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PREPARATION AND CHARACTERIZATION OF GOLGI MEMBRANES FROM RAT LIVER

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SUMMARY

1. A Golgi-rich fraction from rat liver has been isolated by the direct application of the zonal centrifugation method used previously to isolate Golgi-rich membranes from bovine liver.

2. UDP-galactose–*N*-acetylglucosamine galactosyl transferase is concentrated about 100-fold in this fraction compared to the homogenate and appears to be a useful marker enzyme for this organelle in rat liver as well as beef liver. As in bovine liver Golgi, the fraction from rat liver is only slightly contaminated by other organelles as evidenced by low glucose-6-phosphatase, ATPase and acid phosphatase activities. Thiamine pyrophosphatase activity was found present in endoplasmic reticulum and plasma membranes as well as Golgi membranes from rat liver, and was therefore not a useful marker.

3. Some species differences in the Golgi preparations exist. Rat liver Golgi contain little or no rotenone-insensitive NADH– or NADPH–cytochrome *c* reductase activity whereas bovine liver Golgi have significant levels of these activities. In addition, the Golgi fraction from rat liver appears to have a unique and characteristic protein profile after electrophoresis in polyacrylamide gels as compared with endoplasmic reticulum, plasma membranes and mitochondria. Bovine liver Golgi preparations, on the other hand, appear very similar to endoplasmic reticulum after electrophoresis on acrylamide gels.

4. A major protein of band mobility 0.456 ± 0.008, relative to ribonuclease A, is present in rat liver Golgi preparations. This band is also characteristic of bovine liver Golgi preparations and may be due either to serum very low density lipoproteins or serum albumin, or both.

5. Using the information obtained from this type of preparation, a simpler, one-step procedure was devised which increases the yield of Golgi membranes from rat liver to 0.15–0.3 mg Golgi protein per g as compared to 0.05 mg Golgi protein per g obtained by the zonal procedure.

INTRODUCTION

The preparation and properties of Golgi-rich vesicles from beef liver have been described[1]. The preparation appeared to be unique both in morphology and

Abbreviation: Bicine, *N,N*-bis(2-hydroxyethyl)glycine.

in its ability to carry out at least one reaction important in the biosynthesis of glycoproteins, that is, the transfer of galactose from UDPGal to GlcNAc[2,3]. This activity appears to be the first reliable marker enzyme for Golgi membranes. It was of interest therefore to see if the method of preparation outlined for this cell fraction from beef liver could be applied to rat liver, a species more suitable for metabolic studies. The present communication deals with the application of the zonal ultracentrifuge to the isolation of Golgi vesicles from rat liver. The properties of the Golgi fraction isolated from rat liver are compared to that isolated previously from bovine liver. Applying the information obtained from these studies, a modification of the procedure was devised which gave much improved yields of Golgi from rat liver, and allows the isolation of a Golgi fraction from a portion of the liver from a single rat. A preliminary report of this work has been published[4].

MATERIALS AND METHODS

Preparation of Golgi membranes

Male rats, Sprague–Dawley or Holtzman strain, about 200 g each, were used. They were fed *ad libitum* before use. All sucrose solutions were prepared using "Ultra Pure" grade from Mann Research Labs and "% sucrose" (wt./wt. solution) determined using a Bausch and Lomb refractometer at 25°. All centrifugations were made in a Spinco ultracentrifuge and all operations were carried out at 4°.

(*a*) *Preparation of Golgi membranes using zonal centrifugation.* The livers of 25–30 rats were collected, washed briefly in 0.25 M sucrose, blotted, and minced with scissors. The minced liver was suspended in 3 vol. of 0.5 M sucrose containing 0.1 M sodium phosphate (pH 7.2) and 1% Dextran 500 (ref. 5) (Pharmacia Fine Chemicals, New Market, N.J.). The mixture was homogenized (3 full strokes) with a Potter–Elvehjem homogenizer with a Teflon pestle, with a clearance of 0.026 inch at 1000 rev./min. The homogenate was fractionated exactly as described previously for beef liver[1]. The mixture was centrifuged 30 min at 10000 rev./min (8720 $\times$ $g_{av.}$) in a rotor No. 30. The supernatant was poured through 4 layers of cheesecloth and centrifuged as before for 30 min at 20000 rev./min (34880 $\times$ $g_{av.}$). The pellets (R_2) were suspended in 53% sucrose containing 0.1 M sodium phosphate buffer (pH 7.1) using 2–3 strokes of a Dounce homogenizer with a Type A pestle. The final concentration of sucrose was adjusted to 43.7%. Fractionation of R_2 was carried out by means of zonal centrifugation in a B-14 rotor as described previously[1]. Fractions of 20 ml each were collected at a rate of 10–20 ml/min. After mixing each fraction, the concentration of sucrose and protein was determined. Fractions were combined as indicated in the original experiments. The combined fractions and a portion of the R_2 fraction were diluted with an equal volume of cold distilled water and centrifuged 60 min at 30000 rev./min (78480 $\times$ $g_{av.}$). The pellets were suspended finally in 0.25 M sucrose in small Potter–Elvehjem homogenizers.

(*b*) *Preparation of Golgi membranes, using a single step-gradient.* About 4 g of rinsed, blotted and minced liver from an individual rat were suspended in 3 vol. of 53% sucrose containing 0.1 M sodium phosphate buffer (pH 7.1) and homogenized as in (a). The entire homogenate was adjusted to 43.7% sucrose by adding 0.25 M sucrose. 10 ml of the homogenate were carefully placed in the bottom of a SW-25.2 cellulose nitrate tube and overlaid with the following step-gradient: 15 ml of 38.7%

sucrose, 10 ml of 36.0%, 10 ml of 33.0% and 12 ml of 29.0%. The sucrose solutions were unbuffered but were adjusted to pH 7.1 with NaOH before use. The gradient was centrifuged for 53 min at 25000 rev./min ($g_{av.} = 75500$) in a SW-25.2 rotor. The contents of the tube were displaced by infusing 50% sucrose into the bottom of the tube and 2-ml fractions collected from the top.

Other subcellular fractions

Rough and smooth microsomes were prepared from individual rat livers by a modification[6] of the method of DALLNER[7]. Plasma membranes were prepared from a pool of rat livers from 10 rats as described previously for bovine liver[6].

Enzymic assay

Glucose-6-phosphatase, Mg^{2+}-stimulated ATPase, 5'-nucleotidase, and rotenone-insensitive NADH–cytochrome *c* reductase activities were determined as described previously[6,8]. NADPH–cytochrome *c* reductase was also carried out in the presence of rotenone in the same manner as for NADH–cytochrome *c* reductase. Thiamine pyrophosphatase was assayed as described by YAMAZAKI AND HAYAISHI[9] with some modification[1].

Galactosyl transferase was determined as described previously[1] except that sodium cacodylate buffer was used at pH 6.5 rather than pH 6.75 as before. Galactosidase was determined as described previously[5] except that *N*,*N*-bis(2-hydroxyethyl)-glycine (Bicine)–HCl buffer (pH 8.6) was used instead of sodium cacodylate buffer (pH 7.2).

Electron microscopy

Negative staining with phosphotungstic acid was carried out as described previously[10]. For sectioning, samples were fixed overnight in the cold in 1% OsO_4 in 0.1 M veronal–acetate buffer (pH 7.4) containing 2.4 mM $CaCl_2$ and 0.06 M NaCl, block stained in 0.5% uranyl acetate, and dehydrated in a series of increasing ethanol concentrations. Ethanol was replaced with propylene oxide and the samples were embedded in Araldite. Heavy osmication was carried out as described previously[1].

Chemical assays

Protein was determined by the procedure of LOWRY *et al.*[11] and phosphorus by a modification[12] of the method of CHEN *et al.*[13]. UDPGal was obtained from Calbiochem, Los Angeles, Calif. UDP[$^{14}C_6$]Gal, uniformly labeled, was obtained from New England Nuclear Corp., Boston, Mass.

The products of the galactosyl transferase assay were characterized by chromatography on DEAE paper (Whatman No. DE81) using *n*-butanol–*n*-propanol–water (3:1:1, by vol.). Separation was continued for 48 h. At this time, the paper was air-dried and the distribution of radioactivity determined after cutting the paper into 1-cm-wide strips which were counted in 15 ml toluene containing 42 ml Liquifluor (Fisher Scientific, Pittsburgh, Pa.) per l. Counting was done in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

N-Acetyllactosamine was the kind gift of Dr. Mary C. Glick, Department of Therapeutic Research, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Electrophoresis

Electrophoresis of the cell fractions was carried out on polyacrylamide gels using the phenol–acetic acid–urea procedure of TAKAYAMA *et al.*[14] as modified by ZAHLER *et al.*[15]. After adding the sample, containing 15–20 μg of protein, 2 μg crystalline ribonuclease A (beef pancreas, Worthington) was added in 0.05 ml of the phenol solution to serve as a marker protein.

Rat serum albumin (Pentex, Fraction V), purified by repeated electrophoresis on Sephadex G-25 at pH 4.9 and 7.5, was the kind gift of Dr. Theodore Peters, Jr., Mary Imogene Bassett Hospital, Cooperstown, N.Y. Rat serum very low density lipoproteins ($d < 1.006$) were prepared by zonal centrifugation of rat serum and were the kind gift of Dr. H. G. Wilcox, Department of Pharmocology, School of Medicine, Vanderbilt University, Nashville, Tenn.

RESULTS

The method of preparation of Golgi vesicles from bovine liver was directly applicable to the preparation of Golgi vesicles from rat liver. A Golgi-rich fraction (R_2) was obtained from the rat liver by differential centrifugation by the same procedure used to fractionate bovine liver[1]. This sample was then fractionated by zonal centrifugation using the B-14 zonal rotor. The distribution of protein in a typical preparation after centrifugation for 45 min at 35000 rev./min ($g_{max} = 97600$) is shown in Fig. 1. Fractions of 20 ml each were collected from the inner portion of the rotor, which corresponds to the top of a gradient made in a centrifuge tube. The fractions collected in Tubes 10–19 were combined to give Fraction 1, while Tubes 20–23 were combined

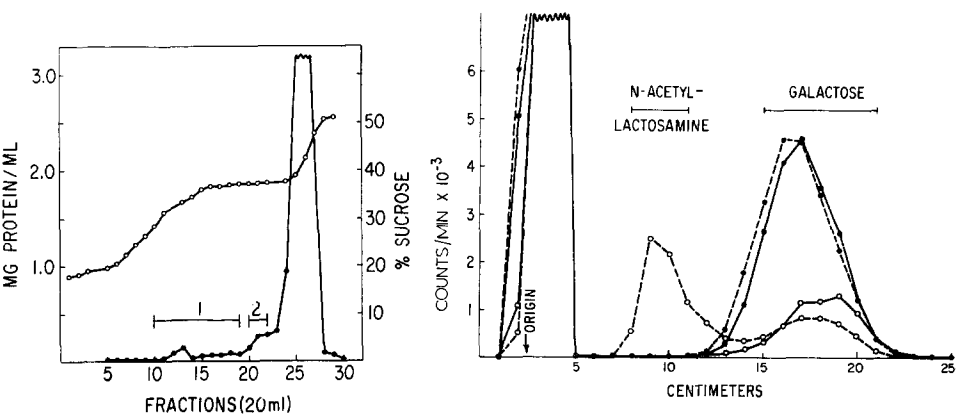


Fig. 1. Fractionation of crude Golgi fraction (R_2) from rat liver by zonal centrifugation. 20-ml fractions were collected. Fraction 1 = Tubes 10–19, Fraction 2 = Tubes 20–22; ○—○, % sucrose; ●—●, mg protein per ml.

Fig. 2. Chromatography of reaction products after incubation of plasma membranes or Golgi-rich fractions of rat liver with UDP[$^{14}C_6$]Gal. ●—●, plasma membranes without added GlcNAc; ●---●, plasma membranes with added GlcNAc; ○—○, Golgi fraction without added GlcNAc; ○---○, Golgi fraction with added GlcNAc. Galactose (1.5 μmoles) and *N*-acetyllactosamine (3 μmoles) were added to the reaction mixture before chromatography. After chromatography they were located with aniline phthalate spray. The migration of the standards are indicated at the top of the figure. Unreacted UDP[$^{14}C_6$]Gal remains at the origin in this system and accounts for the large peak of radioactivity found there.

to give Fraction 2. The fractions were diluted with an equal volume of distilled water and centrifuged at 30 000 rev./min (g_{max} = 78480) for 1 h. The pellets were suspended in 0.25 M sucrose. Analysis of these fractions is shown in Table I. It can be seen that Fraction 1 obtained from the zonal separation is approx. 100-fold enriched in galactosyl transferase activity as compared to the homogenate whereas this fraction is very low in glucose-6-phosphatase activity, a marker enzyme for endoplasmic reticulum. About 10 mg protein was recovered in this fraction from about 200 g wet weight of liver.

The products of the reaction of plasma membranes or the purified Golgi membranes with UDP[$^{14}C_6$]Gal were chromatographed on DEAE-cellulose paper. The results are illustrated in Fig. 2. When no acceptor is added, both plasma membranes and the Golgi vesicles give a product which chromatographs in a similar manner as free galactose. When GlcNAc is added to the reaction mixture together with plasma membranes, the reaction product is unchanged, whereas if added together with Golgi vesicles, a new product is formed which migrates as a disaccharide with an R_F similar to *N*-acetyllactosamine. Free galactose is still formed but in a somewhat reduced quantity.

In order to see if the relatively high hydrolysis rate seen in the absence of acceptor is due to contamination of the Golgi preparations with plasma membranes, the effect of pH on the hydrolysis and transfer reactions was studied. The results are summarized in Fig. 3. The pH response of the hydrolysis of UDPGal to yield free galactose of the two preparations is completely different. In the Golgi preparations the pH optimum for both hydrolysis and transfer occurs at about pH 6.5 in cacodylate buffer, whereas for plasma membrane, the rate of the reaction increases sharply between pH 6.5 and 7.8 in cacodylate buffer. The highest value was obtained at pH 8.6 using Bicine buffer.

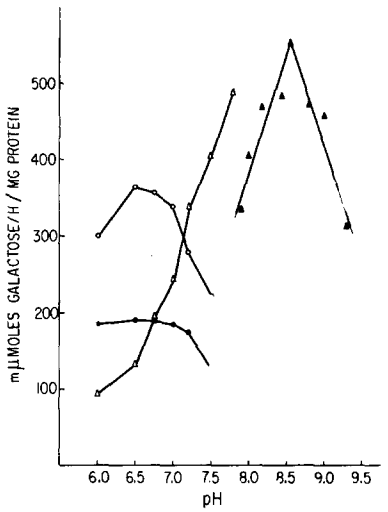
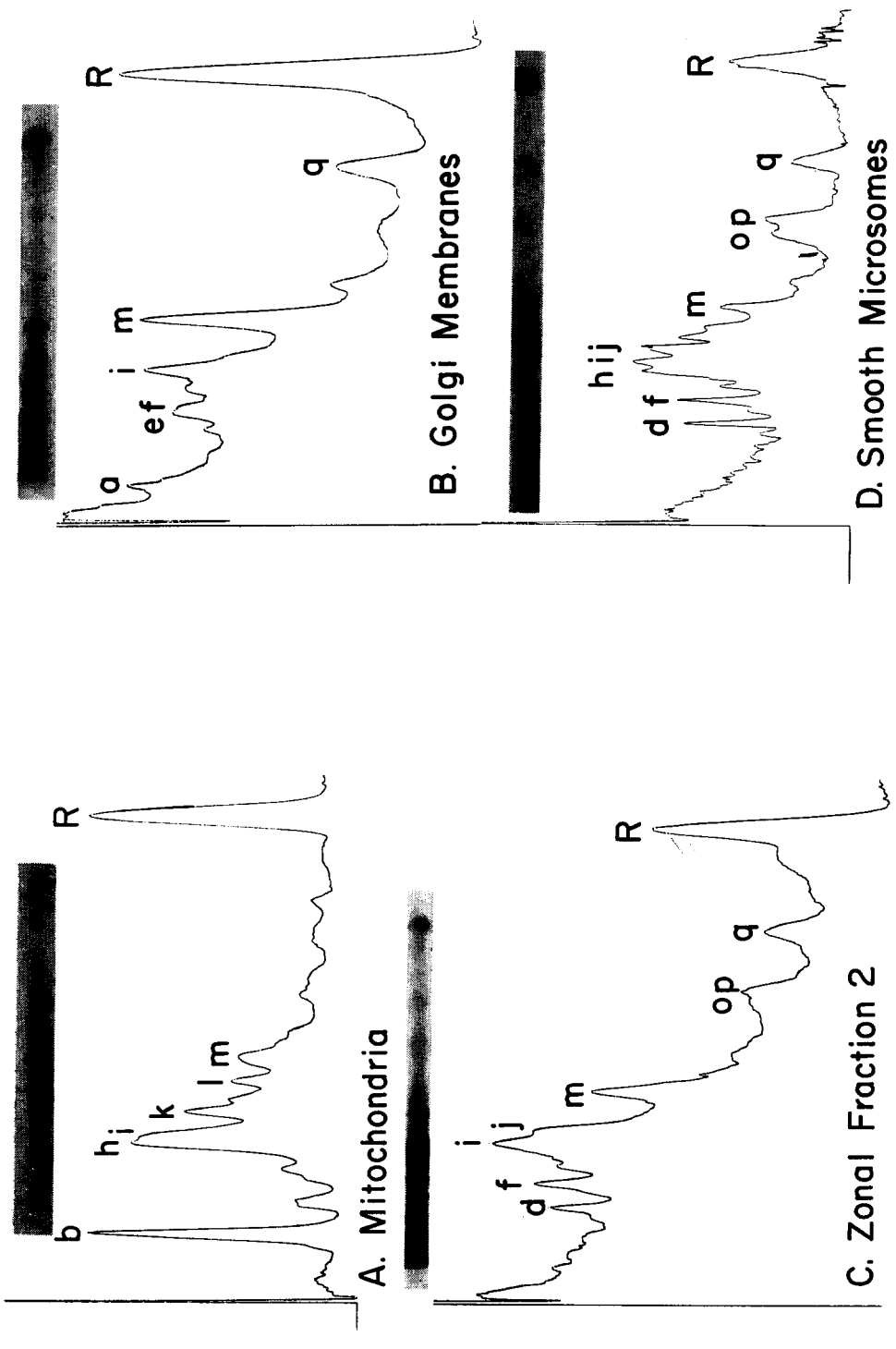


Fig. 3. Release or transfer of [$^{14}C_6$]galactose from UDP[$^{14}C_6$]Gal by purified Golgi fractions and plasma membranes from rat liver as a function of pH. ●—●, Golgi fraction in the absence of added GlcNAc; ○—○, Golgi fraction in the presence of added GlcNAc. The difference between these two curves represents transferase activity. Plasma membranes without added GlcNAc, were run as follows: pH 6.0–7.8 using 0.05 M cacodylate buffer, △—△; pH 7.8–9.2 using 0.05 M Bicine buffer, ▲—▲.


A. Mitochondria
B. Golgi Membranes
C. Zonal Fraction 2
D. Smooth Microsomes
R
b
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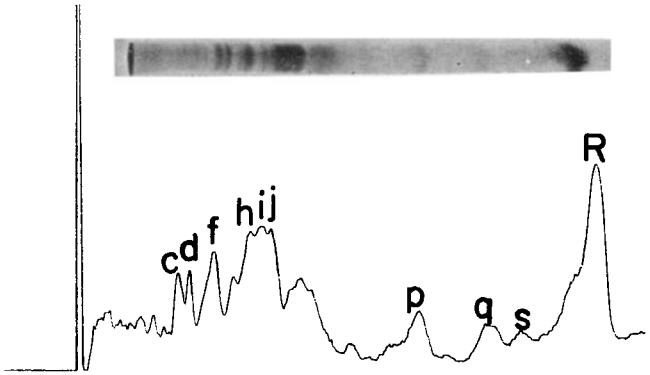
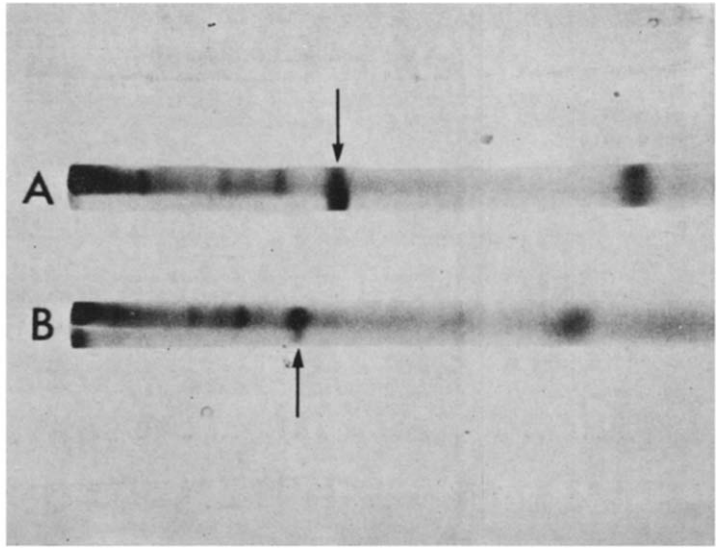




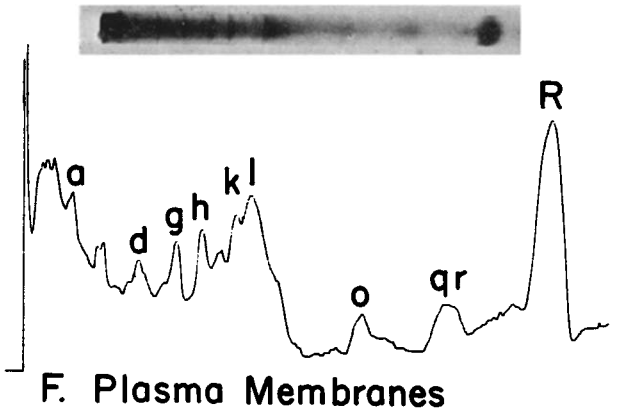


Fig. 4. A–F. Typical disc gel electrophoresis patterns and densitometer tracings of preparations of organelles from rat liver. The major peaks are designated by letters. The mobilities of these peaks relative to ribonuclease are given in Table II. R = ribonuclease standard added as a marker protein.

Fig. 5. Comparison of rat liver Golgi membranes with (A) rat serum very low density lipoproteins and (B) rat serum albumin by the split-gel technique[18]. In each case, Band m is indicated by an arrow.

The preparations were further characterized by electrophoresis in acrylamide gels. Purified rat liver mitochondria, Golgi vesicles, smooth and rough microsomes and plasma membranes were compared with respect to the relative mobilities of their major protein bands. The results are summarized in Fig. 4 and Table II. Characteristic patterns were obtained for all the organelles studied except smooth and rough microsomes which have minor but very reproducible differences in their patterns. The most striking feature found in this aspect of the study is that the Golgi preparations (Fig. 4B) are unique and can easily be distinguished from smooth microsomes (Fig. 4D) by their lack of Bands d, h, j, o, and p and by their large and characteristic

TABLE I

SPECIFIC ACTIVITY OF GALACTOSYL TRANSFERASE IN RAT LIVER FRACTIONS

R_2 is residue after centrifugation at 20000 rev./min for 30 min. Fractions 1 and 2 are the subfractions of R_2 collected by zonal centrifugation in the experiments shown in Fig. 1. Galactosyl transferase is expressed as nmoles galactose transferred to GlcNAc per h per mg protein at 37°. Glucose-6-phosphatase is expressed as μmoles P_i released per min per mg protein at 32°

Fraction	*Total protein (mg)*	*Galactosyl transferase*	*Glucose-6-phosphatase*
Homogenate	36 816	2.0	0.044
R_2	281	6.7	0.114
Fraction 1	10.8	195	0.007
Fraction 2	10.0	19	0.028

TABLE II

RELATIVE MOBILITIES OF MAJOR PROTEIN COMPONENTS OF RAT LIVER ORGANELLES

Electrophoresis was run in duplicate or triplicate on at least two different preparations of each organelle except plasma membranes where only one preparation was run in quadruplicate. Mobilities, given relative to that of added ribonuclease, are averages of at least four gels ± S.E.

Band	*Mitochondria*	*Golgi*	*Microsomes*		*Plasma membranes*
			Rough	*Smooth*	
a		0.087 ± 0.019			0.094 ± 0.007
b	0.142 ± 0.010				
c			0.195 ± 0.003		
d			0.217 ± 0.002	0.216 ± 0.002	0.216 ± 0.015
e		0.246 ± 0.014			
f		0.253 ± 0.003	0.264 ± 0.002	0.260 ± 0.013	
g					0.279 ± 0.019
h	0.329 ± 0.014		0.334 ± 0.011	0.329 ± 0.003	0.324 ± 0.015
i	0.338 ± 0.013	0.345 ± 0.018	0.355 ± 0.006	0.351 ± 0.003	
j			0.374 ± 0.008	0.378 ± 0.003	
k	0.387 ± 0.009				0.389 ± 0.015
l	0.416 ± 0.024				0.428 ± 0.006
m	0.455 ± 0.011	0.456 ± 0.008			
n	0.504 ± 0.013				
o				0.624 ± 0.004	0.635 ± 0.011
p			0.662 ± 0.004	0.664 ± 0.009	
q		0.788 ± 0.015	0.796 ± 0.002	0.784 ± 0.011	0.791 ± 0.007
r					0.811 ± 0.005
s			0.857 ± 0.003		

major Band m. Golgi fractions are also easily distinguished from plasma membranes (Fig. 4F) by the lack of Bands g, k, l, and o. Fraction 2 from the zonal preparation (Fig. 1) was also compared to the Golgi fraction by gel electrophoresis. It can be seen (Fig. 4C) that in this fraction Bands d, f, j, o, and p are more prominent than in Fraction 1 (Fig. 4B). The pattern appears similar to smooth microsomes (Fig. 4E) except that Band m is more prominent.

Two possible candidates for contributing to the intensity of Band m in the Golgi preparations are serum very low density lipoproteins which have been isolated from preparations of rat liver Golgi vesicles[16], and serum albumin, which is secreted by liver, probably through the Golgi apparatus[17]. We compared the mobility of these two proteins with the major band of rat liver Golgi by the split-gel technique of DUNKER AND REUCKERT[18]. The results are shown in Fig. 5. It can be seen that both serum very low density lipoproteins and serum albumin migrate in an identical way

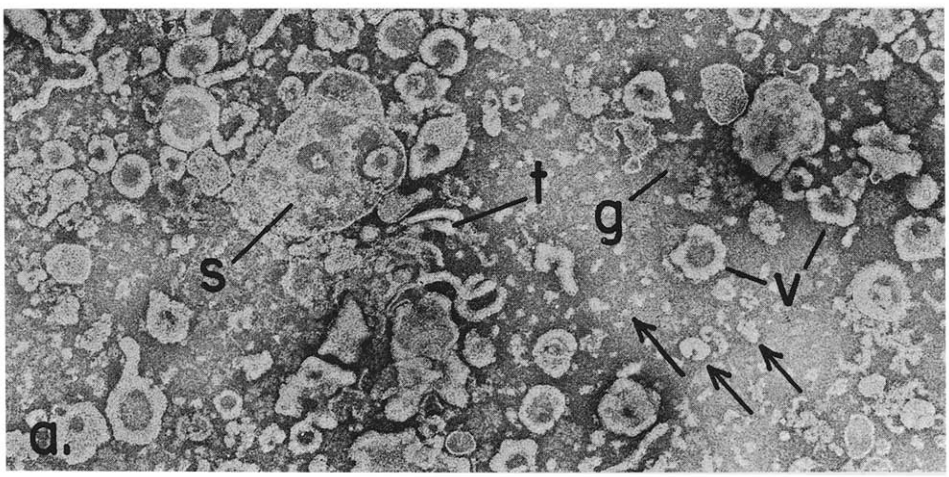
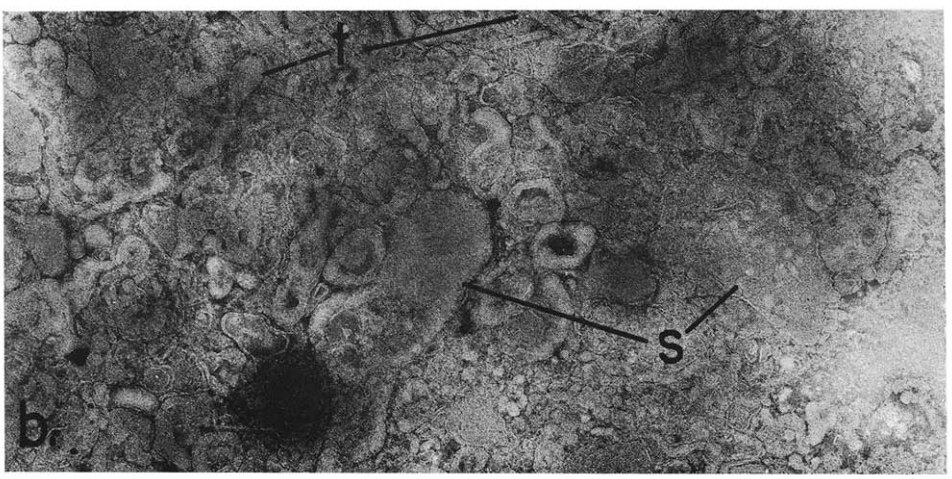


Fig. 6)



(Fig. 6)

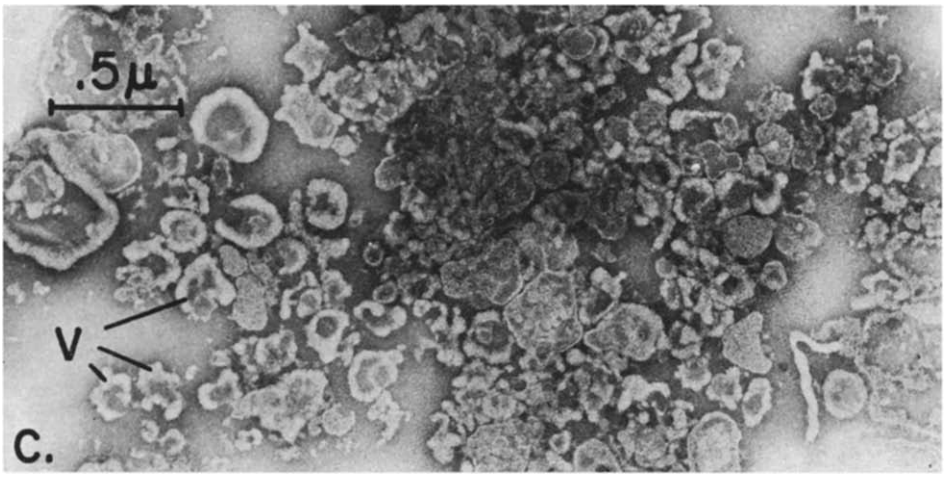


Fig. 6. Electron micrographs of fractions isolated from rat liver homogenate by differential centrifugation followed by zonal centrifugation (see Table I and Fig. 1). All fractions negatively stained with 2% potassium phosphotungstate (pH 7.2). (a) Fraction R_2. (b) Fraction 1. (c) Fraction 2 (all 35000 ×). s, sacs; t, tubules; v, vesicles probably derived from endoplasmic reticulum; g, glycogen; arrows indicate small fragments and vesicles.

with Band m in this system and either one or both could contribute to the intensity of this band in these preparations.

The fractions obtained were also characterized by electron microscopy, both in sections of fixed and embedded samples and by negative staining with phosphotungstic acid. The starting material for fractionation on the zonal rotor, R_2, is shown in Fig. 6a. The fraction is very similar to the R_2 fraction derived from bovine liver described previously[1]. It consists basically of five elements: large sacs (s); smooth tubular profiles (t), often with bulging ends; medium-sized vesicles with irregular electron-opaque edges (v), characteristic of endoplasmic reticulum; glycogen aggregates (g); and small fragments and vesicles (arrows). After zonal centrifugation, these elements are partially resolved. Fractions 1 and 2 are shown in Figs. 6b and 6c, respectively. The Golgi-rich fraction (Fig. 6b) appears to consist mainly of large sacs (s) and tubules (t). Fraction 2 (Fig. 6c), on the other hand, has a large proportion of medium-sized vesicles with irregular, electron-opaque edges (v). These are characteristic of endoplasmic reticulum. Thin sections of OsO_4-fixed and embedded samples of Fraction 1 are shown in Figs. 7a and 7b. The most characteristic features seen in such sections are large vesicles which appear to contain material inside, and associated tubules which are probably connected to the sacs.

The formation of large osmium aggregates upon treatment with unbuffered OsO_4 at elevated temperature is a characteristic property of Golgi apparatus both *in situ*[19] and in the isolated form[1]. Figs. 8a and 8b illustrate the formation of such aggregates in the enriched Golgi fraction isolated after zonal centrifugation of R_2 (Fig. 8b). It can be seen that there is a substantial increase in the deposition of reduced osmium observed in the highly purified zonal fraction compared to that found in R_2.

The information obtained from the large-scale preparation of Golgi vesicles was then utilized to develop a simpler one-step procedure to obtain Golgi vesicles from small amounts of rat liver. A step-gradient was devised which could be used

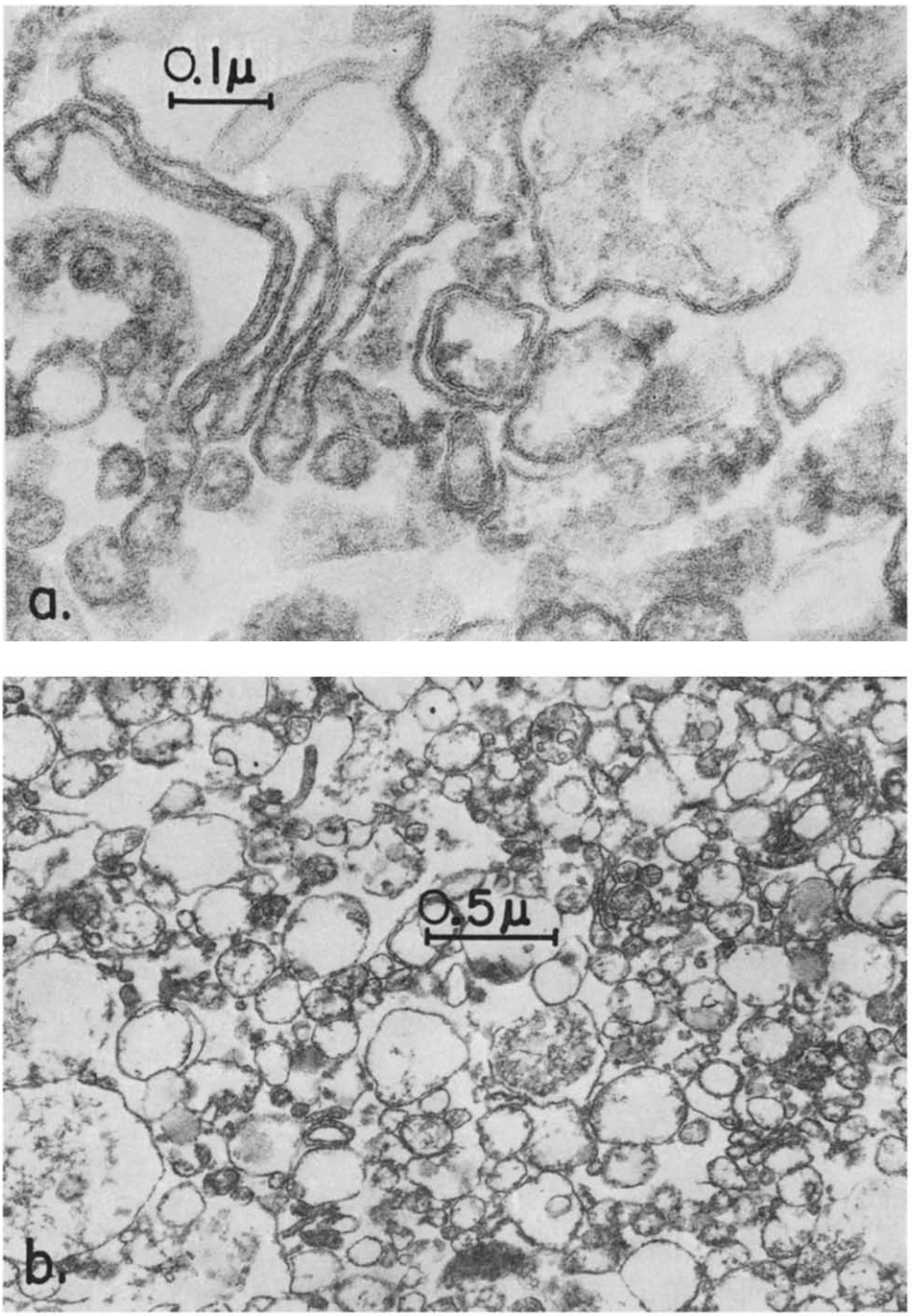


Fig. 7. Electron micrographs of Golgi membranes isolated by zonal centrifugation. Samples were fixed in 1% OsO_4, embedded and sectioned. (a) Characteristic features of this preparation are large sacs with connected tubular profiles (140000 ×). (b) A larger field of material isolated as in (a) (35000 ×).

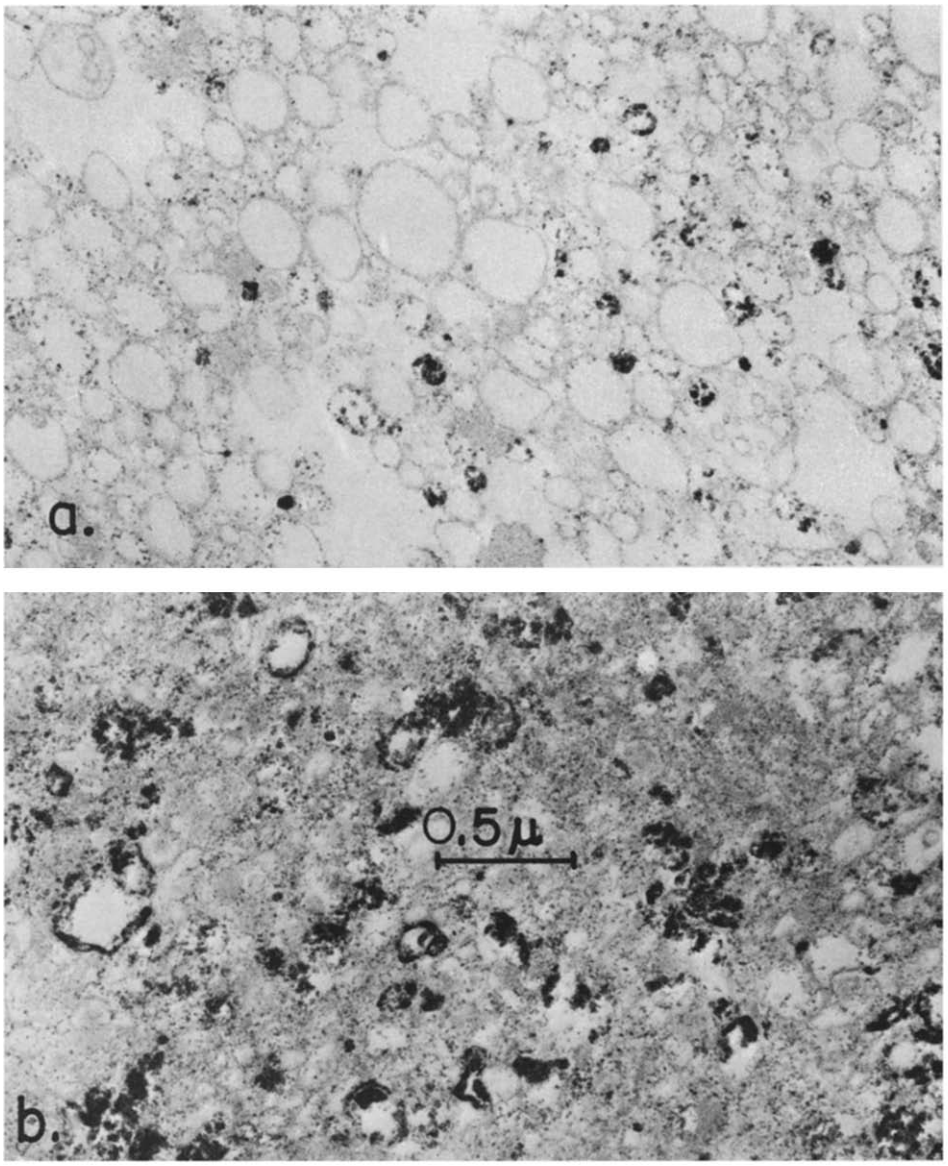


Fig. 8. Deposition of aggregates of osmium in isolated fractions from rat liver. (a) Crude Golgi Fraction R_2. (b) Purified Golgi Fraction 1 (Fig. 1 and Table I. All 35000 ×).

directly with the homogenate to obtain usable quantities of Golgi membranes from 3–4 g of liver. This gradient is illustrated in Fig. 9. The material at the interfaces of the gradient were collected as Fractions 1–3. Galactosyl transferase was measured directly on the fractions obtained from the gradient. The distribution of protein and galactosyl transferase activity in a typical experiment is shown in Table III. About 20–25% of the activity of the homogenate is recovered in the fraction isolated at the interface between 33.0 and 29% sucrose. Significant levels of galactosyl transferase

activity were also seen in Fractions 2 and 3 of the gradient, but the increased levels of NADH–cytochrome *c* reductase found in these fractions indicate an increased contamination with endoplasmic reticulum.

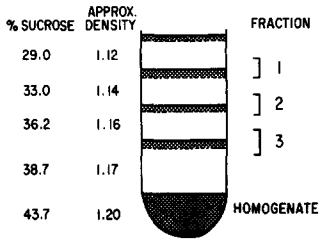


Fig. 9. Fractionation of total rat liver homogenate on a sucrose step-gradient. The material at the surface was predominately lipid and was discarded. Fractions 1 and 2 appeared colorless, while Fraction 3 was pink.

TABLE III

DISTRIBUTION OF PROTEIN AND GALACTOSYL TRANSFERASE AFTER CENTRIFUGATION OF WHOLE HOMOGENATE IN A SUCROSE STEP-GRADIENT

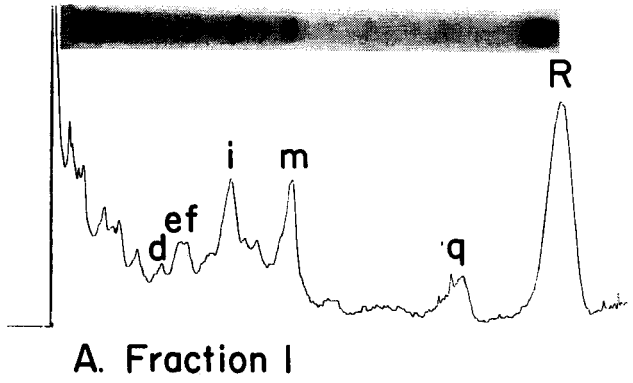
Galactosyl transferase is expressed as in Table I. NADH–cytochrome *c* reductase is expressed as μmoles cytochrome *c* reduced per min per mg protein at 32°. 4 μg rotenone were added to the assay before addition of the substrate.

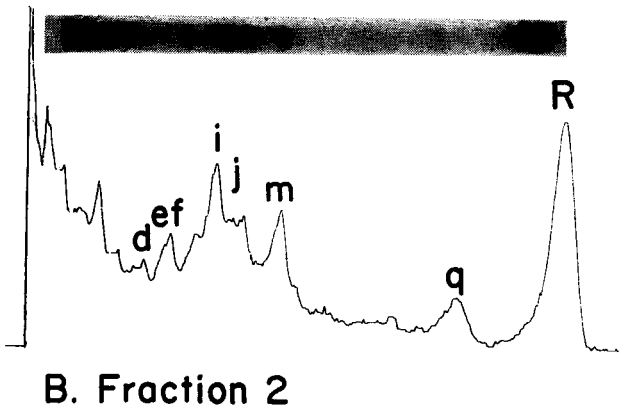
Fraction	*Total protein (mg)*	*Galactosyl transferase*		*NADH–cytochrome c reductase*
		Specific activity	*% total activity*	
Original homogenate (3.3 g liver)	291	1.9	100	—
Gradient fraction				
1	0.51	235	22	0.196
2	0.42	129	10	0.419
3	0.55	109	11	0.994

Electrophoresis of these fractions on acrylamide gels yielded the patterns shown in Fig. 10. Fraction 1 appears to have the same characteristic features seen in the Golgi preparation purified by means of zonal centrifugation (Fig. 4b), *i.e.* lack of Bands d, h, j, o, and p and prominent Bands i and m. Fractions 2 and 3, on the other hand, show increasing levels of endoplasmic reticulum Bands d, h, j, o, and p, and decreasing prominence of Bands i and m.

The effect of pH on the galactosyl transferase and galactosidase activity of the Golgi preparation made by the single-step method is illustrated in Fig. 11. Although these curves have the same general shape as those found for the zonal preparation (Fig. 3), the hydrolytic activity of these preparations is much lower, indicating that this activity is not characteristic of this organelle but is due to contamination of the fraction, most probably with lysosomes.

Fig. 12 shows the dependence of the transferase reaction on added Mn^{2+}. As for bovine Golgi preparations, maximum activity was observed when 3 μmoles Mn^{2+} were added to the assay mixture (final concentration of 40 mM Mn^{2+}).





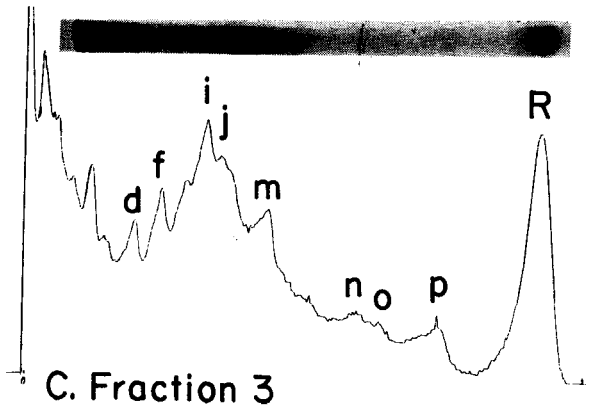


Fig. 10. Disc gel electrophoresis patterns and densitometer tracings of Fractions 1–3 obtained from rat liver homogenate as indicated in Fig. 9. The mobilities of the major peaks, designated by letters, are given in Table II. R = ribonuclease standard added as a marker protein.

Electron microscopic observation of the Golgi fraction obtained by the single-step procedure was limited to negative staining with phosphotungstic acid due to the small amounts of membranes obtained. Fig. 13 shows that, as in the zonal preparation, sacs and tubules are the main components found in this fraction. Enclosed lipoprotein particles are often seen in this type of preparation.

Table IV summarizes the enzymatic profiles found for the two types of Golgi preparations described in this work as compared to smooth and rough microsomes and plasma membranes. The Golgi preparation made with zonal ultracentrifuge is characterized by very low glucose-6-phosphatase, rotenone-insensitive NADH–cytochrome *c* reductase and NADPH–cytochrome *c* reductase activities, indicating less than 1% contamination with endoplasmic reticulum. A rough measure of contamination with plasma membranes, the major contaminant, is given by ATPase and galactosidase activities which are about 11% that found in the plasma membrane preparation studied. Thiamine pyrophosphatase does not appear to be concentrated in the Golgi membranes but is present at a low level throughout the membrane fractions. Significant acid phosphatase activity was found in this type of Golgi preparation indicating some contamination due to lysosomes. The exact level of this contamination is difficult to estimate since the acid phosphatase activity of highly purified rat liver lysosomes has not been reported. A specific activity of 0.8 has been reported[20] for partially purified rat liver lysosomes. This would indicate an upper limit of 7% contamination with lysosomes in this preparation. A level of 2–3% contamination is a more likely estimate based on the number of contaminating lysosomes seen in sections of this fraction in the electron microscope.

Lysosomal contamination is not present at a significant level in the Golgi membranes prepared by the single-step procedure. Plasma membrane contamination

TABLE IV

ENZYME PROFILES OF PLASMA MEMBRANES, GOLGI FRACTIONS AND SMOOTH AND ROUGH MICROSOMES

All phosphatases are expressed as μmoles P_i released per min per mg protein. Cytochrome *c* reductases are expressed as μmoles cytochrome *c* released per min per mg protein. Galactosidase is expressed as nmoles galactose released from UDPGal per h per mg protein. Galactosyl transferase is expressed as nmoles galactose transferred per h per mg protein.

Assay	*Plasma membranes*	*Golgi*		*Microsomes*	
		Zonal	*Step-gradient*	*Smooth*	*Rough*
ATPase	1.24	0.154	—	0.088	0.065
Glucose-6-phosphatase	0.004	0.007	0.013	0.108	0.096
NADH–cytochrome *c* reductase***	0.000	0.008	0.089	2.0	1.4
NADPH–cytochrome *c* reductase***	0.001	0.000	0.012	0.084	0.064
Succinate–cytochrome *c* reductase	0.000	0.002	0.005	0.006	0.004
Acid phosphatase	—	0.054	—	0.035	0.010
Thiamine pyrophosphatase*	0.037**	0.075	—	0.182	0.208
Galactosidase	552	61.2	31.3	17.0	4.0
Galactosyl transferase	0.8	190	316	17.4	0.4
μg P per mg protein	19.2	23.3	23.4	28.3	38.5

* Thiamine pyrophosphatase assayed at 37°, all others at 32°.
** No ATP added since plasma membranes have a very active ATPase activity.
*** 4 μg rotenone added as 1 mg/ml ethanol solution before addition of substrate.

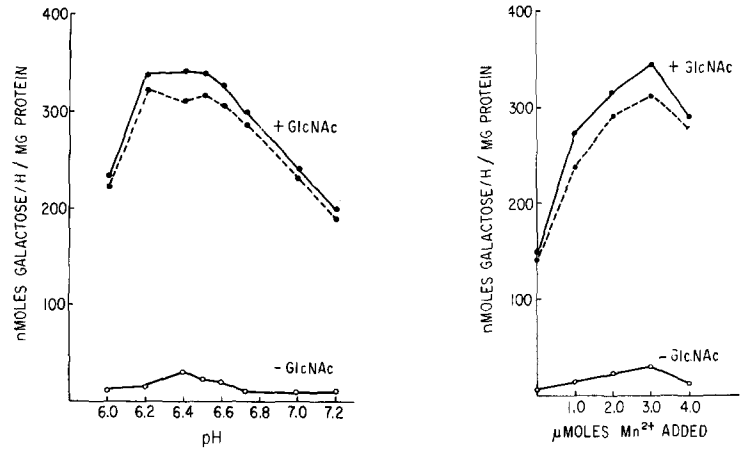


Fig. 11. Release and transfer of [$^{14}C_6$]galactose from UDP[$^{14}C_6$]Gal by purified Golgi fractions from rat liver isolated by the sucrose step-gradient procedure (Fig. 9) as a function of pH. ○—○, without added GlcNAc; ●—●, with added GlcNAc; ●---●, difference between the two curves, which represents galactosyl transferase activity. Cacodylate buffer, 0.05 M, was used throughout.

Fig. 12. Effect of added Mn^{2+} on release and transfer of [$^{14}C_6$]galactose from UDP[$^{14}C_6$]Gal by purified Golgi fractions from rat liver isolated by the sucrose step-gradient procedure (Fig. 9). ○—○, without added GlcNAc; ●—●, with added GlcNAc; ●---●, difference between the two curves, which represents galactosyl transferase activity. 0.05 M cacodylate buffer (pH 6.5) was used throughout. Peak activation was found when 3 μmoles $MnCl_2$ were added to the assay mixture (final concentration 40 mM Mn^{2+}).

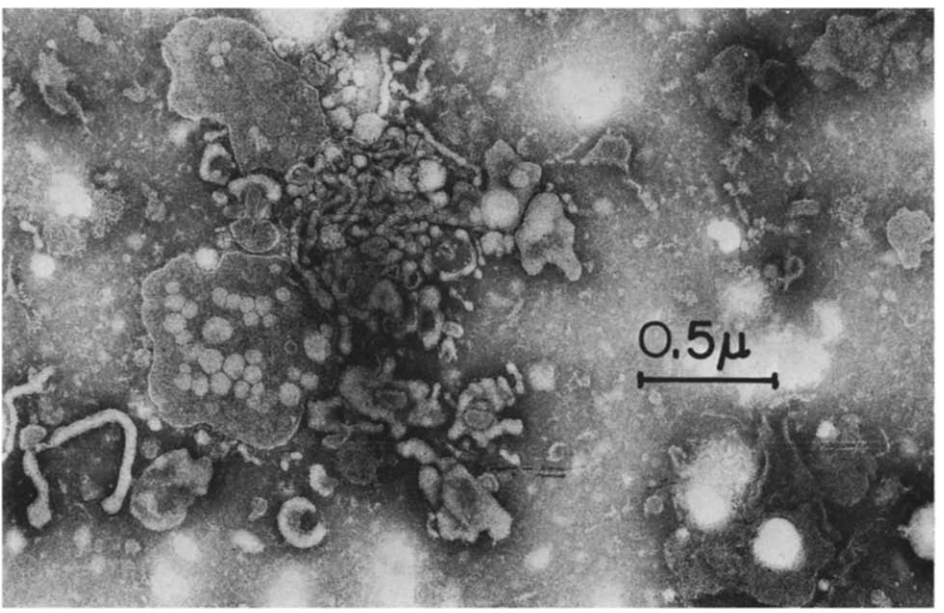


Fig. 13. Electron micrograph of Fraction 1 isolated from rat liver homogenate by the sucrose step-gradient procedure (Fig. 9 and Table III) and negatively stained with 2% phosphotungstic acid, pH 7.2 (35000 ×).

is also reduced, as indicated by the glycosidase activity, and is about 5%. Microsomal contamination, however, is increased and varies from 5 to 15%. Contamination with mitochondria is negligible in both preparations.

DISCUSSION

We have shown that the isolation of a Golgi-rich fraction from rat liver is possible by a direct application of the zonal centrifugation procedure first used to isolate a Golgi-rich fraction from bovine liver. Golgi vesicles from rat liver have many similarities to those from bovine liver. Morphologically, the fraction consists of sacs and tubules containing a considerable amount of material inside. Enzymatically both rat and beef liver Golgi fractions exhibit high UDP-galactose–*N*-acetylglucosamine galactosyl transferase activity, an enzyme which appears to be characteristic of Golgi preparations from various sources[1]. As in bovine liver Golgi, the fraction from rat liver is characterized by low glucose-6-phosphatase, ATPase, thiamine pyrophosphatase and acid phosphatase activities.

Some species differences in the two Golgi preparations from liver have been observed. Rat liver Golgi contain little or no NADH– or NADPH–cytochrome *c* reductase activity, in contrast with beef liver Golgi, which have about 60% of the activity found in endoplasmic reticulum. In addition, the patterns obtained for the two preparations on acrylamide gel electrophoresis are different. Beef liver Golgi appears very similar to endoplasmic reticulum although it can be distinguished from the latter[15]. Rat liver Golgi is easily distinguishable from liver rat endoplasmic reticulum by electrophoresis on acrylamide gels, a property which is extremely useful in monitoring very small quantities of cell fractions.

Electrophoresis in acrylamide gels has shown that a band of relative mobility 0.456 ± 0.008 compared to ribonuclease A, is characteristic of both rat liver and bovine liver Golgi preparations. This band may be due either to serum very low density lipoproteins, which have been shown to be present in rat liver Golgi[16], or to serum albumin, which is synthesized in liver and is probably exported from the cell *via* the Golgi apparatus[17,23], or both. Smooth endoplasmic reticulum, isolated as smooth microsomes from individual rats by the method of DALLNER[7], appears to have a small and variable amount of this band. The band, if present, is a minor component in rough endoplasmic reticulum. Electrophoresis in polyacrylamide gels is a useful tool for discerning contamination of Golgi preparations with either endoplasmic reticulum or mitochondrial membranes. Plasma membrane contamination would be the most difficult to detect with this technique, since no very prominent and unique bands are found in the plasma membranes.

Isolation of very pure Golgi vesicles by the zonal technique appears to be somewhat more easily accomplished from rat liver than from bovine liver. In bovine liver there is more smooth endoplasmic reticulum than rough, whereas in rat liver, there is more rough endoplasmic reticulum than smooth. The latter condition is evidently to be preferred when trying to separate Golgi vesicles from endoplasmic reticulum.

Galactosyl transferase appears to be localized in the Golgi-rich fraction of rat liver. The overall recovery of activity in this fraction by the zonal procedure is only about 3% of the total activity present in the homogenate. The method is extremely

useful, however, as a generalized method for preparing Golgi vesicles from liver tissue and for preparation of 10–20 mg of material at a time. Such quantities are necessary for chemical and enzymological characterization of a new preparation.

The single-step procedure for the isolation of Golgi vesicles from rat liver is extremely useful in situations where small amounts of liver (3–4 g) are all that is available for fractionation, *i.e.*, in metabolic studies on individual animals. The yield is about 0.15–0.3 mg Golgi protein per g wet weight of rat liver as compared to about 0.05 mg Golgi protein per g liver for the zonal procedure. It should be pointed out that the protein concentration of the filtered homogenate prepared in the one-step procedure is only about 90 mg protein per ml as compared to about 180 mg/ml in the homogenate prepared for the zonal procedure even though the same quantity of homogenizing medium is used. This is referable to the difficulty in homogenizing and filtering the more dense sucrose used in the one-step procedure. The yield may be improved further in the one-step procedure by using a more vigorous homogenizing step at this stage of the preparation but at the risk of greater mechanical damage to the preparation. Contamination with endoplasmic reticulum is increased, varying from 5 to 15% of the preparation on a protein basis. Since only small amounts of material are available, rotenone-insensitive NADH–cytochrome *c* reductase is a very useful marker enzyme for quantitating the extent of contamination with endoplasmic reticulum in these preparations. Electrophoresis in acrylamide gels also needs very little material and has also proven a useful diagnostic tool for monitoring small-scale preparations of rat Golgi.

It may be noted that the specific activity of galactosyl transferase for Golgi vesicles obtained by the one-step procedure, though somewhat variable, is consistently higher than that obtained by the zonal procedure. Yet, the level of known contaminants, as judged by assay of known marker enzymes, is about the same for the two preparations. One possible explanation is that the Golgi apparatus is somewhat heterogeneous in its content of transferase activity and that the two isolation procedures used select slightly different populations of particles derived from the Golgi. There is considerable evidence of heterogeneity in the Golgi membranes *in situ* in staining for complex carbohydrates[21], in ordinary osmium staining[22] and in heavy osmication[19]. We have also observed that Golgi vesicles isolated by the zonal procedure have a higher content of serum albumin than those prepared by the flotation procedure[23] which lends support to the idea of a somewhat heterogeneous population of vesicles.

While this work was in progress, MORRÉ *et al.*[24] published a different isolation procedure for obtaining Golgi-rich fractions from rat liver. This method of preparation differs from that presented here in that their material is sedimented from the homogenate at very low *g* forces (2000 × *g* for 30 min). This is probably due to the high concentration of divalent cations used in the homogenizing medium (5 mM $MgCl_2$). It has been shown previously by DALLNER AND NILLSSON[25] that 10 mM Mg^{2+} causes extensive aggregation of isolated smooth microsome fractions from rat liver and that this aggregation is essentially irreversible. Added to the initial homogenate, however, the effect of Mg^{2+} may be more specific. Galactosyl transferase was shown to be present in this type of Golgi preparation[26] at about the same level as we have found in our preparations. Further, SCHACHTER *et al.*[27] have recently shown that such preparations contain other sugar transferases such as *N*-acetylglucosaminyl and sialyl

transferase and suggest that the Golgi apparatus in liver is equipped with a multienzyme system for the synthesis of the terminal sugars in glycoproteins.

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