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ISOLATION OF GOLGI FRACTIONS FROM COLCHICINE-TREATED RAT LIVER

I. MORPHOLOGICAL AND ENZYMIC CHARACTERIZATION

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Summary

1. Three Golgi fractions, GF-1, GF-2 and GF-3, were isolated from the livers of rats pretreated with colchicine, which gave better yields of the fractions than ethanol treatment of rats.

2. Electron microscopic observation showed that GF-1 was composed mainly of secretory vesicles, GF-3 consisted predominantly of small tubules and flattened cisternae, and GF-2 was an intermediate fraction composed of secretory vesicles and cisternal elements.

3. Among these three fractions the highest activity of galactosyl transferase, marker enzyme of the Golgi complex, was found in GF-3 and the lowest activity was in GF-1, although a different distribution of the enzymes was observed in fractions obtained from ethanol-treated rat liver.

4. Enzymatic characterization of these fractions showed that no significant contamination with other subcellular components occurred in GF-1 and GF-2.

Introduction

The Golgi complex is composed of morphologically heterogeneous elements; stacks of lamellae (cisternae) and associated vesicles and vacuoles. In

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Abbreviations used: SDS, sodium dodecyl sulphate; VLDL, very low density lipoprotein. In the Golgi fraction, $\rho < 1.006$ g/ml (Mahley, R.W., Bersot, T.P., LeQuire, V.S., Levy, R.I., Windmueller, H.G. and Brown, W.V. (1970) Science 168, 380—382); otherwise ρ ñ 0.94—1.006 g/ml (Lloyd, J.K. and Forsbrooke, A.S. (1974) in Structure and Function of Plasma Proteins (Allison, A.S., ed.), Vol. 1, pp. 1—33, Plenum Press, New York-London).

hepatocytes secretory vesicles are morphologically characterized by numerous VLDL particles enclosed in them and their location in a cell can be easily traced by electron microscopy. Recently the biosynthesis and secretion of VLDL have been extensively studied [1—5]. Secretory vesicles are often found in the proximity of one side of the Golgi stack (the maturing or trans side). They migrate from the Golgi area to the sinusoidal face of the plasma membrane and finally fuse with it to discharge their contents such as VLDL [6—8] and albumin [9,10].

In spite of the above evidence revealed by morphological observation, the biochemical and molecular mechanisms underlying the formation of the secretory vesicles, directional movement and fusion with plasma membranes have yet to be determined. In addition to these problems associated with secretion, the fate of the assembled membrane is also interesting in regard to the formation of plasma membranes [11]. For a biochemical approach to these problems, it seems prerequisite to isolate the secretory vesicles separated from other subcellular components including the Golgi cisternae.

Isolation of the Golgi complex from rat liver has been reported independently by Morré et al. [12], Fleischer et al. [13] and Leelavathi et al. [14]. However, these methods could not be directly applied to the preparation of the secretory vesicles, since the fractions obtained by these methods contain all the Golgi elements in various proportions.

Recently, separation from ethanol-treated rat liver of a fraction rich in secretory vesicles has been reported by Merritt and Morré [15] and by Ehrenreich et al. [16]. In these studies advantage was taken of the fact that ethanol administration enhances the synthesis of VLDL and results in an increase of the secretory vesicles in a hepatocyte. Secretory vesicle-rich fractions obtained by the two laboratories were shown to be morphologically homogeneous. However, they reported conflicting results concerning the distribution of galactosyltransferase among the Golgi elements. Furthermore, data presented so far are certainly not enough to characterize the biochemical feature of the secretory vesicles.

In the present study we attempted to isolate the secretory vesicles with other Golgi components from colchicine-treated rat livers in order to characterize these elements, especially with respect to galactosyltransferase activity.

Materials and Methods

Materials. The reagents used were obtained from the following sources: colchicine and β-glycerophosphate from E. Merck, Darmstadt, F.R.G.; 5′-AMP, from Boehringer GmbH., Mannheim, F.R.G.; glucose-6-phosphate, *p*-nitrophenyl-β-*N*-acetylglucosamide and UDP-galactose, from Sigma Chemical Co., St. Louis, MO; NADH and NADPH, from Oriental Yeast Co., Tokyo; dl-α-tocopherol, from Esai Co., Tokyo; UDP-[^{14}C]galactose, from New England Nuclear, Boston, MA; all other chemicals were reagent grade and of commercial origin.

Isolation of three hepatic Golgi fractions from rats treated with colchicine or ethanol. Male Sprague-Dawley rats (300—400 g) were used. Rats were starved overnight and colchicine (0.05 mg/100 g body weight) was intraperitoneally

injected twice, at 180 and 90 min before killing [7]. The isolation procedure of Ehrenreich et al. [16] was used with some modifications as follows: (a) livers were crushed through stainless steel meshes (100 meshes; Iida Seisakusho, Osaka, Japan) [17], suspended in 0.25 M sucrose and homogenized in a Potter-Elvehjem homogenizer by hand. The resultant 20% homogenate was centrifuged at 10 000 × *g* for 10 min. (b) An additional 1.15 M sucrose layer was inserted between the microsomal suspension and 0.86 M sucrose layer as described by Redman et al. [10] and centrifuged at 73 000 × *g* for 180 min in a Spinco SW 25.2 rotor. Three fractions (GF-1, GF-2 and GF-3, floating at the interfaces of 0.25/0.6 M, 0.6/0.8 M and 0.86/1.15 M sucrose, respectively) were collected by pipette, diluted with cold water to give a final sucrose concentration of 0.25 M and centrifuged at 105 000 × *g* for 60 min. In the case of ethanol treatment, 1.5 ml of 50% ethanol solution per 100 g body weight was orally administered before sacrifice. In the combination study rats were treated with both colchicine and ethanol as described above.

Isolation of hepatic Golgi fractions from non-treated rats. Isolation was carried out according to the method of Tsuji et al. [18] with a slight modification described below. In the second sucrose density gradient centrifugation, 0.25 M sucrose was layered on the top of the gradient instead of the 28% (w/w) sucrose originally used. The band floating between 0.25 M and 32% (w/w) sucrose layers is referred to as the total Golgi fraction (GF-T). In order to separate the Golgi cisternae from the secretory vesicles, the third sucrose density centrifugation was performed using the system of Ehrenreich et al. [16]. The collected total Golgi fraction (GF-T) was adjusted to 1.15 M sucrose concentration with 2.1 M sucrose, overlaid with 0.86, 0.6 and 0.25 M sucrose layers and then spun at 73 000 × *g* for 180 min in a Spinco SW 25.2 rotor. The two bands floating at the 0.6/0.86 M and 0.86/1.15 M sucrose interfaces were separately collected, diluted to a sucrose concentration of 0.25 M, and centrifuged at 20 000 × *g*, these are referred to as the Golgi-light (GF-L) and Golgi-heavy (GF-H) fractions, respectively.

Osmotic shock treatment and washing with 0.1 M KCl. Each Golgi fraction was suspended in 0.025 M sucrose, allowed to stand in ice for 30 min and centrifuged at 105 000 × *g* for 60 min. Each pellet was suspended in 0.1 M KCl with a small glass homogenizer and centrifuged again. After the second supernatant was removed, the resultant pellet was resuspended in 0.25 M sucrose. Since β-glucuronidase in the Golgi fraction was unstable, dl-α-tocopherol was added to the freshly prepared Golgi fraction (50 μM).

Enzyme assays. Galactosyltransferase was assayed with *N*-acetyl-glucosamine as acceptor according to the method of Morré et al. [19] with a slight modification [20]. The following enzyme activities were determined as previously described: β-glucuronidase [21], β-*N*-acetyl-glucosaminidase [22], acid phosphatase with *p*-nitrophenylphosphate [23] or β-glycerophosphate [24] as substrate, glucose-6-phosphatase [25], 5′-nucleotidase [26], NADH-cytochrome *c* reductase [27], NADH-ferricyanide reductase [28], NADPH-cytochrome *c* reductase [29]. Inorganic phosphate was determined by the method of Fiske and SubbaRow [30]. Glucose-6-phosphatase and β-glucuronidase were assayed immediately after the isolation of the Golgi fractions. Other enzymes were assayed within 20 h after the isolation, during which no significant loss of activ-

ity was observed. All samples were kept at 0—4° C. Protein was determined by the method of Lowry et al. [31] using bovine serum albumin as the standard.

Electron microscopy. Each Golgi fraction was fixed, dehydrated and stained according to Ehrenreich et al. [16]. Entire depth of pellet specimens was examined in a Nihon Denshi JEM 100B electron microscope.

Results

Morphological observation of the Golgi fractions isolated from colchicine-treated rat liver

Since no marker enzyme to discriminate the secretory vesicles from the Golgi cisternae is known, only morphological observation can give a criteria of purity of the fractions. VLDL-particles enclosed in the Golgi elements are known to be reliable marker for the secretory vesicles [16]. As shown in Fig. 1a, GF-1 was composed of various size of vacuoles containing numerous electron dense particles. Almost the entire depth of the pellet prepared for electron microscopy had a similar appearance to Fig. 1a. The upper part (Fig. 1b) and lower part (Fig. 1c) of the pellet of GF-2 had different appearances showing that the upper part consisted of almost the same components as GF-1, while the lower part contained many Golgi cisternae as well as the secretory vesicles. Thus, GF-2 is a heterogeneous fraction containing both secretory vesicles and Golgi cisternae. GF-3 was composed of Golgi cisternae, tubules and small vesicles (Fig. 1d). Only a small number of the secretory vesicles were trapped in this fraction. The electron microscopic appearance described above is in good agreement with that reported by Ehrenreich et al. [16] or Redman et al. [10], and no significant differences could be found between the Golgi fractions from colchicine- and ethanol-treated rat livers.

Yields of the Golgi fractions

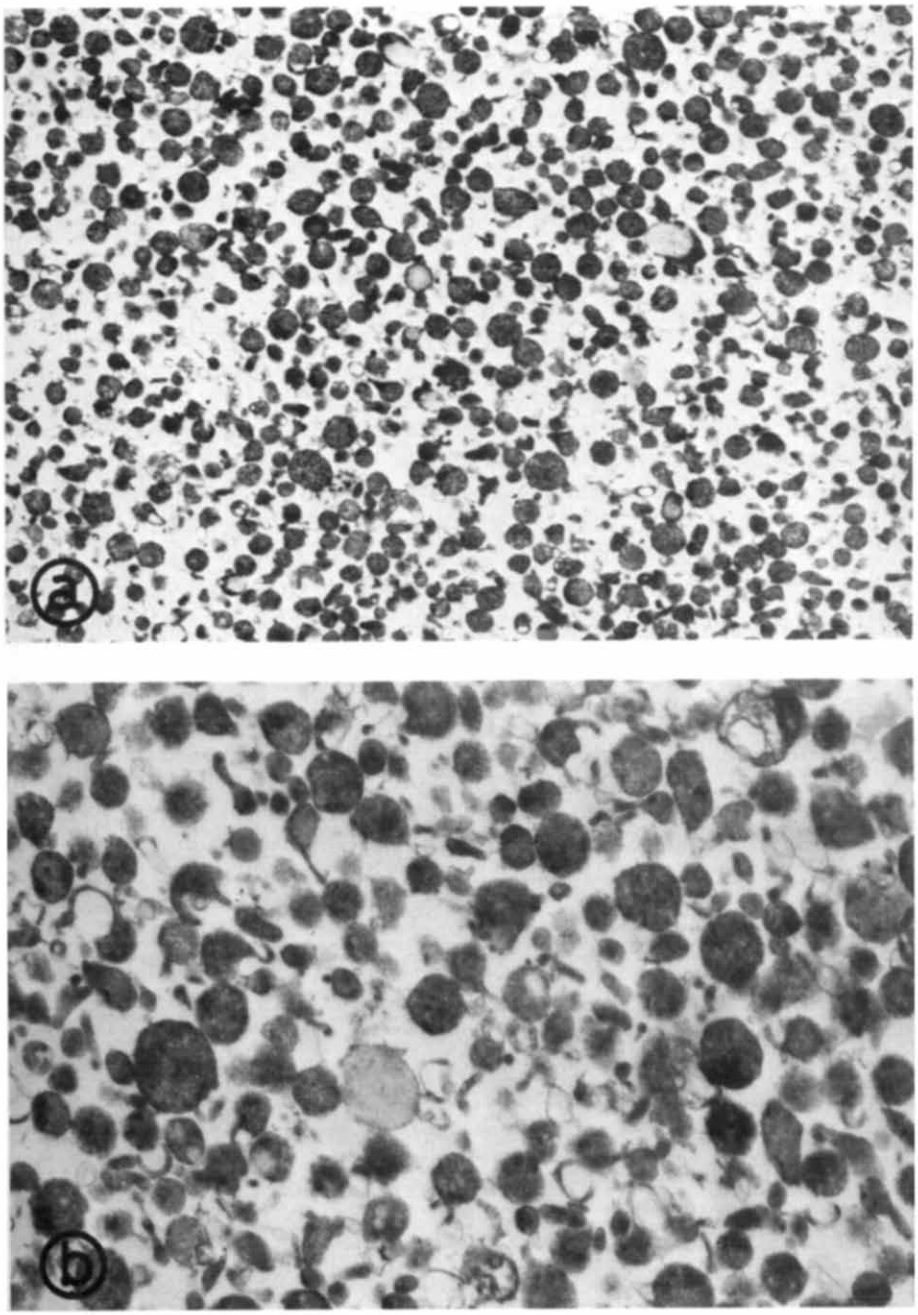
Table I shows the yields of Golgi fractions of livers from 4 groups of rats with or without pretreatments. Ethanol administration caused marked increase of the yields of both GF-1 and GF-2, as compared with those from control liver. Pretreatment with colchicine increased more effectively the yields of all three fractions. A much better yield of GF-1 was obtained by the combined use of ethanol and colchicine, as would be expected from the different mechanisms

TABLE I

YIELDS OF THE GOLGI FRACTIONS ISOLATED FROM CONTROL AND TREATED RAT LIVERS (mg PROTEIN/g LIVER)

Values in parentheses represent the number of experiments.

Fraction	Treatments of rat			
	None	Ethanol	Colchicine	Colchicine + ethanol
GF-1	0.014 (2)	0.07 ± 0.007 (4)	0.11 ± 0.013 (8)	0.16 ± 0.029 (10)
GF-2	0.067 (2)	0.13 + 0.019 (4)	0.21 ± 0.030 (8)	0.21 ± 0.044 (9)
GF-3	0.220 (2)	0.20 ± 0.037 (4)	0.31 ± 0.057 (8)	0.36 ± 0.066 (10)



of effects of the two drugs, stimulation of VLDL synthesis [16,33] and inhibition of its secretion [6–9], respectively.

Galactosyltransferase activity of the Golgi fractions isolated from colchicine- and ethanol-treated rat livers

Galactosyltransferase is now well accepted as a marker enzyme for the Golgi

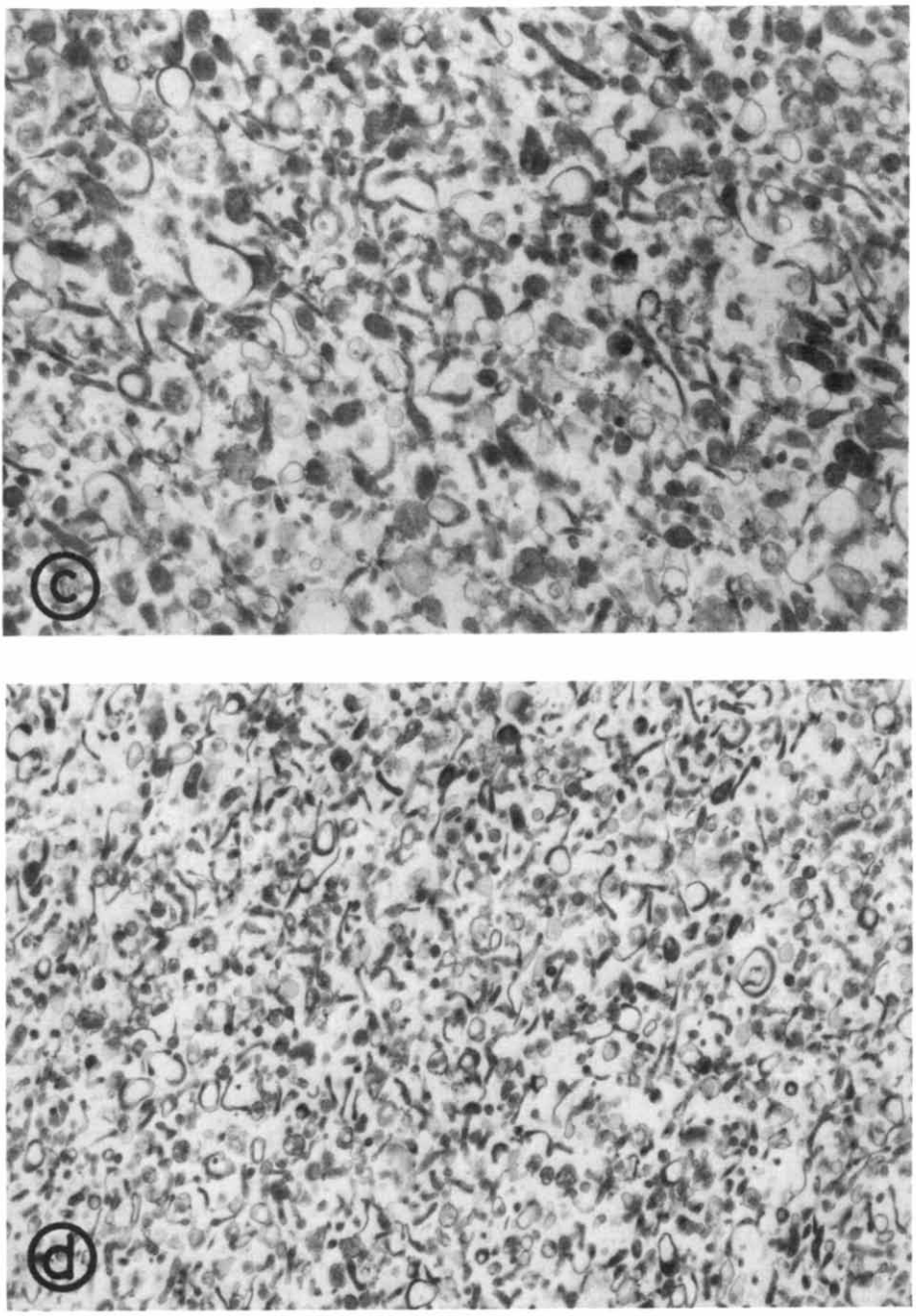


Fig. 1. Electron micrographs of the Golgi fractions isolated from the colchicine-treated rat liver. (a) GF-1, middle part of the sectioned pellet (X10 000); (b) GF-2, upper part of the sectioned pellet (X20 000); (c) GF-2, lower part of the sectioned pellet (X20 000) and (d) GF-3, middle part of the sectioned pellet (X20 000).

TABLE II

SPECIFIC ACTIVITY OF GALACTOSYLTRANSFERASE IN HOMOGENATE AND GOLGI FRACTIONS ISOLATED FROM TREATED RAT LIVER

Values are expressed as specific activity of galactosyltransferase (nmol/h/mg protein) with *N*-acetylglucosamine as acceptor. Values in parentheses represent the percentages of galactosyltransferase activity in the Golgi fractions recovered from the whole homogenate activity in each group.

Fraction	Treatment of rat		
	Ethanol	Colchicine	Colchicine + ethanol
Homogenate	6.0 ± 0.7 (100%)	6.1 ± 1.1 (100%)	5.5 ± 0.8 (100%)
GF-1	263 ± 7.7 (2.4)	83 ± 20 (1.4)	92 ± 34 (2.0)
GF-2	243 ± 32 (3.5)	103 ± 19 (2.8)	136 ± 23 (3.5)
GF-3	187 ± 52 (4.5)	174 ± 52 (6.0)	204 ± 50 (8.2)
Number of experiment	3	6	6

complex owing to its high specific activity and high recovery in the Golgi fraction [32,34]. Since this enzyme is involved in glycosylation of secretory proteins, it is very interesting to ascertain whether this enzyme is localized in a specific locus of the heterogeneous Golgi complex, implying that there exists functional polarity or differentiation among morphologically different components. From this point of view we examined the distribution of galactosyltransferase activity among the Golgi fractions prepared from ethanol- and colchicine-treated rat livers (Table II). Among the Golgi fractions from ethanol-treated rat livers, fraction GF-1 had the highest specific activity of the enzyme and GF-3 had the lowest activity. These results are contrary to those reported by Bergeron et al. [32], but compatible with those obtained by Merritt and Morré [15]. In the case of colchicine-treated rat livers, however, the highest specific activity was found in GF-3 and lowest in GF-1. Furthermore, in the combined treatments with ethanol and colchicine we obtained a similar distribution of the enzyme to that obtained with colchicine alone. At present we cannot explain the reason for the discrepancy between the results obtained from two different treatments.

Light and heavy Golgi fractions from non-treated rat livers

Two fractions, GF-L and GF-H, which correspond in density to GF-2 and GF-3, respectively, were obtained from non-treated rat livers. Morphological profiles of GF-L and GF-H are shown in Fig. 2. Fig. 2a shows that GF-L is mostly composed of secretory vesicles. Fig. 2b shows GF-H, in which many typical Golgi cisternae are observed instead of secretory vesicles. Table III shows the distribution of galactosyltransferase activity between GF-L and GF-H. The specific activity of GF-T increased more than 70-fold over that of the homogenate and about 90% of its activity was recovered in GF-H, whose specific activity increased more than 110-fold compared with that of the homogenate. In contrast, the specific activity of GF-L was only 30-fold above that of the homogenate. This result, together with morphological findings, strongly suggests that galactosyl transferase is mainly localized in the Golgi cisternae. The polarized localization of galactosyltransferase between the Golgi

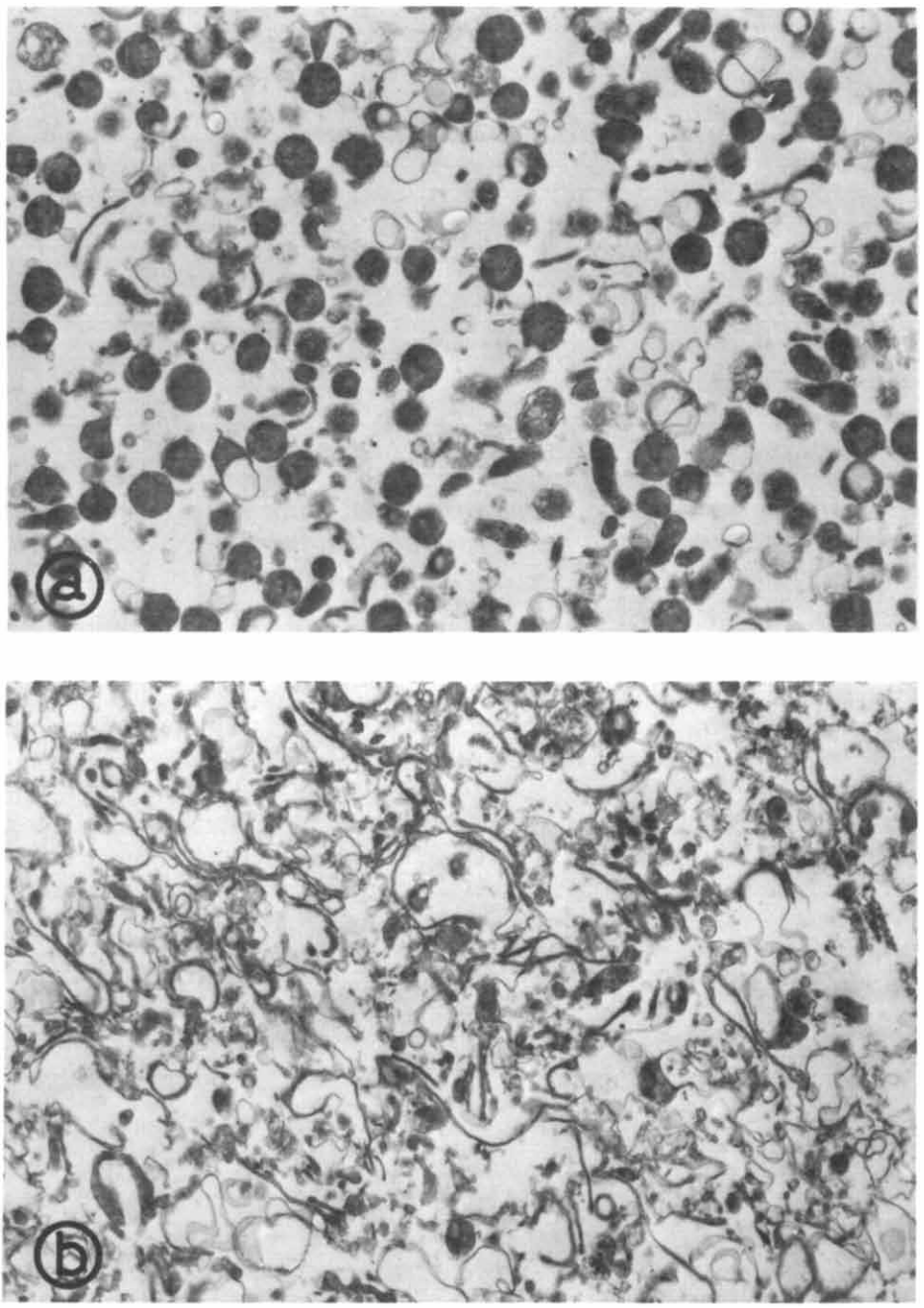


Fig. 2. Electron micrographs of the Golgi fractions isolated from non-treated rat liver. (a) Golgi-light fraction (X20 000); (b) Golgi-heavy fraction (X20 000).

cisternae and the secretory vesicles accords with the result obtained from colchicine-treated rat livers, indicating that colchicine does not affect the distribution of this enzyme and confirming the validity of this drug for the preparation of the secretory vesicle-rich fraction.

TABLE III

DISTRIBUTION OF GALACTOSYLTRANSFERASE ACTIVITY AMONG THE GOLGI FRACTIONS ISOLATED FROM NON-TREATED RAT LIVER

Values are expressed as specific activity (nmol/h/mg protein) with *N*-acetylglucosamine as acceptor. The recoveries of galactosyltransferase in the Golgi-total fraction was 15—25% of the whole homogenate activity. Values in parentheses represent the percent distribution of activity in the Golgi subfractions (Golgi-light and Golgi-heavy) recovered from the Golgi-total fraction.

Experiment	Specific activity of galactosyltransferase			
	Homogenate	Golgi-total	Golgi-light	Golgi-heavy
1	6.1	477	122 (5.7%)	688 (94.3%)
2	4.1	474	145 (14.0%)	630 (86.0%)
3	4.7	424	159 (6.7%)	640 (93.3%)

Other enzyme activities in the Golgi fractions from colchicine-treated rat livers

To estimate the purity of the Golgi fractions, activities of several enzymes known as markers specific for other subcellular structures were determined, as shown in Table IV. 5′-Nucleotidase activity associated with GF-1 and GF-2 was relatively low, while GF-3 showed considerably high activity. 5′-Nucleotidase has been used as a marker for plasma membranes, though Widnell reported the presence of this enzyme also in the endoplasmic reticulum [35]. Furthermore cytochemical study by Farquahr et al. [36] revealed that 5′-nucleotidase is indigenous to the secretory vesicles as well as the Golgi cisternae. Thus the low activity detected in GF-1 and GF-2 are probably due to indigenous enzyme. However, considerably high activity of 5′-nucleotidase in GF-3 seems to be partly due to a contamination by plasma membranes, because the activity found in GF-H, the typical Golgi cisterna fraction, was 100—120 U/mg protein.

TABLE IV

ENZYMATIC CHARACTERIZATION OF THE GOLGI FRACTIONS ISOLATED FROM COLCHICINE-TREATED RAT LIVER

Values are expressed as nmol of substrate catalyzed/min/mg protein ± standard errors. Each value was obtained from 4 experiments. n.d., not detectable.

Enzyme	Specific activity			
	GF-1	GF-2	GF-3	
5′-Nucleotidase	84.3 ± 6.8	146 ± 12.7	361 ± 30.0	(1200) [a]
Glucose-6-phosphatase	n.d.	10.8 ± 2.8	62.7 ± 9.4	(250) [b]
NADH-cytochrome *c* reductase	27.5 ± 9.0	74.8 ± 21.2	450 ± 135	(1730) [b]
NADPH-cytochrome *c* reductase	3.8 ± 1.8	4.2 ± 1.8	10.2 ± 2.7	(78.5) [b]
NADH-ferricyanide reductase	407 ± 67	695 ± 69	1320 ± 50	(3350) [b]
Acid phosphatase				
p-nitrophenylphosphate	64.8 ± 6.5	109 ± 21.5	151 ± 38.1	(2648) [c]
β-glycerophosphate	38.7 ± 3.1	68.9 ± 6.1	46.9 ± 7.7	—
β-Glucuronidase	13.1 ± 2.9	10.7 ± 0.4	6.8 ± 2.1	(236) [c]
β-*N*-Acetylglucosaminidase	22.6 ± 5.0	39.8 ± 4.2	24.4 ± 1.9	(689) [c]

Values in the parentheses at the right column represent the specific activities of the following fractions for which the respective enzymes are used as marker; a, plasma membrane; b, microsomes; c, lysosomes (tritosomes).

The isolation procedure of Ehrenreich et al. [16] is based on the separation of the Golgi elements from a total microsomal fraction by virtue of their different densities, so the contamination by microsomes seems most probable. Table IV shows four microsomal enzyme activities in three Golgi fractions. Except for NADH-ferricyanide reductase, other enzyme activities in GF-1 and GF-2 were very low, indicating little if any contamination of GF-1 and GF-2 by microsomes. However, the considerably high activities detected in GF-3 are probably due to contamination by microsomes, which might result in lower galactosyltransferase activity in GF-3 as compared with that in GF-H (Tables II and III). In contrast to other enzymes, NADH-ferricyanide reductase showed relatively higher activity even in GF-1 where glucose-6-phosphatase activity was not detected.

Several acid hydrolases were assayed in order to estimate possible contamination by lysosomes and to determine whether acid hydrolases reside in a certain Golgi element or not. As shown in Table IV, activities of acid hydrolases were low in all Golgi fractions. If these activities were totally due to lysosomes trapped in the Golgi fractions, contamination would be very small. Cytochemical study on acid phosphatase by Farquahr et al. [36] demonstrated that this enzyme was indigenous to the secretory vesicles but not to the Golgi cisternae. Thus, activities detected in GF-1 and GF-2 are presumably due to indigenous enzyme locating in the secretory vesicles. However, our results showed the presence of acid hydrolases in GF-3, too. This was evident especially for acid phosphatase (*p*-nitrophenylphosphate as substrate) which was found with higher activity in GF-3 than in GF-1 and GF-2. It is one of the characteristics of lysosomes that β-glucuronidase and acid phosphatase are

TABLE V

RELEASE OF ACID PHOSPHATASE AND β-GLUCURONIDASE FROM THE GOLGI FRACTIONS BY HYPOTONIC TREATMENT FOLLOWED BY KCl WASHING

Acid phosphatase was determined with *p*-nitrophenylphosphate as substrate. Specific activity is given in nmol/min/mg protein.

Fraction	Protein Recovery	Acid phosphatase		β-glucuronidase	
		Spec. act.	Recovery	Spec. act.	Recovery
GF-1 suspension	100%	55.1	100%	10.1	100%
1st-sup	16.6	12.8	3.8	3.5	5.9
KCl washing	11.2	35.7	7.2	9.3	10.2
Pellet	60.1	72.2	79.3	12.9	76.6
GF-2 suspension	100%	105.7	100%	10.4	100%
1st-sup	12.4	39.2	4.6	2.7	3.3
KCl washing	9.4	72.4	6.4	8.3	25.9
Pellet	69.1	106.4	71.3	22.6	70.4
GF-3 suspension	100%	133.1	100%	17.7	100%
1st-sup	6.6	139.5	6.5	5.8	2.1
KCl washing	7.9	150.4	9.0	40.0	17.9
Pellet	80.8	138.0	83.8	15.3	69.8
GF-H suspension	100%	171.0	100%	32.2	100%
1st-sup	4.9	145.4	4.2	19.6	3.0
KCl washing	13.1	169.2	13.0	60.5	24.6
Pellet	82.5	129.5	62.5	23.5	60.2

easily released by hypotonic treatment [18]. To examine the lysosomal contamination, three Golgi fractions were subjected to osmotic shock followed by KCl washing (Table V). The percentages of acid hydrolases released from GF-3 were very low and similar those of GF-1, in which acid hydrolases are thought to be the indigenous ones, suggesting that acid hydrolases in GF-3 are resistant to hypotonic treatment and are associated with structures other than lysosomes, probably with the Golgi cisternae, because the same treatment released 80% of β-glucuronidase and 60% of acid phosphatase from lysosomes [18]. The fact that GF-H showed similar percentages of enzyme release as GF-1 and GF-2 further supports the presence of these enzymes in the Golgi cisternae and rules out the possible effect of colchicine on acid hydrolases of GF-3.

These enzymatic characterizations as well as morphological observations of the Golgi fractions exclude the possibility of significant contamination by other subcellular structures such as plasma membranes, microsomes, lysosomes and mitochondria into GF-1 and GF-2, confirming that these fractions are derived from the Golgi complex, though their galactosyltransferase activity was considerably lower than that of GF-H.

Discussion

With colchicine treatment at low dosage better yields of the Golgi fractions were obtained compared with ethanol administration. Colchicine caused 1.5-fold increase of yields of GF-1 and GF-3, and combination of colchicine and ethanol resulted in more than 2-fold increase of GF-1 compared with ethanol alone. It is evident that administration of colchicine was more advantageous than that of ethanol at least in obtaining GF-1 in quantity.

The possibility that treatment with ethanol or colchicine may evoke some serious alteration in liver which is not observed in non-treated rat liver should be considered. For example, in ethanol-treated rat liver almost all Golgi elements including cis-located cisternae are heavily loaded with VLDL-particles, but this is not the case in non-treated starved rat liver [16]. As for colchicine treatments the situation may be similar to ethanol treatment in that it may affect Golgi elements directly or indirectly. According to Stein and Stein [7], 0.025 mol of colchicine per 100 g body weight did not inhibit the protein synthesis and 0.25 mol of the drug caused only a slight inhibition of protein synthesis. Since this result was based on overall incorporation rates of the labeled leucine into total liver proteins, the conclusion could not be extended to possible effect on any particular proteins. Indeed Dorling et al. [37] reported that the synthesis of albumin in rat liver was inhibited by colchicine even at a concentration which gave no apparent inhibition of overall protein synthesis. Therefore, in the present study we used the minimum dose (0.025 mol/100 g body weight) required to increase the number of secretory vesicles and circumvent possible other effects of colchicine on the Golgi elements. Recently Redman et al. [10] have also isolated Golgi fractions from colchicine-treated rat liver by a similar method. However, the dose of colchicine used in their study was 100-fold higher than that used in the present study, and such a high dose of the drug would be undesirable for the characterization of the Golgi elements.

Comparison of the specific activities of galactosyltransferase in homogenates showed no difference between rat livers with and without treatment by ethanol or colchicine (Tables II and III). The Golgi fractions isolated from ethanol-treated rat liver showed higher activities than those from colchicine-treated rat liver. In spite of an apparent morphological similarity, GF-1 from ethanol-treated rat liver showed 3-fold higher activity than that from colchicine-treated rat liver, suggesting that in the former many cisternae highly packed with VLDL-particles floated up with the secretory vesicles and resulted in the elevated activity of galactosyltransferase in GF-1. However, we cannot rule out the possibility that the localization of galactosyltransferase among the Golgi elements may change in response to increased rate of lipid synthesis on ethanol administration. While this study was in progress, Kishore and Carubelli [38] reported similar effects of ethanol and colchicine on the distribution of galactosyltransferase among the three Golgi fractions, corroborating our results.

Polarized localization of galactosyltransferase among the Golgi elements was further confirmed among the fractions prepared from nontreated rat liver. As shown in Table III, most of the enzyme activity of the original total fraction (GF-T) was recovered in GF-H and its specific activity elevated more than 100-fold over that of the homogenate, indicating that galactosyltransferase is really enriched in the Golgi cisternae. One might argue that a large number of secretory proteins contained in the secretory vesicles make the specific activity of this fraction apparently lower. As shown in the accompanying paper, the separation of each fraction into its membrane and content made a comparison of the membrane-bound activity possible, confirming the polarized localization of galactosyltransferase.

Although the localization of NADH-ferricyanide reductase in all Golgi elements has been revealed by the aid of cytochemical staining and partly ascertained in the isolated Golgi fraction by Morré et al. [11], our result proved the existence of this enzyme in the secretory vesicle-rich fraction. Recently Hino et al. [39,40] indicated that NADH-cytochrome *c* reductase as well as NADPH-cytochrome *c* reductase are localized at a restricted region of the Golgi complex. The presence of a common membrane protein in different subcellular membranes may provide useful information as to the biogenetic relationship between these membranes.

The existence of several acid hydrolases in the Golgi fractions is very interesting from the point of view that localization of enzymes may be a clue to understanding the formation of lysosomes. Comparison of activity levels of acid hydrolases revealed that these enzymes were more concentrated in GF-3 and GF-H than GF-1 (Table V). We cannot exclude the possibility that acid hydrolases are highly concentrated in such a limited number of cisternae, as thiamine pyrophosphatase is localized in a few trans cisternae [34,36]. In addition it is not certain whether acid hydrolases detected in the Golgi fractions are really the enzymes destined to be finally segregated into lysosomes or enzymes localized in the Golgi complex [18]. Considering that most lysosomal enzymes are glycoproteins, it is likely that the Golgi cisternae might be involved in transport of acid hydrolases to lysosomes.

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