ASSOCIATION OF THE TWO GLYCOSYL TRANSFERASE ACTIVITIES OF GLYCOPROTEIN SYNTHESIS WITH LOW EQUILIBRIUM DENSITY SMOOTH MICROSONES

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SUMMARY

Smooth microsomes of rat liver were subfractionated on a continuous sucrose gradient to isopycnic equilibrium density. All the microsomal electron-transport enzymes and phosphatases exhibit a broad distribution in the various microsomal subfractions. On the other hand, the N-acetylglucosaminyl and galactosyl transferase activities, which are known to participate in the synthesis of the oligosaccharide chain of the plasma glycoproteins, exhibit a markedly different distribution pattern. They are concentrated to a small fraction having an isopycnic equilibrium density of 1.10. These membranes represent a separate entity of the smooth microsomal fraction, the exact cellular origin of which remains to be clarified.

INTRODUCTION

The microsomal fraction of the rat liver contains glycoproteins (Patterson & Touster, 1962; Izumi et al. 1963). There appear to be at least two main components: one is tightly attached to the membrane and participates in its structural make-up (Miyajima, Tomikawa, Kawasaki & Yamashina, 1969), and the other consists of plasma proteins for export purposes, which mainly, but not exclusively, are enclosed in the luminal compartment (Spiro, 1959; Robinson, Molnar & Winzler, 1964; Redman & Cherian, 1972). These plasma proteins are synthesized in the liver endoplasmic reticulum and discharged into the blood. The biosynthesis of these export proteins has been thoroughly investigated and it is widely held that the oligosaccharide units of the plasma glycoproteins are assembled by the stepwise transfer of the component monosaccharide residues to the polypeptide chain. The steps involved are not thought to be confined to one subcellular site. This hypothesis is the conclusion of extreme investigations both in vivo and in vitro. Thus it appears that while the 'linkage' glucosamine residue may be attached directly to the ribosome-bound polypeptide (Molnar, Robinson & Winzler, 1965; Lawford & Schachter, 1966), additional hexosamine units are incorporated within the membranes of the rough and smooth endoplasmic reticulum (Spiro & Spiro, 1966; Molnar & Sy, 1967; Wagner & Cynkin, 1969a, b). In addition, radioautographic experiments (Peterson & Leblond, 1964; Neutra & Leblond, 1966) and in vitro studies (Wagner & Cynkin, 1969a, b; Fleischer, Fleischan & Ozawa, 1969; Schachter, Jabbal, Hudgin & Pinteric, 1970) suggest that some terminal sugar residues are incorporated within the Golgi apparatus. A plausible
model of glycoprotein biosynthesis would therefore envision the nascent glycoprotein as passing from ribosomes to the Golgi apparatus via rough and smooth endoplasmic membranes, with individual sugar residues being transferred successively at all sites along the route.

The interest in the subcellular site of glycoprotein synthesis has increased a great deal recently because of the newly established procedures for the isolation of Golgi membranes (Fleischer et al. 1969; Morré et al. 1970). Since certain steps of the synthetic process are concentrated in the smooth microsomal fraction, the possibility arose that these activities are not homogeneously distributed but are connected with specific sections of the smooth membranes. A heterogeneous distribution of various enzymes has in fact been found previously in smooth microsomal subfractions by using a stabilizing gradient (Glaumann & Dallner, 1970).

In the present study smooth microsomes were centrifuged to isopycnic equilibrium on a sucrose gradient and the subfractions were analysed for the activity of 2 glycosyl transferases with parallel correlation for the occurrence of microsomal electron-transport enzymes and phosphatases.

MATERIALS AND METHODS

Rats

Adult male albino rats weighing 150—250 g were used. The animals were starved 20 h before sacrifice.

Fractionation

Rough and smooth microsomes used in the enzyme distribution experiments were prepared as described previously (Bergstrand & Dallner, 1969). The subfractionation of smooth microsomes by density-gradient centrifugation required a large amount of total smooth microsomes, which were therefore prepared in an SW 27 rotor of the Spinco-Beckman ultracentrifuge. The liver was homogenized in 0.25 M sucrose, 2 g weight in 10 ml. Non-microsomal large components were removed by centrifugation at 10,000 g for 20 min. The supernatant was decanted and supplemented with CsCl to a final concentration of 15 mM; 27 ml of this supernatant was layered on 8 ml 1.3 M sucrose—15 mM CsCl in an SW 27 tube. Centrifugation was performed at 95,000 g for 90 min. Total smooth microsomes at the 0.25 M /1.3 M sucrose interface were taken out after removal of the upper 0.25 M sucrose, which was discarded. The smooth microsomes obtained by this procedure contained 5—6 mg protein/g liver, had an RNA:protein ratio of 0.05, and were in non-aggregated form since the suspension completely passed the 0.45-um Millipore filter. The purity and content of the intermediate phase and pellet were not controlled, and so the above procedure should not be used for preparing rough microsomes without additional check.

The total smooth microsomes were subfractionated by density-gradient centrifugation. The microsomal suspension was diluted with an identical volume of 0.05 M sucrose, which was added dropwise with the use of a magnetic stirrer; 6 ml of this suspension were layered on a linear sucrose gradient (Britten & Roberts, 1960) ranging from 0.59 to 1.28 M in a volume of 27 ml. Centrifugation was performed in SW 27 rotor at 67,000 g for 16 h. Fractions were removed by puncturing the bottom of the tube, just above the pellet. For measurement of electron-transport enzymes and phosphatases, the gradient was divided into 11 fractions (8 times 2 ml, 2 times 4 ml, plus all the remainder). These fractions were used directly for measurement of enzyme activities. The pellet was resuspended in 0.25 M sucrose. When the amounts of cytochrome b₅ and P-450 were measured, the liver was perfused with 0.25 M sucrose. For determining the activities of the glycosyl transferases, the gradient was divided into 7 fractions (6 times 4 ml, plus the remainder). The appropriate subfractions from 6 gradients were
Glycoprotein synthesis in smooth microsomes

Fig. 1. Schematic illustration of the subfractionation procedure for total smooth microsomes of rat liver by density gradient fractionation.

collected, made up with distilled water, and centrifuged at 192 000 g for 90 min in a 50-T rotor. All pellets were suspended in 0.05 M Tris-maleate buffer, pH 6.5, in a final volume of 0.5–1 ml. The above fractionation procedure is illustrated in Fig. 1.

Chemical and enzymic measurements

Protein was measured according to Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard.

The activities of NADH- and NADPH-cytochrome c reductase, inosine diphosphatase (IDPase), glucose-6-phosphatase (G6Pase), and ATPase were estimated as before (Dallner, Siekevitz & Palade, 1966). The amounts of cytochrome b5 and P-450 were determined by difference spectroscopy (Dallman, Dallner, Bergstrand & Ernster, 1969).

The transfer of [14C]glycosyl groups to protein was measured as radioactivity precipitated with 5% trichloroacetic acid (TCA). Incubation mixtures contained, in a total volume of 0.2 ml: 1–2.5 mg microsomal proteins, 2 μmol MnCl2, 0.4 μmol EDTA, 9 μmol Tris-maleate buffer, pH 6.5, and, as indicated, 3 nmol (61 000 cpm) UDP-N-acetyl-glucosamine (GlcNAc)-14C, or 4 nmol (53 000 cpm) UDP-galactose (Gal)-14C. Incubations were carried out at 37 °C, and reactions were terminated by the addition of 3 ml cold 5% TCA. TCA precipitates were washed (at room temperature) 3 times with 3 ml 5% TCA, once with 2 ml absolute ethanol, 2 ml ethanol/ether (1:1), and 2 ml ether. Dried precipitates were suspended in 1 ml hydroxide of hyamine and heated at 55–65 °C until dissolved. The digested samples were added to 10 ml toluene-phosphor solution, and radioactivity was determined by liquid scintillation spectrometry. Zero time controls (determined by adding the radioactive sugar nucleotide after TCA precipitation) were subtracted from all values.

RESULTS

Distribution of glycosyl transfer activity in rough and smooth microsomes

Table 1 shows the distribution of N-acetyl-glucosaminyl and galactosyl transfer activities among various subcellular fractions. It must be borne in mind that these activities are dependent upon the simultaneous presence of both transferase enzymes and the appropriate acceptors within a particular preparation, and thus do not necessarily reflect the total transferase activity of the cell or its subfractions.

The smooth microsomal fraction is the most active in catalysing the transfer of both GlcNAc and Gal from their sugar nucleotide precursors to TCA-precipitable endogenous acceptors, accounting for approximately one-quarter of the original activities of the homogenate, in the case of both sugars. The activity of the rough microsomes is comparatively low, the smooth fraction typically displaying 8–14 times the total activities, and 10–23 times the specific activities of the rough. The intermediate
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Table 1. Recovery of GlcNAc- and Gal-transferases, and G6Pase activities in rough and smooth microsomes

Rough and smooth microsomes were prepared as described previously (Bergstrand & Dallner, 1969). The intermediate fraction in the 1.3 M sucrose layer was also recentrifuged in order to estimate total recovery.

<table>
<thead>
<tr>
<th></th>
<th>Homogenate</th>
<th>Rough microsomes</th>
<th>Intermediate fraction</th>
<th>Smooth microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, mg/g liver</td>
<td>204.6</td>
<td>11.2</td>
<td>3.0</td>
<td>4.5</td>
</tr>
<tr>
<td>GlcNAc transferase, cpm/g liver</td>
<td>11915</td>
<td>611</td>
<td>227</td>
<td>2045</td>
</tr>
<tr>
<td>% of homogenate</td>
<td>100</td>
<td>27.2</td>
<td>22.7</td>
<td>2267</td>
</tr>
<tr>
<td>Gal transferase, cpm/g liver</td>
<td>9300</td>
<td>272</td>
<td>227</td>
<td>2267</td>
</tr>
<tr>
<td>% of homogenate</td>
<td>100</td>
<td>29.3</td>
<td>24.3</td>
<td></td>
</tr>
<tr>
<td>G6Pase, μmol Pi/20 min/g liver</td>
<td>198.1</td>
<td>71.6</td>
<td>51.1</td>
<td>40.4</td>
</tr>
<tr>
<td>% of homogenate</td>
<td>100</td>
<td>36.1</td>
<td>26.6</td>
<td>20.4</td>
</tr>
</tbody>
</table>

Fig. 2. Time course of GlcNAc-transferase activity of total smooth microsomes.

fraction contained only a minor portion of the total activities comparable to that present in the rough, and the activities of the 100,000 g supernatant fraction were undetectable.

It can also be seen from Table 1 that the rough and smooth microsomal fractions account for about 55% of the original G6Pase activity of the homogenate. The corresponding activity of the intermediate and supernatant fractions is negligible.

Fig. 2 shows the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to TCA-insoluble material as a function of time. The activity is seen to level off relatively rapidly, perhaps indicative of exhaustion of endogenous acceptors, or of sugar nucleotide, or both.
Glycoprotein synthesis in smooth microsomes

Fig. 3. Distribution of smooth microsomal protein at isopycnic equilibrium density. For further details see Materials and Methods. $p =$ pellet, fraction 7 = top of the gradient. The numbers above the figure denote the median equilibrium density of the appropriate subfractions.

Distribution of protein on the density gradient

Total smooth microsomes, isolated on a CsCl-containing discontinuous sucrose gradient, display a broad distribution after density-gradient equilibration (Fig. 3). Utilizing the gradient and the centrifugation conditions described in Materials and Methods, 16 h of centrifugation are required to reach the equilibrium. Only a small part of the protein is localized in the 1.08–1.12 density region, and the large majority of the protein is found in the 1.13–1.18 density region. This distribution pattern is in agreement with previous investigations (Rothschild, 1963; Glaumann & Dallner, 1970).

Electron transport enzymes in the subfractions

In an earlier study, using zone centrifugation procedure, electron-transport enzymes were found to be enriched in the low sedimentation velocity smooth microsomes (Glaumann & Dallner, 1970). The distribution pattern after density-gradient centrifugation, however, is very different (Fig. 4). The activity or amount of electron-transport enzymes is similar in all subfractions with the exception of the upper part. The low-protein-containing top fractions are very poor regarding these enzymes. In spite of the fact that both cytochrome $c$ reductases have a broad distribution pattern, the fractions which exhibit the highest specific activities are slightly separated: the vesicles richest in NADH-cytochrome $c$ reductase activity have low density, while the NADPH-cytochrome $c$ reductase activity enriched subfractions are in the higher density region. Although the distribution of cytochrome $b_5$ follows the distribution of its functional flavoprotein counterpart (NADH-cytochrome $c$ reductase), the
Fig. 4. Distribution of electron-transport enzymes in smooth microsomes at isopycnic equilibrium density. The plot is made in the manner adapted by de Duve et al. (1955). Relative protein content of fractions is shown on the abscissa and relative specific activity on the ordinate. The column at the far left represents the pellet. The next corresponds to fraction 1, deriving from the lowest part of the gradient, since it was removed first by puncturing the bottom of the tube. In this way, the fractions to the right originate from the upper parts of the gradient. The values for the individual fractions represent the mean values of 6 experiments.

Fig. 5. Distribution of phosphatases in smooth microsomes at isopycnic equilibrium density. Distribution as presented in Fig. 4.
Glycoprotein synthesis in smooth microsomes

Fig. 6. Distribution of glycosyl transferase activities in smooth microsomes at isopycnic equilibrium density. Distribution as presented in Fig. 4.

cytochrome P-450 content of the subfractions is slightly different from the activity distribution of the NADPH-cytochrome c reductase.

Phosphatases in the subfractions

As with the electron transport enzymes, the recovery of the three microsomal phosphatase activities (ATPase, G6Pase, and IDPase) was high, or around 90% (Fig. 5).

There is no characteristic distribution pattern for any one of the 3 phosphatases. They appear in all subfractions, but G6Pase and IDPase activities display significant tendencies to be lower both in the pellet and in the upper part of the gradient.

Glycosyl transfer activity of sucrose gradient fractions

\(N\)-acetylglucosaminyl and galactosyl transferase activities exhibit a distribution on the equilibrium density gradient which is in marked contrast to that of the other enzymes studied (Fig. 6). Thus both glycosyl transfer activities are recovered predominantly in fraction 6, while the pellet and fractions 1-4 are almost completely devoid of these activities. This is particularly noteworthy in view of the fact that the fractions characterized by high transferase activities have an extremely low protein content compared to the fractions with low activity. The \(N\)-acetylglucosaminyl transferase activity of fraction 6 is enriched 18-fold in comparison to total smooth microsomes, and the galactosyl transferase of this fraction is enriched 8-fold.

Values observed for the glycosyl transfer activities of both the subcellular and gradient fractions might not be valid if destruction of the sugar nucleotide substrates were to occur to a greater extent in one fraction than in another. Such a degradation of sugar nucleotides in subcellular glycosyl transfer systems has been observed (Wagner & Cynkin, 1971), and is presumably due to pyrophosphatase enzymes. This possibility was examined in the case of UDP-GlcNAc, and the results are presented in Tables 2 and 3. It will be seen that sufficient percentages of the original substrates
Table 2. Amount of substrate left after incubation of rough and smooth microsomes for the GlcNAc-transferase reaction

Aliquots of incubation mixtures were withdrawn at various intervals and subjected to chromatography under conditions which separate GlcNAc-1-P from the intact sugar nucleotide. The solvent system employed (Roseman, Distler, Moffatt & Khorana, 1961) was ethanol:0.5 M ammonium acetate, pH 3.9 (5:2). The samples were applied to 25-cm wide strips of Whatman 3 MM chromatography paper, and after drying were overspotted with 0.3 μmol of non-radioactive UDP-GlcNAc. After subjecting to descending chromatography overnight the chromatograms were dried, the u.v.-absorbing areas were cut out, and their radioactivity was measured in 10 ml of Bray's solution (Bray, 1960). (Attempts to separate UDP-Gal from Gal-1-P by similar methods were unsuccessful.)

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>Incubation time, min</th>
<th>UDP-GlcNAc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cpm</td>
</tr>
<tr>
<td>Homogenate</td>
<td>0</td>
<td>4139</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2272</td>
</tr>
<tr>
<td></td>
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<td>Rough microsomes</td>
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<td>1471</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>495</td>
</tr>
<tr>
<td>Intermediate fraction</td>
<td>0</td>
<td>1573</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>375</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>150</td>
</tr>
<tr>
<td>Smooth microsomes</td>
<td>0</td>
<td>1770</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>258</td>
</tr>
</tbody>
</table>

The data in this paper demonstrate that the high capacity of smooth microsomes in rat liver to participate in certain steps of glycoprotein biosynthesis is concentrated to a very limited number of membranes characterized by low equilibrium density. Glucosamine and galactose are transferred from their sugar nucleotide derivatives to TCA-precipitable endogenous acceptors within subcellular fractions obtained from rat liver. Considerably greater percentages of both of these activities are found associated with the smooth rather than the rough microsomal fraction, and they are

DISCUSSION

The data in this paper demonstrate that the high capacity of smooth microsomes in rat liver to participate in certain steps of glycoprotein biosynthesis is concentrated to a very limited number of membranes characterized by low equilibrium density. Glucosamine and galactose are transferred from their sugar nucleotide derivatives to TCA-precipitable endogenous acceptors within subcellular fractions obtained from rat liver. Considerably greater percentages of both of these activities are found associated with the smooth rather than the rough microsomal fraction, and they are
Table 3. *Amount of substrate left after incubation of smooth microsomal subfractions for the GlcNAc-transferase reaction*

Percentages of UDP-GlcNAc remaining after various periods of incubation determined as in legend to Table 2.

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>Incubation time, min</th>
<th>UDP-GlcNAc cpm</th>
<th>% of total</th>
</tr>
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<tbody>
<tr>
<td>Total smooth microsomes</td>
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<td>3262</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>664</td>
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<td></td>
<td>15</td>
<td>194</td>
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<td>Pellet</td>
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<tr>
<td></td>
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<td></td>
<td>15</td>
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<td>Fraction 1</td>
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<tr>
<td></td>
<td>15</td>
<td>538</td>
<td>11.1</td>
</tr>
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</table>

more enriched within the fraction. These results are in close agreement with those of several previous studies (Lawford & Schachter, 1966; Spiro & Spiro, 1966; Molnar & Sy, 1967; Wagner & Cynkin, 1969a, b). The distributions, recoveries, and enrichments of glycosyl transferase activities observed are believed to be quantitatively valid, since paper chromatographic analysis of incubation mixtures revealed that at least some substrate remained in all fractions over the interval examined, although destruction of sugar nucleotide, presumably due to pyrophosphatase activity (Wagner & Cynkin, 1971), did occur.

When smooth microsomes were applied to a sucrose density gradient and centrifuged to equilibrium, a marked enrichment and apparent concentration of N-acetylglucosaminyl and galactosyl transfer activities were observed in the fractions collected.
from the upper part of the gradient. This distribution is in sharp contrast to that of other enzymes studied, i.e. electron-transport enzymes and phosphatases, which exhibited a broad distribution but were virtually absent in fraction 6. While the enrichment of glycosyl transferase activities is indeed noteworthy (in the case of glucosamine 18-fold, and in the case of galactose 8-fold over those of total smooth microsomes), the apparent distribution of these activities should be interpreted with some caution. Thus, destruction of UDP-GlcNAc was observed in the fractions having low glycosyl transfer activity, while destruction was only slight in the fractions characterized by high transfer activities. Nevertheless, the recoveries of the activities were relatively high (65–80% of the glucosaminyl transferase, and 70–80% of the galactosyl transferase), which speaks against artifacts of distribution pattern.

The results of the present study are in agreement with the hypothesis of a stepwise completion of glycoproteins during their transport in the endoplasmic reticulum, and further suggest that there may be specific sites within the smooth microsomal fraction, capable of being isolated by density gradient fractionation, which transfer one or more sugars to glycoprotein acceptors. The exact subcellular sites of the various steps involved in glycoprotein biosynthesis are yet to be elucidated, progress being hampered by various limitations inherent in the approaches so far applied. Thus, in vivo tracer and radioautographic studies may not be able to distinguish between actual sites of biosynthesis and transport of completed product. This is largely because the various plasma glycoproteins may have one sugar present at several distinct locations in the oligosaccharide chain, and a given sugar may be in any one of several different configurational or positional linkages.

In vitro studies may also give misleading results if important but often overlooked considerations are not taken into account. One of these, already mentioned, is the presence of antagonistic enzymic activities (such as that of pyrophosphatase). If such activities were sufficiently higher in one fraction than in another, the distribution of glycosyl transferase activity between the two could be only apparent. Another limitation involved in in vitro methods of analysis is encountered when transferase activities are measured with endogenous acceptors. If an acceptor was to become separated from the transferase enzyme during the course of isolation, the actual glycosyl transfer may appear to be of lesser extent, or even be absent entirely. This difficulty has been circumvented in some cases by measuring glycosyl transfer to exogenous glycoprotein acceptors (Schachter et al. 1970).

When reasoning in the above way, one has to be aware that the method utilized in these experiments does not measure the enzyme itself but determines the transferase activity, e.g. requires both the enzyme and the acceptor. The situation is analogous to what is occurring in the analysis of many microsomal enzyme activities (NADH-cytochrome reductase, oxidative demethylation) and mitochondrial enzyme activities (NADH-coenzyme Q reductase, succinate oxidase). Therefore, it cannot be excluded that the low activity in a subfraction may be partly explained by the limitation of the available endogenous acceptor rather than by the absence of the transferase enzyme itself. In this case, most of the subfractions may contain glycosyl transferases but not the sugar acceptor. Low-density membranes would thus participate in the oligo-
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saccharide biosynthesis on the basis of their capacity to bind acceptor, obligatory to
the establishment of the enzyme-acceptor complex.

Intensive work on the isolation of the Golgi system from liver (Fleischer et al. 1969;
Morré et al. 1970) aroused great interest, and within a short time numerous papers
were published on the chemical and enzymic composition of this subcellular organelle.
The main result of this effort was the finding that some of the glycosyl transferases are
highly concentrated in this fraction (Wagner & Cynkin, 1969b; Fleisher et al. 1969;

The median equilibrium density was found to be 1.13, but the chemical and
enzymic composition of this fraction is not clear mainly because of the non-competitive
findings of the various authors. Contamination by other cytoplasmic membranes in
the Golgi fraction, as isolated so far, may influence the results. The specific activities
of glycosyl transferases in fraction 6 are at the levels found in the isolated Golgi
fraction. Fraction 6 is also poor in electron transport enzymes. It is therefore possible
that the low-density membranes, as isolated in our density-gradient experiments,
are not simply a part of the smooth endoplasmic reticulum but are purified Golgi
membranes. The median equilibrium density of vesicles in fraction 6 is, however,
significantly lower than that given for the isolated Golgi membranes.

One cannot exclude the possibility that the isolation procedure employed altered
the density of the individual vesicles or membranes in the upper part of the gradient,
which are not identical with those of higher density isolated previously. In the isolation
procedures described so far, only a few per cent of the total Golgi fraction are re-
covered. Judging from the total glycosyl-transferase activity of the homogenate, it is
conceivable that the Golgi system, like the endoplasmic reticulum, consists of a
collection of heterogeneous membrane elements. In electron-microscopical pictures
(Palade, Sickevitz & Caro, 1962), and particularly in pictures after electron-micro-
scopic autoradiography (Jamieson & Palade, 1967), the morphological and functional
heterogeneity of the Golgi complex is evident, and one can differentiate at least 2
components: peripheral small vacuoles and large elongated cisternae. If the mem-
branes rich in transferase activity represent the Golgi system, the amount present in
total smooth microsomes is about 0.1 mg protein/g liver. In this case, the smooth
microsomes from our preparation procedure would contain about 3% Golgi elements.

In the experiments described here, the various electron-transport enzymes and
phosphatases exhibit a broad distribution pattern and are lacking only in membranes
localized at the very top of the gradient. This pattern is very different from that
taken by smooth microsomes when subfractionated by zone centrifugation on a
stabilizing sucrose gradient (Glaumann & Dallner, 1970). With that procedure, 3
separate peaks could be differentiated: one for electron-transport enzymes, one for
G6Pase, and one for ATPase. Zone centrifugation separates particles according to
their sedimentation velocity, where size and, indirectly, permeability are the decisive
factors and particle density is of no importance. In density-gradient centrifugation,
the final position of the particle is determined by its isopycnic equilibrium density.
Since the microsomal membrane after centrifugation is completely permeable to
sucrose (Nilsson, Pettersson & Dallner, 1971), the density of a vesicle will be given
only by the density of the membrane itself including the hydration water. It is obvious that the 2 physicochemical properties, density and size distribution, bear no relationship to each other in the various smooth microsomal vesicles under the conditions employed.

It can be concluded that there is a pronounced heterogeneity among smooth membranes as regards equilibrium density, possibly caused by a difference in membrane composition and/or structural arrangement. The recognized heterogeneous distribution of most microsomal enzymes in the smooth microsomal fraction is not apparent after density-gradient centrifugation except for a limited number of membranes, those with the lowest density, in which a concentration of the glycosyl transferase activities occurs. The data available are not sufficient to decide whether these membranes originate from the smooth endoplasmic reticulum or derive from another subcellular organelle.

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REFERENCES


Glycoprotein synthesis in smooth microsomes


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