ER (17). The membrane-bound enzyme activities first appear in rough and later in smooth microsomes. A similar phenomenon has also been demonstrated to occur after phenobarbital induction (18). The transfer of completed enzyme molecules from the rough to the smooth membranes is further supported by turnover studies of isolated NADPH-cytochrome *c* reductase and cytochrome $b_5$ (19).

Our investigations suggest that macromolecules may be inserted into microsomal membranes from the cytoplasmic side. This and similar ideas have been proposed several times previously (20, 21). Subunits of several mitochondrial enzymes are believed to be synthesized on cytoplasmic ribosomes and to be subsequently transferred to their locations in the mitochondria (22). Cytoplasmic pools of polypeptides play an important role in the



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biosynthesis of plasma membranes, as has been demonstrated previously for reticulocytes (23), and in the assembly of the viral envelope (24).

The role of free ribosomes in the synthesis of various intracellular proteins is much discussed. In addition to the above mentioned membrane proteins, ferritin (25), and catalase (26, 27), some microsomal components, such as NADPH-cytochrome *c* reductase (28) and cytochrome P-450 (29), are believed to be synthesized at least partially on free ribosomes. The ribosomes themselves rapidly exchange their proteins with equivalent proteins in the cytoplasm (30).

Similar to our observations are those described by Spatz and Strittmatter (31) and by Enomoto and Sato (32) for cytochrome $b_5$. The form of this enzyme isolated after solubilization with detergent, though not the form isolated after solubilization with trypsin, can be incorporated into microsomal membranes by incubation. Taylor et al. (33) proposed that microsomal membrane proteins exchange rapidly with equivalent proteins in the cytoplasm; such an exchange is necessary for explaining how components of the ER membrane turn over rapidly and at different rates.

The mode of transfer of a protein unit from one organelle to another is a much discussed subject. Our findings indicate that lipoglycoprotein units may be involved in such transfers. The autoradiographic and histochemical observations of Bennett et al. (10) suggested the transfer of small morphological units from the Golgi system to the plasma membrane; and Hirano et al. (34) postulated the involvement of a membrane unit in transferring oligosaccharide chains to the plasma membrane. On the basis of experiments with liver and pancreas cells, Palade (35) concluded that in spite of extensive membrane fusions in biological systems there is no evidence for the mixing of lipid and protein components.

Several cytoplasmic glycoproteins could be incorporated into microsomes; but it is difficult to establish the natural state of lipoglycoproteins *in situ*. The protein fraction eluting with the void volume upon chromatography of the supernate on Sephadex G-100 (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) was very actively incorporated into microsomes. Thus, the lipoprotein present in the soluble phase has a mol wt exceeding 150,000. On the other hand, lipoproteins released from Golgi or found in the supernate which could be purified by centrifugation in KBr solutions and incorporated into microsomes contained proteins of smaller molecular weight (70,000 daltons and smaller by SDS-gel electrophoresis). Proteins of these smaller molecular weights were found in the microsomal fraction after incorporation. The most probable explanation for these findings is that the gel electrophoretic procedure dissociated lipoprotein units to yield smaller individual proteins. For these reasons, it is not possible at present to define the size of the lipoprotein that can be incorporated into microsomes and that appears in the membrane as a constitutive component.

The synthesis and transport of microsomal membrane glycoproteins begins with the glycosylation of these proteins in the ER. It was proposed by Eylar that membrane glycoproteins are glycosylated by different transferases than are the serum proteins (36). Recent experiments indicate that, in contrast to serum proteins, membrane glycoproteins newly mannosylated with GDP-mannose, with synthetic dolichol phosphate-mannose, or with dolichol pyrophosphate-oligosaccharide-mannose are localized on the outer surface of the rough and smooth microsomal membranes and can be removed by protease and phospholipase treatment of intact vesicles (37).

In fact, the sialoproteins of the Golgi membranes that were released during sucrose incubation also appear to be localized on the cytoplasmic surface of the isolated Golgi vesicles. These findings suggest that the protein moves in the lateral plane of the ER membrane during its biosynthesis. This suggestion, together with the experiments involving treatment with a French press seem to exclude the possibility that the lipoprotein is moving in the lumen like secretory proteins. The mechanism which regulates the release of the sialolipoprotein from the Golgi into the cytoplasm is not understood at present; but specificity is indicated by the fact that the protein is not effectively incorporated into Golgi membranes or mitochondria.

Conclusive evidence for the pathway described above could be provided by following the movement of labeled proteins. Another possibility would be to find an activity associated with one of the proteins released from Golgi and incorporated into microsomes and to follow this activity. A large number of microsomal enzymes were measured during the various purification steps, but none were associated with specific fractions. However, it is quite possible that membrane enzymes



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are present in the cytoplasm as apo-enzymes lacking prosthetic groups or that lipid-requiring enzymes are inactive until incorporated into the ER membrane. Alternatively, the isolation procedure could inactivate enzymes.

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