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ISOLATION OF A GOLGI-RICH FRACTION FROM RAT LIVER

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SUMMARY

A fraction rich in membranes of the Golgi apparatus was isolated from rat liver by discontinuous density gradient centrifugation. Electron microscopic analysis of the fraction revealed the presence of structures very similar to those of the Golgi apparatus in intact cells, namely stacked cisternae, secretory vesicles, and tubular elements. The Golgi-rich fraction contained over 90 % of the UDP-galactose: *N*-acetylglucosamine galactosyltransferase, about 2 % of the glucose-6-phosphatase and 12 % of the AMP phosphohydrolase present in the post-nuclear supernatant of liver homogenates.

INTRODUCTION

In a previous communication from this laboratory[1], marked alterations in the ultrastructural appearance of the Golgi apparatus of hepatocytes were reported to occur in Sprague–Dawley rats fed a choline-deficient diet. We undertook subsequently to isolate the Golgi apparatus from the liver of such animals in order to study its alterations from a biochemical point of view. In earlier attempts we tried the methods described by KUFF AND DALTON[2] and by MORRÉ AND MOLLENHAUER[3]. These methods, however, proved unsatisfactory in our hands, since we could obtain only Golgi fractions which were contaminated with several other cell structures, as judged by both electron microscopic and enzymatic criteria. The same difficulty was encountered in the isolation of the Golgi apparatus from the liver of Sprague–Dawley rats fed a commercial chow. After several modifications of the above methods, we have devised a relatively simple procedure whereby a Golgi fraction of high purity can be obtained consistently, and in high yield, from the liver of rats fed a commercial chow. The description of such a procedure is the subject of the present report.

MATERIALS AND METHODS

Male Sprague–Dawley rats (Sprague–Dawley, Madison, Wisc.) weighing 100–200 g and fed *ad libitum* a commercial chow (Wayne Lab Block, Allied Mills, Chicago, Ill.) and water were used.

Isolation of the Golgi-rich fraction

Sucrose solutions were prepared in 0.1 M potassium phosphate buffer (pH 6.65) 5 mM in $MgCl_2$. The following solutions were used: A, homogenization medium: 0.5 M

Abbreviation: DPT, thiamine pyrophosphate.

sucrose; B, first discontinuous gradient: 5 ml of 1.3 M sucrose at the bottom of cellulose acetate tubes for the 40 rotor of a Spinco Model-L centrifuge; C, second discontinuous gradient: 6 ml each of 1.4 M, 1.3 M and 1.25 M sucrose, starting from the bottom of cellulose acetate tubes for the SW-25.1 rotor of the same centrifuge; D, 1.1 M sucrose.

The animals were decapitated and the livers quickly removed, chilled in cold homogenizing medium, blotted and weighed. 4 g of tissue were transferred into 20 ml of homogenizing medium, minced with scissors, and homogenized for 30 sec using a Polytron (Model PT 10, Brinkman Instruments Westbury, N.Y.) at tap 1. The homogenate was then centrifuged at 600 × *g* for 10 min in an International refrigerated centrifuge model PR-2. The supernatant was aspirated, and the sediment (unbroken cells, red blood cells and nuclei) was discarded. A known volume (6 ml) of post-nuclear supernatant was then layered on the first sucrose gradient (B) and centrifuged at 105000 × *g* for 60 min. This resulted in the formation of: (1) an upper fat layer; (2) a clear reddish supernatant; (3) a thick membrane felt above the 1.3 M sucrose interphase; (4) a turbid 1.3 M sucrose infranatant; and (5) a sediment at the bottom of the tube. The fat layer and supernatant were carefully removed and the membrane felt was aspirated with the aid of a Pasteur pipette. The 1.3 M sucrose infranatant and the pellet were also collected.

The membrane felt was used for the preparation of the Golgi-rich fraction. It was gently but thoroughly resuspended with a glass rod, and the molarity of the sucrose adjusted to 1.1 M with the aid of a Bausch and Lomb refractometer. The suspension was then brought to a known volume (usually 8 ml) with 1.1 M sucrose, and 6 ml were layered on top of the second discontinuous sucrose gradient (C), followed by 6 ml of homogenizing medium. The gradient was centrifuged for 90 min at 25000 rev./min. This resulted in two main membrane fractions, one at the interface between 1.1 M sucrose and the homogenizing medium, and the other at the interface between 1.25 and 1.1 M sucrose. The two fractions were collected separately and designated Golgi fraction and smooth endoplasmic reticulum fraction, respectively. Barely discernible bands formed also at the other interfaces (1.25–1.3 and 1.3–1.4 M sucrose) and were collected and pooled (Fraction 3).

Analytical procedures

Proteins and RNA were precipitated from the collected fractions by addition of trichloroacetic acid to a final concentration of 10%. Protein was determined by the method of LOWRY *et al.*[4], or with a biuret reagent[5], using bovine serum albumin as standard; RNA was estimated by the Schmidt–Thannhauser procedure as modified by HUTCHINSON AND MUNRO[6], using yeast RNA as standard. P_i was determined according to MARTIN AND DOTY[7].

Enzyme assays

The following substrates and cofactors were used: AMP, DPT, glucose 6-phosphate, *N*-acetyl-D-glucosamine, UDP-D-galactose (Sigma Chemical Co., St. Louis, Mo.), and UDP-[^{14}C]galactose (uniformly labeled, 252 mg/mole; New England Nuclear Corp., Boston, Mass.). A sample of *N*-acetyllactosamine was kindly supplied by Dr. Akira Kobata, National Institutes of Health, Bethesda, Md.

Prior to enzyme assay, the collected membrane fractions were washed by repeated centrifugation and dispersion in, and aliquots of the post-nuclear supernatant

dialyzed overnight against the buffer required for the specific assay. The following activities were measured: AMP phosphohydrolase (EC 3.1.3.5)[8], DPT phosphohydrolase (EC 3.6.1.–)[9], glucose-6-phosphate phosphohydrolase (EC 3.1.3.9)[10], and succinate–cytochrome *c* reductase (EC 1.3.99.1)[11]. UDP-galactose:*N*-acetylglucosamine galactosyltransferase (*N*-acetyllactosamine synthetase, EC 2.4.1.–) was assayed as described by WATKINS AND HASSID[12]. Assay mixtures contained the following components (μmoles, in a final volume of 15 μl): UDP-D-[^{14}C]galactose, 0.01 (12 000 counts/min); *N*-acetyl-D-glucosamine, 0.2; $MnCl_2$, 0.6; Tris–HCl buffer (pH 7.4), 0.2; 2-mercaptoethanol, 0.01; and enzyme (0.1 mg protein). After 1 h of incubation at 37°, the mixture was dried on the startline of a paper chromatogram (Whatman No. 1) and subjected to chromatography in *n*-propanol–ethyl acetate–water (7:1:2, by vol.). The radioactive reaction product was shown to have the same mobility of authentic *N*-acetyllactosamine by radioautography. The distribution of radioactivity was determined by counting successive 1-cm-wide strips cut from the paper perpendicular to the direction of chromatography. The strips were wetted with 0.5 ml water in counting vials, and after addition of 10 ml of Bray scintillation mixture[13] were counted in a Packard scintillation spectrometer. The activity was calculated from the counts/min associated with the *N*-acetyllactosamine area, and the specific activity was expressed as nmoles D-galactose transferred per h per mg protein.

Electron microscopy

Glutaraldehyde, 3.5% in 0.2 M phosphate buffer (pH 7.2–7.4)[14], was added to aliquots of the membrane fractions and these were then centrifuged and pelletted. The pellets were fixed overnight in 3.5% glutaraldehyde in 0.2 M phosphate buffer at 4° and post-fixed in unbuffered 2% OsO_4 for 2 h at 4°. Dehydration and embedding in Vestopal were performed as previously reported[15]. Silver sections were cut with glass or diamond knives and stained with lead citrate[16]. The sections were lightly carboned either before or after staining. For negative staining, aliquots of the membrane fractions were washed and resuspended in ammonium formate buffer[17] and stained with 2% phosphotungstic acid (pH 7.0–7.2). Sections and negatively stained preparations were examined and photographed with a Philips EM-300 electron microscope at 80 kV.

RESULTS

Electron microscopy

Fraction 1 (Golgi fraction). Examination of negatively stained preparations and of sections revealed the presence in this fraction of only smooth-membrane structures. Rough endoplasmic reticulum vesicles and mitochondria are conspicuously absent. Three elements of the Golgi apparatus are particularly abundant and recognizable, namely, cysternae, secretory vesicles and tubular profiles (Fig. 1). In negatively stained preparations (Fig. 2), cysternae appear as intricate arrays of anastomosing tubules of various diameter, some of which, at the periphery of the array, are dilated or sac-like (secretory part of cysternae, ref. 18). When sectioned tangentially, the cysternae appear as "fenestrated plates"[18] having a central disk-like region surrounded by a tight network of anastomosing tubules (Figs. 1, 4 and 5; see also Fig. 1 in ref. 18).

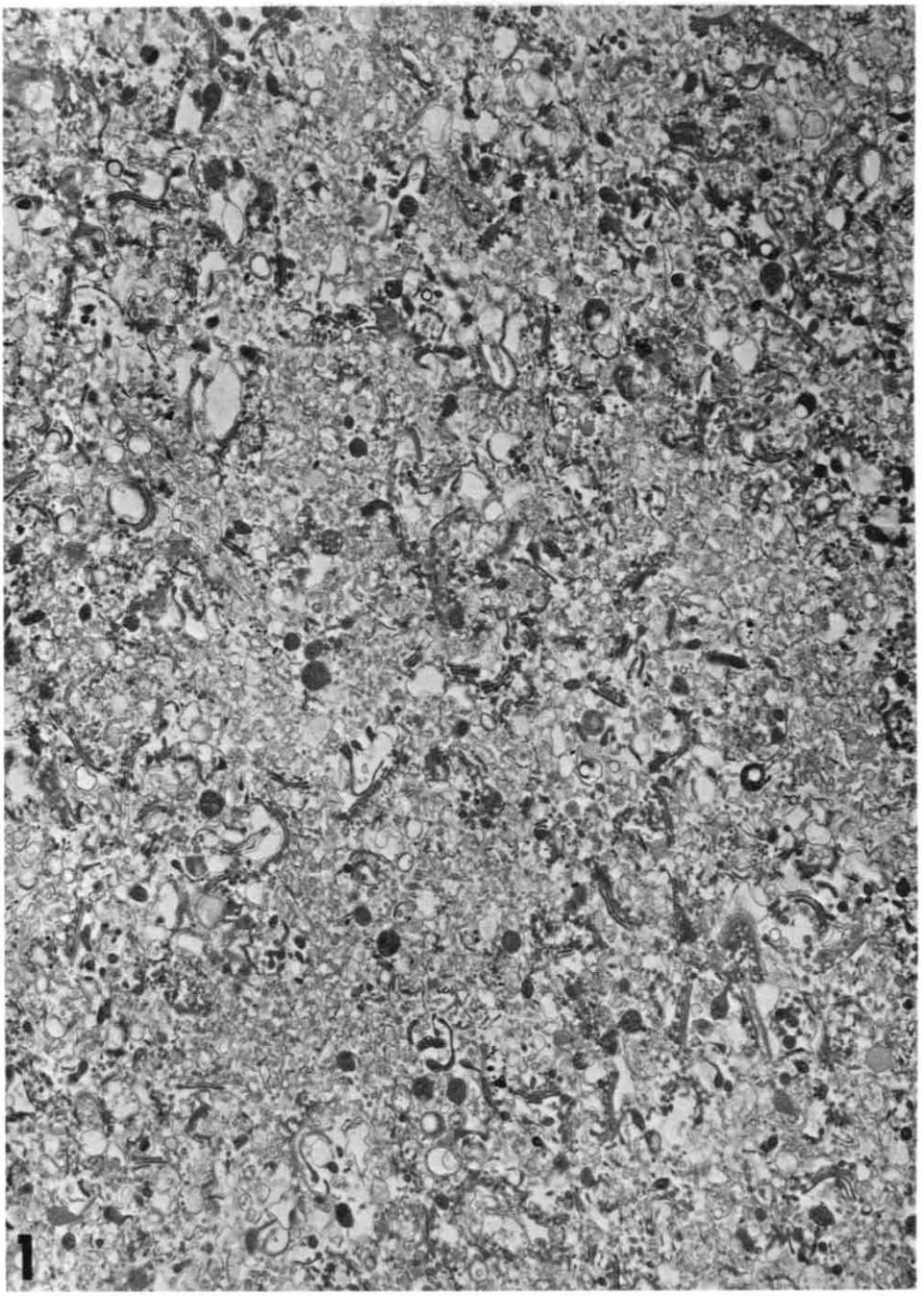
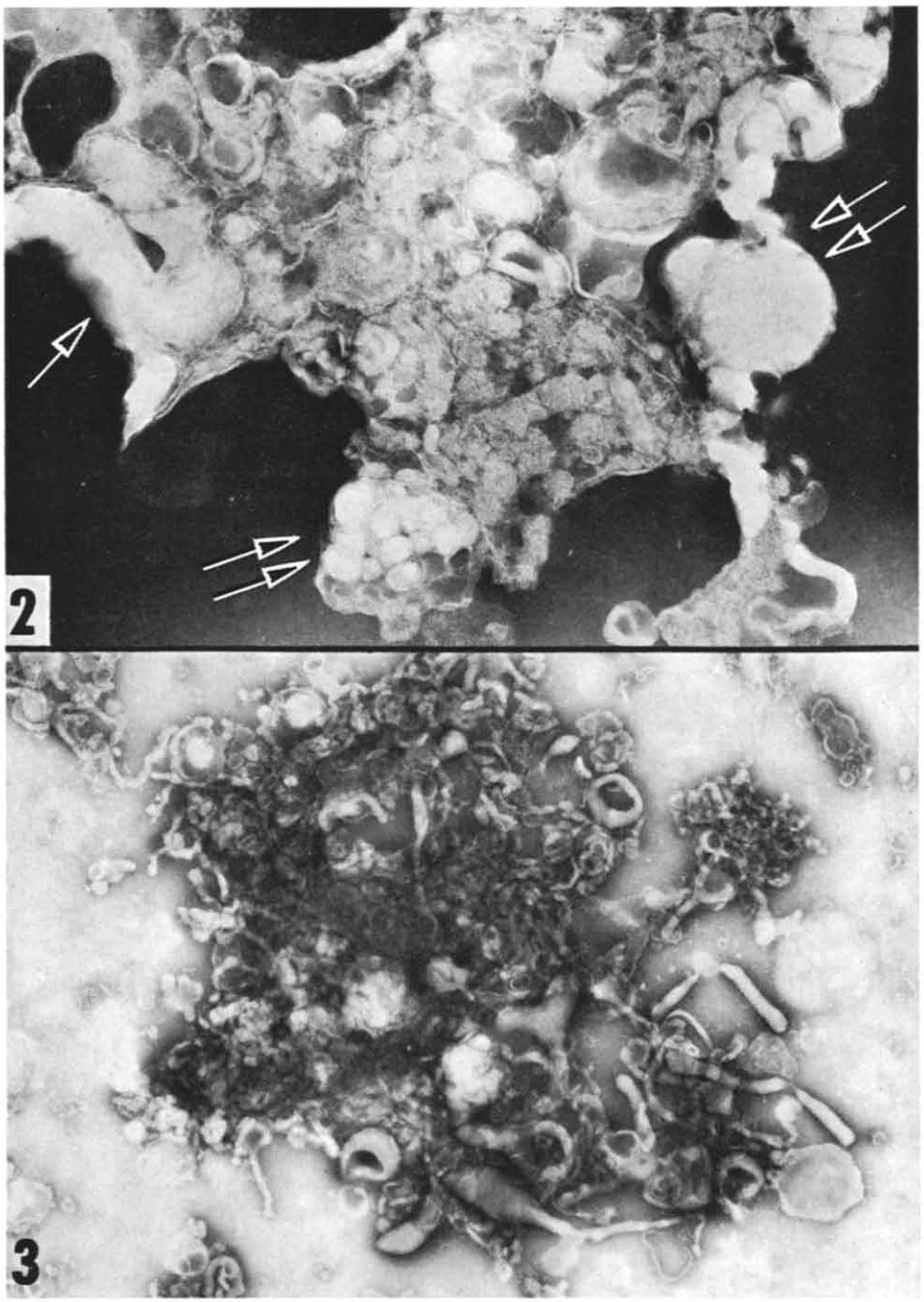


Fig. 1. Golgi fraction. Low magnification view. Thin section, 11 200 ×.



Figs. 2 and 3. Golgi fraction. Intricate arrays of anastomosing tubules (cysternae). Tubules (arrow) filled with and distended by a particulate material (probably plasma lipoproteins) can be seen in Fig. 2 at the periphery of the cysternae and may represent the secretory part of cysternae. Other tubules are connected with secretory vesicles (double arrow). Negative stains: Fig. 2, 68 500 ×; Fig. 3, 34 900 ×.

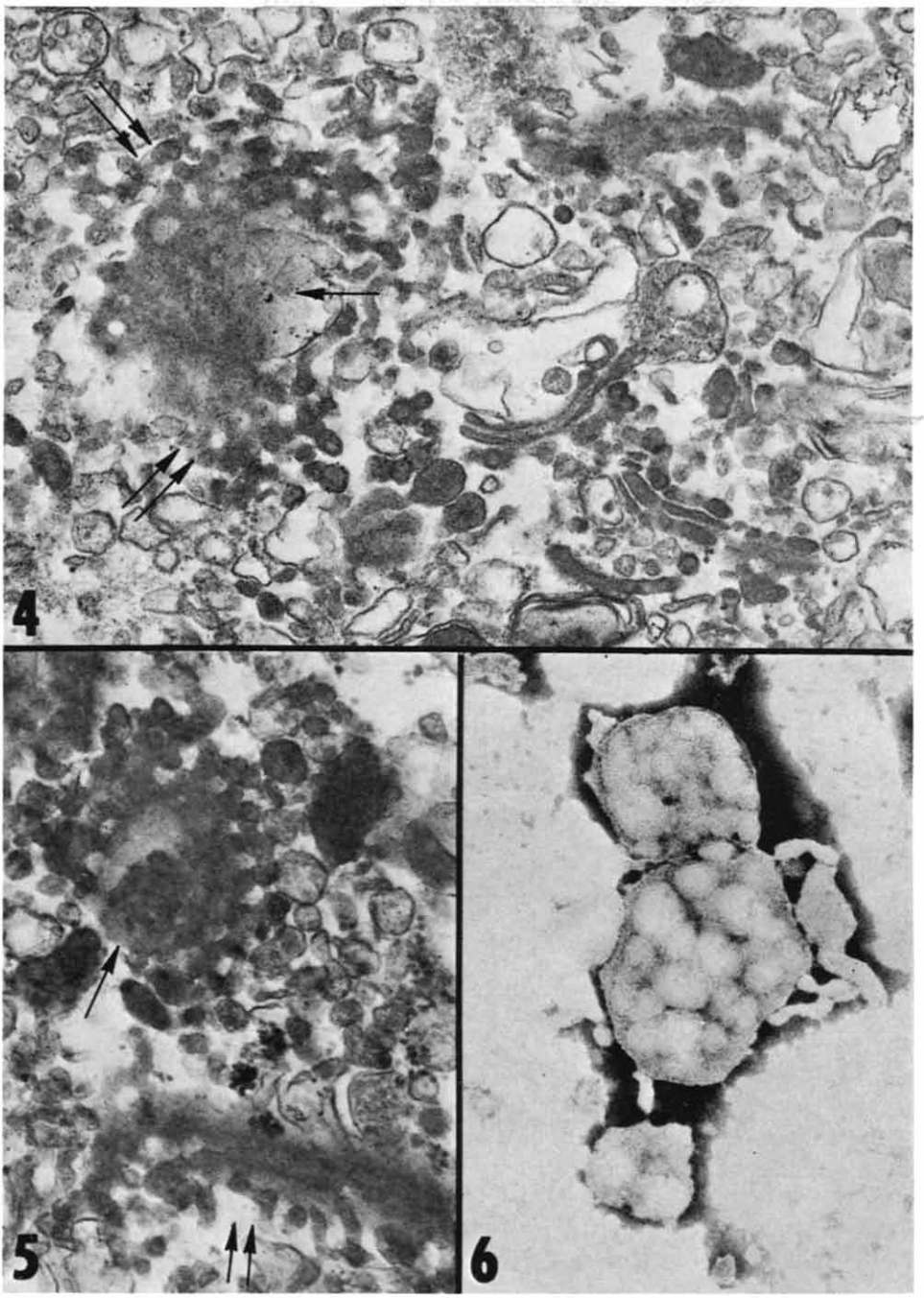
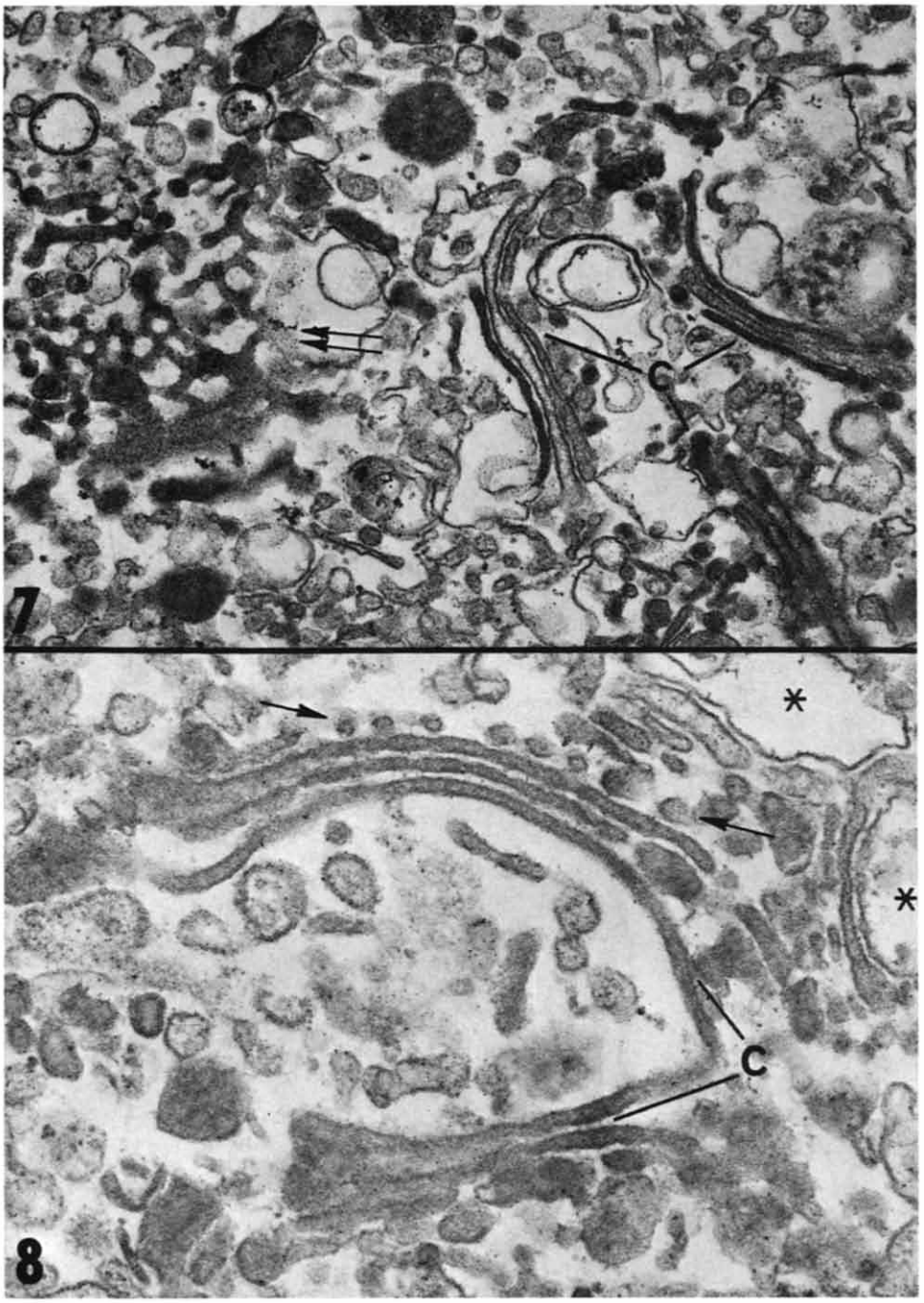


Fig. 4. Golgi fraction. Tangential section of a cysternae showing a central, disk-like region (arrow) surrounded by a network of anastomosing tubules (double arrows). Thin section, 52 300 ×.
Fig. 5. Golgi fraction. Two cysternae cut tangentially (arrow) and obliquely (double arrow). Thin section, 44 525 ×.
Fig. 6. Golgi fraction. Secretory vesicles filled with a particulate material. Negative stain, 77 000 ×.



Figs. 7 and 8. Golgi fraction. Cross sections of cysternae showing elongated and flattened sacs (C), or large and distended vesicles (*). Tubules (double arrows), or tubular profiles (arrows), are always seen in the vicinity of the cysternae. Thin sections: Fig. 7, 47000 ×; Fig. 8, 81000 ×.

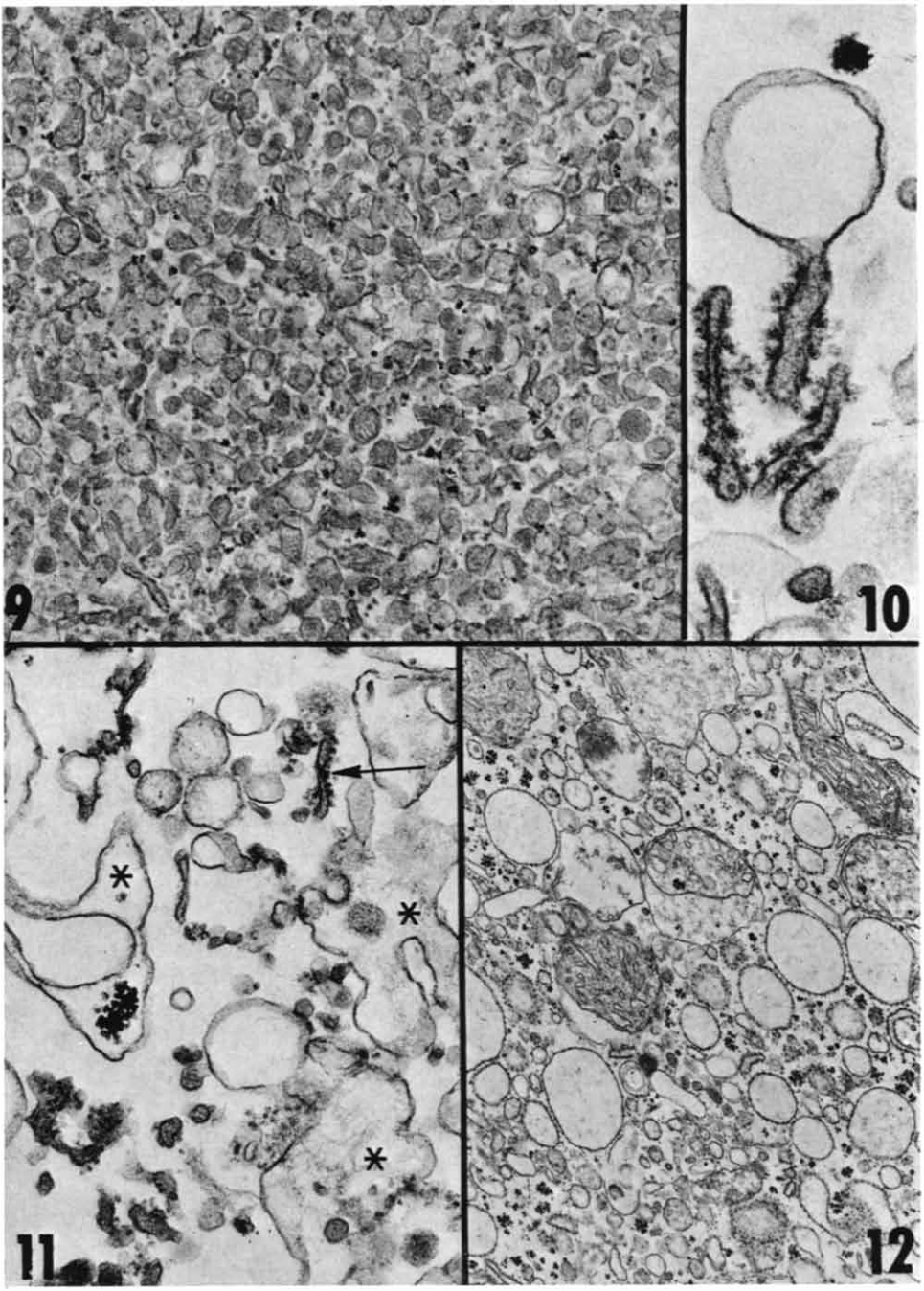


Fig. 9. Smooth endoplasmic reticulum fraction. Smooth surfaced vesicles. Thin section, 38 300 ×.

Fig. 10. Fraction 3. High magnification view of a transitional element of endoplasmic reticulum. Thin section, 80 400 ×.

Fig. 11. Fraction 3. Long, sheet-like smooth-surfaced membranes (*) resembling plasma membranes, and a transitional element of endoplasmic reticulum (arrow). Thin section, 38 350 ×.

Fig. 12. 1.3 M sediment. Mitochondria, rough microsomes and glycogen particles are easily recognized. Thin section, 17 450 ×.

In cross sections, instead, cysternae show as elongated and flattened sacs, often stacked (dictyosomes, ref. 18) and curved, and with pear-shaped or dilated ends (Figs. 1, 7 and 9). According to MOLLENHAUER AND MORRÉ[18], these elongated sacs are cross sections of the central, disk-like region of the dictyosome cysternae. Around these, or in their vicinity, tubular profiles are always present (Figs. 4, 7 and 8). Frequently, one or more of the stacked cysternae is dilated (Fig. 8). Secretory vesicles (Figs. 1, 2, 4, 6 and 7) appear as round or oval sacs, of various size, limited by a smooth membrane and filled with and distended by a material which, more often than not, has a globular or spherical shape (Figs. 2 and 6). This material may represent plasma lipoproteins[19–21]. Frequently, secretory vesicles have one or more tubules connected to their periphery (Figs. 4 and 6). Tubular profiles and small vesicles are then seen in great abundance in both negatively stained preparations and sections of this fraction (Figs. 1, 4, 5, 7 and 8). Most of these tubules and vesicles are associated with either cysternae or secretory vesicles and seem to be, at least in part, elements of, or cross section of, the tubular network connecting the cysternae of dictyosomes (Fig. 7). However, the identity of other tubules and small vesicles is less clear, and it is likely that they are elements of smooth endoplasmic reticulum.

Fraction 2 (smooth endoplasmic reticulum). In both negative stain preparations and sections of pellets, this fraction appeared to be fairly homogeneous, with small vesicles bounded by a smooth membrane (smooth microsomes) as the prevalent element (Fig. 9). However, tubular profiles similar in appearance to those associated with the Golgi cysternae in the previous fraction are present in discrete amounts. Transitional elements of rough endoplasmic reticulum and glycogen particles were also seen occasionally.

Fraction 3. This fraction appeared to be quite heterogeneous and to consist of: smooth microsomes, transitional elements of rough endoplasmic reticulum (Fig. 10), plasma membranes (Fig. 11), dense bodies resembling lysosomes, glycogen particles and an occasional disrupted mitochondrion.

Other fractions. Three of the layers obtained after centrifugation of the post nuclear supernatant over a cushion of 1.3 M sucrose were also examined in thin sections. The membrane felt consists of a mixture of the structural elements seen in Fractions 1–3. The 1.3 M infranatant contains mostly smooth microsomes, glycogen particles, free ribosomes and to a less extent rough microsomes and mitochondria. Mitochondria, glycogen and rough microsomes are the major components of the pellet forming at the bottom of the tubes (Fig. 12).

Protein and RNA content

The protein and RNA content of various subcellular fractions was determined in order to estimate the yield of the Golgi fraction, and its contamination with rough endoplasmic reticulum membranes and/or free ribosomes. As can be seen from Table I, the yield for the Golgi fraction and for the smooth endoplasmic reticulum fraction is about 3 mg of protein per g of liver. Approx. 40% of the whole-homogenate proteins is lost in the preparation of the post nuclear supernatant, while about 8% of the post nuclear supernatant proteins sediment in the membrane felt. The RNA data (Table I), show that the membrane felt contains only about 4% of the RNA present in the post nuclear supernatant. Thus, centrifugation of post nuclear supernatant over a cushion of 1.3 M sucrose is fairly effective in removing rough microsomes and

TABLE I

PROTEIN AND RNA CONTENT OF VARIOUS SUBCELLULAR FRACTIONS

Each figure represents the mean ± S.E. (3 preparations).

Fraction	*Protein (mg/g liver)*	*RNA (mg/g liver)*
Whole homogenate	152.0 ± 10.2	12.61 ± 2.10
Post nuclear supernatant	95.7 ± 2.7	7.56 ± 0.20
Membrane felt	7.2 ± 0.1	0.27 ± 0.03
1.3 M infranatant	9.5 ± 0.7	1.74 ± 0.11
1.3 M sediment	21.7 ± 1.0	6.60 ± 0.39
Golgi fraction	2.9 ± 0.1	0.098 ± 0.001
Smooth endoplasmic reticulum fraction	3.1 ± 0.1	0.140 ± 0.001

ribosomes from the felt; most of the RNA is in the 1.3 M sediment and infranatant, where the abundant presence of rough microsomes was revealed by electron microscopy (Fig. 12). Another redistribution of RNA occurs during centrifugation of the felt in the second discontinuous gradient, so that the Golgi fraction and the smooth endoplasmic reticulum fraction each contain 1.5–2% of the RNA in post nuclear supernatant. No attempt was made at estimating the relative contribution of bound and free ribosomes to the RNA contaminants in the Golgi and smooth endoplasmic reticulum fractions.

Enzyme activities

The activities of the following enzymes were measured: *N*-acetyllactosamine synthetase and DPT phosphohydrolase, as markers for Golgi membranes; AMP phosphohydrolase, as a marker for plasma membranes; glucose-6-phosphate phosphohydrolase for endoplasmic reticulum membranes, and succinate–cytochrome *c* reductase for mitochondria. As can be seen from Table II, essentially all the *N*-acetyllactosamine synthetase activity of post nuclear supernatant is membrane bound, and is almost quantitatively recovered in the membrane felt. The Golgi fraction contains more than 90% of the post nuclear supernatant activity, and the smooth endoplasmic reticulum fraction about 6%. The specific activity of the enzyme in the Golgi fraction is over 30 times greater than that in post nuclear supernatant*, more than twice that of the membrane felt and about 15 times as great as that in the smooth endoplasmic reticulum fraction. On the other hand, the specific and the total activity are very low in the 1.3 M infranatant and 1.3 M sediment, respectively, where rough microsomes collect according to the electron microscopic and RNA data. These activities may well be accounted for by the sedimentation of Golgi membrane in these layers. It seems likely, therefore, that *N*-acetyllactosamine synthetase is localized only in smooth membranes, and essentially in those of the Golgi fraction[22]. However, no evidence for such a localization was obtained for DPT phosphohydrolase (Table II). This

* The assay method used in this work is based on a direct determination of *N*-acetyllactosamine formed, rather than on subtraction of the radioactivity of D-galactose from that of the reaction product. Furthermore, we have observed that with the addition of Triton X-100 (0.5%) to the enzyme preparation considerably higher specific activities can be obtained, of the order of 150 nmoles of D-galactose transferred per h per mg protein.

TABLE II

N-ACETYLLACTOSAMINE SYNTHETASE AND DPT PHOSPHOHYDROLASE ACTIVITY OF VARIOUS SUBCELLULAR FRACTIONS

Each figure represents the mean ± S.E. (3 preparations).

Fractions	*N-Acetyllactosamine synthetase*		*DPT phosphohydrolase*	
	Total activity (nmoles galactose transferred per h per g liver)	*Specific activity (nmoles galactose transferred per h per mg protein)*	*Total activity (μmoles P_i released per h per g liver)*	*Specific activi (μmoles P_i released per h per mg protei*
Post nuclear supernatant	113.10 ± 1.51	1.12 ± 0.10	94.91 ± 0.98	0.98 ± 0.04
Supernatant*	0.95 ± 0.34	0.015 ± 0.001	4.77 ± 3.55	0.08 ± 0.05
Sediment**	111.07 ± 2.4	2.92 ± 0.21	87.70 ± 1.60	2.20 ± 0.15
Membrane felt	102.87 ± 1.81	14.37 ± 0.39	19.08 ± 0.65	2.71 ± 0.15
1.3 M infranatant	9.14 ± 1.97	0.90 ± 0.12	22.18 ± 0.38	2.22 ± 0.15
1.3 M sediment	1.24 ± 0.38	0.06 ± 0.01	27.98 ± 1.48	1.33 ± 0.04
Golgi fraction	102.67 ± 4.60	35.05 ± 1.73	8.22 ± 0.86	2.78 ± 0.15
Smooth endoplasmic reticulum fraction	7.50 ± 0.48	2.41 ± 0.22	9.04 ± 1.16	2.82 ± 0.19

* Supernatant obtained after centrifugation of post nuclear supernatant at 105000 × *g* for 60 min.

** Sediment obtained after centrifugation of post nuclear supernatant at 105000 × *g* for 60 min.

enzyme is definitely membrane-bound, since it is almost completely sedimented by centrifugation of the post nuclear supernatant at 105000 × *g* for 60 min. However, after centrifugation of the post nuclear supernatant over a cushion of 1.3 M sucrose roughly equal activity is found in the membrane felt, the 1.3 M infranatant and the 1.3 M sediment. Approx. 9 % of the DPT phosphohydrolase activity of post nuclear supernatant is present in either the Golgi or the smooth endoplasmic reticulum fraction. The specific activity of the enzyme in the latter two fractions is not very different from that in the membrane felt, 1.3 M infranatant or the post nuclear supernatant sediment, while in the 1.3 M sediment the specific activity is about 50 % of that in these fractions. Localization of DPT phosphohydrolase in the Golgi apparatus of rat liver cells had been suggested on the basis of histochemical evidence[23]. Recently, however, FLEISCHER *et al.*[22] measured this enzyme in different subcellular fractions and found that it is widely distributed. Our data support the findings of FLEISCHER *et al.*[22]. Data on AMP phosphohydrolase and glucose-6-phosphate phosphohydrolase are presented in Table III. The Golgi and smooth endoplasmic reticulum fractions contain 12 and 14 %, respectively, of AMP phosphohydrolase activity of the post nuclear supernatant. However, AMP phosphohydrolase activity has been reported to be present not only in plasma membrane but also in endoplasmic reticulum[24]. Furthermore, the specific activity of AMP phosphohydrolase for highly purified plasma membrane is about 47 μmoles P_i released per h per mg protein[25]. Thus, from the specific activity data of Table III, it can be calculated that the contamination of the Golgi and smooth endoplasmic reticulum fraction with plasma membrane is about 4 %. On the other hand, contamination of the Golgi fraction with smooth endoplasmic reticulum appears to be significant. Indeed, if one assumes that Golgi membranes have no glucose-6-phosphate phosphohydrolase, and given a specific activity 3.8 for this enzyme in smooth endoplasmic reticulum (Table III), then the Golgi

TABLE III

5'-AMPase AND GLUCOSE-6-PHOSPHATASE ACTIVITY OF VARIOUS SUBCELLULAR FRACTIONS

Each figure represents the mean ± S.E. (2–3 determinations).

Fractions	*AMPase*		*Glucose-6-phosphatase*	
	Total activity (μmoles P_i per h per g liver)	*Specific activity (μmoles P_i per h per mg protein)*	*Total activity (μmoles P_i per h per g liver)*	*Specific activity (μmoles P_i per h per mg protein)*
Post nuclear supernatant	42.47 ± 3.99	0.44 ± 0.02	231.80 ± 15.60	2.38 ± 0.08
Membrane felt	12.64 ± 0.33	1.80 ± 0.08	27.18 ± 4.76	3.83 ± 0.80
1.3 M infranatant	10.62 ± 0.60	1.06 ± 0.03	57.15 ± 10.2	5.89 ± 0.30
1.3 M sediment	21.87 ± 0.06	1.05 ± 0.03	127.2 ± 18.5	5.79 ± 0.41
Golgi fraction	4.95 ± 0.08	1.65 ± 0.03	4.56 ± 0.97	1.61 ± 0.37
Smooth endoplasmic reticulum	6.06 ± 0.46	1.89 ± 0.03	12.12 ± 0.60	3.80 ± 0.06

fraction appears to be contaminated with smooth endoplasmic reticulum membranes to the extent of 42 %. However, there is no evidence for the absence of glucose-6-phosphate phosphohydrolase in membranes of the Golgi apparatus which are thought to derive from those of the endoplasmic reticulum[26]. Therefore, the significance of these results is at present not clear. It is of interest to note that similar glucose-6-phosphate phosphohydrolase activities were found in Golgi preparations isolated with other methods[22,27]. Most of the glucose-6-phosphate phosphohydrolase activity of the post nuclear supernatant is recovered in the 1.3 M sediment and infranatant, where the enzyme shows also the highest specific activity.

The Golgi fraction and the 1.3 M sediment contained less than 0.5 % and more than 85 %, respectively, of the succinate–cytochrome *c* reductase activity present in the post nuclear supernatant (data not shown). The specific activity of the enzyme was 0.13 mμmole of cytochrome *c* reduced per min per mg protein in the Golgi fraction and 3.9 mμmoles in the 1.3 M sediment.

DISCUSSION

The Golgi apparatus of mammalian cells is a complex organelle, the components of which are constituted of smooth membranes and are closely associated with smooth endoplasmic reticulum[28]. It thus, seems likely that in preparing subcellular fractions, by more or less conventional methods, membranes of the Golgi apparatus are recovered in the so called smooth microsome fraction. Therefore, the latter would seem to be a particularly suitable starting material for the isolation and purification of Golgi membranes. The method herein described is based on such considerations. A felt of smooth membranes is first obtained by centrifugation of a post nuclear supernatant of rat liver over a cushion of 1.3 M sucrose[29], and is then fractionated in a discontinuous sucrose gradient. Two fractions, essentially, are thus obtained: one at the interphase between 0.5 and 1.1 M sucrose, and the other at the 1.1–1.25 M sucrose interphase. By both morphologic and enzymatic criteria, Fraction 1 appears to be quite rich in elements of the Golgi apparatus, while Fraction 2 consists mostly of smooth microsomes.

As pointed out by MORRÉ *et al.*[27], the initial homogenization of the tissue is quite critical for obtaining a good morphologic preservation of the Golgi elements. A mild homogenization is required, even though this leads to a sizeable loss of homogenate proteins (unbroken cells, *etc.*) during preparation of the post nuclear supernatant (Table I). Despite this loss, however, the yield of protein (3 mg/g liver, Table I) in the Golgi fraction is substantive, and considerably greater than that reported for other methods[22,27].

Work on the isolation of the Golgi apparatus of mammalian cells has to date been greatly hampered by the lack of specific enzyme markers. DPT phosphohydrolase is not suitable for this purpose (Table II). While this work was in progress, FLEISCHER *et al.*[30] reported the localization of *N*-acetyllactosamine synthetase in the membranes of a Golgi-rich fraction isolated from bovine liver, and suggested the use of this enzyme as a marker for Golgi membranes. Our data (Table II), as well as similar ones presented recently by MORRÉ *et al.*[27], support the findings and conclusions of FLEISCHER *et al.*[22,30], and extend them to rat liver. Indeed, it was found (Table II) that all the *N*-acetyllactosamine synthetase activity present in a post nuclear supernatant of rat liver is membrane bound, and that more than 90 % of it can be recovered in a single fraction, Fraction 1, which on electron microscopic examination was found to be very rich in elements of the Golgi apparatus. In this fraction the concentration of the enzyme was more than 30-fold greater than that in the post nuclear supernatant. A small amount of enzyme of low specific activity, was also present in the smooth endoplasmic reticulum fraction. This finding can probably be accounted for by contamination of the smooth endoplasmic reticulum fraction with Golgi membranes, and is not indicative of a localization of *N*-acetyllactosamine synthetase also in the membranes of smooth endoplasmic reticulum. It appears thus that this enzyme can indeed be used as a specific marker for Golgi membranes: on this basis Fraction 1 is quite rich in this type of membrane.

As for purity, the Golgi fraction seems to be contaminated with plasma membranes to an extent of only 4%, assuming an exclusive localization of AMPase in plasma membranes[31]. On the other hand, contamination with smooth endoplasmic reticulum appears to be more significant on the basis of glucose-6-phosphate phosphohydrolase activity while that with mitochondria and rough endoplasmic reticulum was negligible. Thus, the degree of overall contamination of the Golgi fraction compares quite favorably with that reported for other methods[22,32,33]. The procedure described here represents a relatively simple means whereby elements of the Golgi apparatus can be reproducibly isolated in a good yield and degree of purity. Although it has been applied so far only to rat liver, there seems to be no *a priori* reason why it should not prove successful for the isolation of Golgi structures from other sources.

Worthy of note is the presence in Fraction 3 of discrete numbers of transitional elements of endoplasmic reticulum (Fig. 10). These elements are characterized by small smooth-surfaced vesicles at the end of fragments of rough endoplasmic reticulum. *In situ*, they are seen along the boundary between the Golgi apparatus and the rough endoplasmic reticulum, and they are thought to play a role in the intracellular transport of proteins synthesized for export[23]. The amount of membranous material collecting in Fraction 3 is, however, very small, and was examined only by electron microscopy.

Since the work herein presented was completed, four pertinent papers were

published. FLEISCHER *et al.*[22] have described *in extenso* their method for the isolation of a Golgi-rich fraction by zonal centrifugation, as well as their original findings on the localization of *N*-acetyllactosamine synthetase. MORRÉ *et al.*[27] have reported the localization of the same enzyme in a Golgi-rich fraction isolated with their method from rat liver, as well as the lipid composition of such a fraction[35]. In the fourth paper, MOLLENHAUER *et al.*[36] state that they too met with difficulties and poor success in the isolation of a Golgi fraction from the liver of fed Sprague–Dawley rats. The difficulties were said to be overcome by feeding the animals a choline deficient diet, with or without 20% ethanol in the drinking water, for up to 7 months. The efficiency and nature of this regimen, however, are open to question since none of the known pathological alterations induced by either choline deficiency or ethanol were observed in the liver of these rats.

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