Biochimica et Biophysica Acta, 769 (1984) 171–178
Elsevier

BBA 71948

ISOLATION OF HIGHLY PURIFIED GOLGI MEMBRANES FROM RAT LIVER

USE OF CYCLOHEXIMIDE IN VIVO TO REMOVE GOLGI CONTENTS

JANET A. TAYLOR [a], ANTHONY R. LIMBRICK [b], DAVID ALLAN [a] and JACOB D. JUDAH [a]

[a] *Department of Experimental Pathology, The School of Medicine, University College London, University Street, London WC1E 6JJ and* [b] *Department of Biophysics, University College London, Gower Street, London WC1E 6BT (U.K.)*

(Received June 22nd, 1983)

Key words: Golgi membrane; Cycloheximide; Secretory product; Membrane lipid

Following administration of cycloheximide to rats in order to deplete the liver of secretory products, Golgi membranes have been isolated largely free of internal contents. These membranes have a high specific activity of galactosyltransferase (400 times that of the homogenate) and are thought to be derived from the *trans* Golgi. Their phospholipid and polypeptide composition resembles that of Golgi membranes prepared by other procedures but their triacylglycerol and cholesterol contents are greatly reduced. These results conflict with previous reports that *trans* Golgi membranes are rich in cholesterol.

Introduction

Although the membranes of the Golgi apparatus are known to be a vital part of the mechanism by which secretory proteins are exported from cells, analysis of the composition and enzyme activity of these membranes is not straightforward, largely because it is hard to isolate the membranes free of contents. These contents (mainly albumin and lipoproteins) usually account for the bulk of the protein and lipid of Golgi vesicles and mask the polypeptides and lipids which are characteristic of the Golgi membranes.

Jamieson and Palade [1,2] showed that continuing protein synthesis was not necessary for translocation of secretory proteins in the exocrine pancreas. This was also found to be true of rat-liver [3–5] from which approx. 80% of proalbumin was secreted within 30 min of the injection of cycloheximide [6]. These observations suggested to us that we could use cycloheximide to get rid of secretory proteins from the Golgi apparatus and thus make it possible to isolate Golgi membranes. The work reported here describes such a method for the isolation of highly purified Golgi membranes which unlike those prepared by alkaline extraction [7] retain high activity of the characteristic Golgi enzyme, galactosyltransferase, and are greatly depleted in cholesterol and triacylglycerol.

Materials and Methods

Preparation of Golgi fractions. The method of Bergeron [8] or of Quinn and Judah [9] was used to prepare light vesicle fractions (GF 1 + 2) and a heavy cisternal fraction, GF3 [10]. In most experiments, the method of Hino et al. [11] was used, except that the livers were first passed through a stainless steel press (Harvard apparatus Co., Dover, MA, U.S.A.) and homogenised in 0.5 M sucrose, containing 1% dextran (M_r 250000) and 50 mM Tris-maleate, pH 6.75, in a Dounce homogeniser (Blaessig Glass Co., Rochester, NY, U.S.A.) with five strokes of the loose pestle, followed by five strokes of the tight pestle. The homogenate was centrifuged at $1000 \times g_{max}$ for 5 min in the HB-4

rotor of the RC2B Sorvall centrifuge (Dupont Instruments, Hitchin, Herts., U.K.) to yield a 'nuclear' pellet. The supernatant together with a loosely packed superficial layer was poured off and centrifuged at $5000 \times g_{max}$ for 10 min (SS 34 rotor of the above centrifuge). The pellet was gently suspended in homogenising buffer and layered over 1.2 M sucrose in tubes of the SW41 Ti rotor (Beckman Instruments Ltd., Glenrothes, Scotland, U.K.) and centrifuged at $100000 \times g_{av}$ for 60 min. The Golgi fraction floated on the 1.2 M sucrose layer. It was removed and washed as described by Hino et al. [11]. This preparation is called BY-1 following the usage of Ref. 11 or BY-1C if obtained from a rat treated with cycloheximide.

Cycloheximide treatment. Cycloheximide (1.5 mg/kg, dissolved in 150 mM NaCl) was administered by intravenous injection through a tail-vein. The animals were killed 90 min later.

Purification of Golgi fractions on Ficoll gradients. In tubes of the SW41 Ti rotor, 12 ml linear Ficoll gradients were generated from 5% (w/w) and 15% (w/w) Ficoll 400 containing 1 mM EGTA and 5 mM Tris-HCl, pH 7.4 (measured at 20°C). On top of the gradient were layered 0.5 ml suspensions of Golgi fractions and the tubes then centrifuged at $200000 \times g_{av}$ for 16 h. The major Golgi fraction floated as a sharp band near the top of the gradient (approx. 8% (w/w) Ficoll). It was removed, diluted 5-fold with 250 mM sucrose, containing 1 mM EGTA and 5 mM Tris-HCl, pH 7.4 (at 20°C) and pelleted by centrifugation at $100000 \times g_{av}$ for 30 min. All centrifugations were done at 2°C.

Sodium carbonate extraction of Golgi vesicles was done as described by Howell and Palade [7].

Plasma membranes were prepared by the method of Dorling and Le Page [12]. Endoplasmic reticulum-enriched fractions (microsomes) were obtained as a by-product of the Golgi vesicle preparation [8].

Serum albumin was assayed by the 'rocket-plate' method [13].

Enzyme assay. UDPgalactose: ovalbumin galactosyltransferase was assayed by the method of Hino et al. [11], in the presence of 0.5% (w/v) Triton X-100. In the absence of detergent the activities of BY-1C were 5–10% of maximum. The activities of the membranes from Ficoll gradients (BY-1F and BY-1CF) were approx. 50% indicating a loss of latency after the long centrifugation. 5'-Nucleotidase, glucose-6-phosphatase and succinate dehydrogenase were assayed as described by Wisher and Evans [14], β-*N*-acetylhexosaminidase as described by Barrett and Heath [15] and thiamine pyrophosphatase as described by Cheetham et al. [16]. Protein was measured by the method of Lowry et al. [17], using bovine serum albumin as standard.

SDS-polyacrylamide gel electrophoresis was by the method of Laemmli [18].

Lipid analysis. Lipids were extracted according to Allan and Cockcroft [19]. Phospholipids and cholesterol were determined as described by Allan et al. [20]. Triacylglycerol was separated by TLC using hexane/diethyl ether/acetic acid (80 : 20 : 1, v/v) as solvent and was quantitated densitometrically after spraying plates with 20% ammonium sulphate and charring at 190°C for 30 min. A Joyce-Loebl Chromoscan was employed for densitometry.

Electron microscopy. Samples were fixed in suspension in 2.5% (w/v) glutaraldehyde for 60 min on ice. They were pelleted ($30000 \times g$ for 20 min) and post-fixed in 1% (w/w) osmic acid for 2 h. The pellet was dehydrated in ethanol and embedded in Araldite, and sections were stained in methanolic uranyl acetate followed by Sato's lead stain.

Animals. Female Wistar rats (about 180 g body wt.) were obtained from A Tuck & Sons, Battlesbridge, Essex, U.K.

Glucose-6-phosphate, AMP, UDPgalactose, ovalbumin, cytochrome *c*, sodium succinate and 4-nitrophenyl-*N*-acetyl-β-D-glucoseaminide were obtained from Sigma Chemical Co., Poole, Dorset, U.K.

UDP-[^{3}H]galactose and [^{3}H]AMP were obtained from Amersham International, Amersham, Bucks, U.K. Bovine serum albumin, A Grade, was from Calbiochem-Behring Corp., C P Laboratories, Bishops Stortford, Herts, U.K. Ficoll 400 was obtained from Pharmacia (Great Britain) Ltd., Hounslow, Middlesex, U.K. Dextran Grade A, (M_r (200–275)$\cdot 10^3$) was obtained from BDH Chemicals Ltd, Poole Dorset, U.K.

Results

Effect of cycloheximide on the separation of Golgi subfractions

Ehrenreich et al. [10] and Bergeron [8] have described methods for the preparation of Golgi vesicles rich in contents and in galactosyltransferase activity. Three fractions were described, called GF1 (the lightest) GF2 and GF3 (the heaviest) which differed in their density on sucrose gradients. The specific activity of galactosyltransferase was highest in GF1 and lowest in GF3 [8]. The vesicles of GF1 and GF2 are full of secretory proteins including albumin and lipoprotein whilst GF3 contains numerous collapsed cisternae. The effect of cycloheximide in vivo on the isolation of these subfractions is shown in Table I. GF1 + 2, the combination of the two light fractions has largely failed to appear: Only 15% of the galactosyltransferase and approx. 50% of the protein, as compared to the controls, was found in this fraction. The GF3 fraction, on the other hand, appears to be unchanged. Since the homogenate activity is unchanged (Table II), this result implies that the galactosyltransferase activity normally associated with GF1 + 2 is sedimenting to a higher density after cycloheximide treatment.

The above procedure was clearly unsatisfactory since it led to the disappearance of a well-defined GF1 + 2 fraction. An alternative method [11] for the isolation of a Golgi-rich fraction was therefore investigated and this gave much better results (Table II). The control (untreated) preparations were quite similar in composition to those reported [11]. Yields were lower due probably to a different method of homogenisation. Table III shows the recoveries of galactosyltransferase at different stages of the preparation. It appears that lysosomal contamination in our preparations was rather higher than in the original preparation but this could be due in part to our use of hexosaminidase as marker, whereas Hino et al. [11] used acid phosphatase, which may well have extralysosomal localisation [15]. In contrast to the results described above where the yield of galactosyltransferase in the GF1 + 2 fraction was drastically reduced when livers were pre-treated with cycloheximide, the yield of this Golgi marker enzyme in Golgi vesicles isolated by the method of Hino et al. [11] was actually increased following cycloheximide treatment. The specific activity of galactosyltransferase was also increased, presumably due to the decrease in secretory protein content. Additional purification of the preparations on Ficoll gradients resulted in a further increase in the specific activity of galactosyltransferase in both control and treated preparations. Approximately 60% of the galactosyltransferase applied to the Ficoll gradients was recovered in well-defined bands near the top of the gradient (approx. 8% (w/w) Ficoll). The purified Golgi fractions (BY-1F and BY-1CF) retain only 10–15% of the other four marker enzymes. These results are not due to a differential loss of enzyme activity since 85–100% of all the marker enzyme activities are recovered when the whole gradient is analysed.

From cycloheximide-treated rats, we recovered in the Ficoll-purified BY-1CF membrane fraction 20% of the total galactosyltransferase of the homogenate with only 0.05% of the protein i.e. a 400-fold increase in specific activity compared with the homogenate.

TABLE I

GALACTOSYLTRANSFERASE AND ALBUMIN CONTENT OF LIGHT (GF1+2) AND HEAVY (GF3) GOLGI FRACTIONS FROM CONTROL AND CYCLOHEXIMIDE-TREATED RATS

The Golgi fractions were obtained as described under Methods. Galactosyltransferase activity is expressed as nmol galactose transferred per h per mg protein.

	Control		Cycloheximide-treated	
	GF1+2	GF3	GF1+2	GF3
Galactosyltransferase	595 ±107	261 ±35	90 ±21	299 ±45
Protein (mg/rat liver)	1.7± 0.19	2.67± 0.27	0.89± 0.07	2.67± 0.37
Albumin (mg/mg protein)	0.16	0.05	< 0.02	0.03

TABLE II

ENZYME MARKERS OF GOLGI FRACTIONS FROM NORMAL AND CYCLOHEXIMIDE-TREATED RATS

The Golgi fractions BY-1 and BY-1C were prepared by the method of Hino et al. [11]. The purified Golgi fractions BY-1F and BY-1CF were obtained from these by centrifugation on Ficoll gradients. Enzyme activities are expressed as μmol product formed per h per mg protein, except galactosyltransferase which is expressed as nmol galactose transferred per h per mg protein. The values are generally means ± S.E. of eight determinations. Where S.E. values are not given, the values are the means of two analyses on different preparations. The figures in parentheses are the recoveries (%) from the homogenates.

	Control			Cycloheximide-treated		
	Homogenate	BY-1	BY-1F	Homogenate	BY-1C	BY-1CF
Galactosyltransferase	4.58 ± 0.4	306 ± 61 (17)	1100 (10)	5.5 ± 0.3	720 ± 73 (35)	2229 ± 95 (20)
Thiamine pyrophosphatase	0.15	1.9 (3.2)	3.7 (1.0)	0.17	2.0 (3.3)	4.4 (1.3)
5'-Nucleotidase	1.2 ± 0.13	4.9 ± 0.6 (1.0)	1.4 (0.05)	1.31 ± 0.1	7.0 ± 1.4 (1.5)	2.57 ± 0.19 (0.1)
Glucose-6-phosphatase	9.6 ± 2.2	5.85 ± 0.39 (0.15)	2.5 (0.01)	9.8 ± 1.0	6.0 ± 0.6 (0.17)	4.6 ± 0.6 (0.02)
Succinate dehydrogenase	1.11	0.27 (0.06)	0.09 (0.003)	1.28	0.32 ± 0.09 (0.07)	0.10 (0.004)
Hexosaminidase	2.40 ± 0.51	16.6 ± 2.6 (1.8)	2.1 (0.04)	2.75 ± 0.35	11.8 ± 1.1 (1.2)	2.53 ± 0.22 (0.05)
Protein (mg/rat liver)	1182 ± 74	3.00 ± 0.30 (0.25)	0.5 (0.04)	1022 ± 61	2.85 ± 0.29 (0.28)	0.50 ± 0.07 (0.05)
Albumin (mg/mg protein)	0.005	0.13	0.18	0.002	< 0.01	< 0.02

In two separate experiments (with and without cycloheximide in each case) thiamine pyrophosphatase activity was measured in the BY-1 fractions and again after centrifugation on Ficoll gradients. About 3% of the enzyme activity was recovered in the BY-1 with 10-fold enrichment over the parent homogenate, in good agreement with previous reports for rat liver [16,21]. After purification on Ficoll gradients the specific activity was increased a further 2-fold (Table II). This compares with a 3-fold increase in specific activity of galactosyltransferase after the Ficoll gradients.

TABLE III

RECOVERIES OF GALACTOSYLTRANSFERASE AT VARIOUS STAGES DURING ISOLATION OF GOLGI FRACTIONS

Each fraction was as described in Materials and Methods. Recoveries are expressed as percentage of galactosyltransferase activities in the homogenate, see Table II.

	Control	Cycloheximide-treated
Total homogenate	100	100
Nuclear pellet	36	43
5000 × *g* supernatant	47	17
5000 × *g* pellet	22	40
BY-fractions	17	36

Albumin content of Golgi fractions

Albumin content was used as a measure of the effectiveness of the cycloheximide. The albumin content of the various Golgi fractions was markedly reduced, by about 90% in GF1 + 2, BY-1C and BY-1CF as compared to controls, as shown in Tables I and II. Table II gives the albumin content of the homogenates for comparison.

Polyacrylamide gel electrophoresis

Fig. 1 shows densitometric scans of electrophoretograms derived from (a) control Golgi preparations, (b) the same after Na_2CO_3 extraction

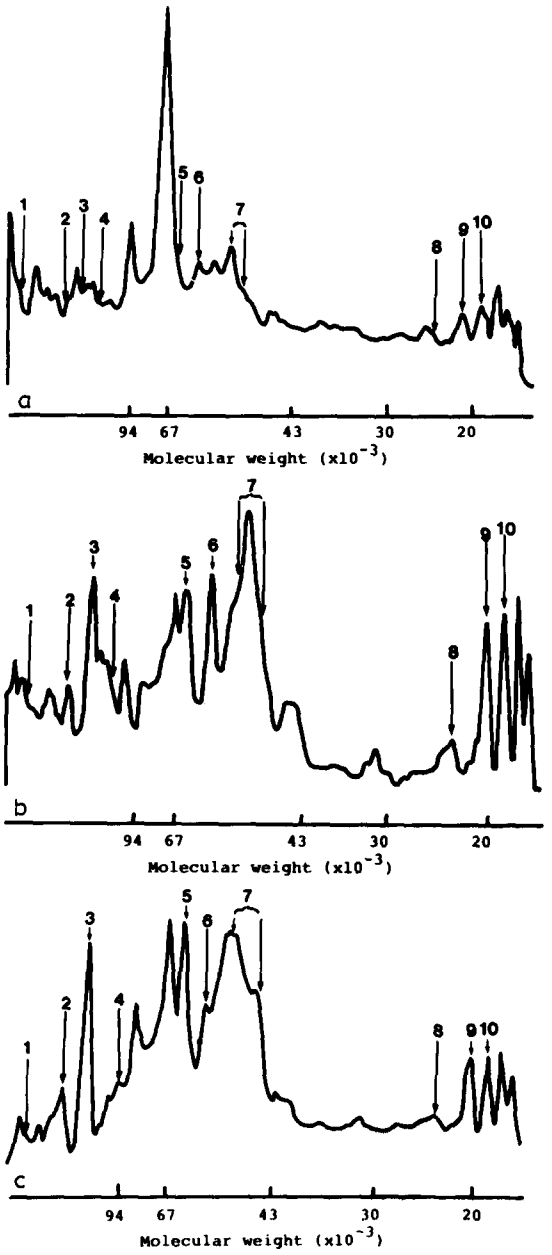

Fig. 1. Densitometric scans of SDS-polyacrylamide gel electrophoresis of Golgi proteins. (a) Control Golgi vesicles; (b) membranes obtained after Na_2CO_3 extraction of this fraction. (c) Golgi membranes prepared from cycloheximide-treated rats, and purified by centrifugation on Ficoll gradients. Arrows point to proteins which appear to be enriched in the Golgi membranes, as compared to track (a).

and (c) a Golgi membrane fraction obtained from a cycloheximide-treated rat and purified on a Ficoll gradient. As expected, the serum albumin band is much reduced both in the carbonate-extracted membranes and in those depleted in vivo. Bands in the two tracks which have increased in intensity when compared to controls may represent true Golgi membrane proteins. The results agree quite well with those reported for Golgi vesicles extracted with 100 mM Na_2CO_3 [7].

Lipid composition of Golgi membranes

The phospholipid and cholesterol contents of purified Golgi membranes derived from cycloheximide-treated rat liver (BY-1CF) have been compared with those of control Golgi vesicles, plasma membranes and endoplasmic reticulum (Table IV). The results show that the BY-1CF fraction has a lipid composition broadly similar to that of endoplasmic reticulum and very distinctly different from that of plasma membrane particularly with regard to cholesterol and sphingomyelin levels. This is not simply due to the effect of cycloheximide since the controls (BY-1 and BY-1F) are also lower in cholesterol and sphingomyelin than than GF1 + 2.

The phospholipid composition of Golgi membranes prepared by the cycloheximide procedure resembles the composition reported for Golgi membranes produced by other methods [7,22]. However, the cycloheximide procedure gives a product with much lower contents of cholesterol, triacylglycerol, sphingomyelin and lysophosphatidylcholine than either GF1 + 2 or Na_2CO_3-extracted GF1 + 2 (Table IV and Ref. 7).

Electron Microscopy

In the electron microscope it was clear that the Golgi-rich fraction (BY-1) consisted largely of tubular stacks of membrane with dense contents, together with a variety of larger dense vesicles (Fig. 2a). The same fraction derived from cycloheximide-treated livers (BY-1C) was not obviously different in morphology but there was a very pronounced decrease in the internal density of the membrane-bounded structures (Fig. 2b). This was consistent with the idea that cycloheximide treatment caused a reduction in the amount of secretory content material in the Golgi fraction.

Similarly, after centrifugation on Ficoll gradients the numerous vesicles and budding cisternae filled with secretory contents in the control BY-1F

TABLE IV

LIPID COMPOSITION OF MEMBRANE SUBFRACTIONS FROM NORMAL AND CYCLOHEXIMIDE-TREATED RATS

Samples of endoplasmic reticulum and plasma membranes were isolated from normal rat liver as described under Methods. BY-1 and BY-1F are Golgi fractions from normal rat livers before (BY-1) and after (BY-1F) purification on Ficoll gradients, BY-1C and BY-1CF are the corresponding preparations from cycloheximide-treated rats. Lipid determinations were as described under Methods and values are the means of duplicate measurements on a single preparation. Two further analogous experiments gave closely similar results. n.m., not measured.

	Lipid composition of membrane subfractions from rat liver							
	Endoplasmic reticulum	Plasma membrane	GF1+2	GF1+2 (Na_2CO_3)	BY-1	BY-1F	BY-1C	BY-1CF
Phospholipid (μg/mg protein)	573	521	602	1165	431	556	595	690
Cholesterol (μg/mg protein)	61	175	204	316	79	96	63	57
Triacylglycerol (μg/mg protein)	n.m.	n.m.	2240	965	995	1390	266	183
Cholesterol/ phospholipid (mol/mol)	0.20	0.63	0.64	0.51	0.35	0.32	0.17	0.18
Phospholipid classes (mol%)								
Lysophosphatidyl-choline	2	1	4	3	2	1	2	1
Sphingomyelin	7	17	6	9	3	2	2	2
Phosphatidylcholine	56	39	57	53	62	63	61	61
Phosphatidylserine	6	13	6	6	4	5	4	4
Phosphatidylinositol	10	6	8	8	7	8	7	8
Phosphatidyl-ethanolamine	20	24	21	21	21	21	23	24

(Fig. 2c) can be compared to the empty vesicles and cisternae of BY-1CF (Fig. 2d).

Discussion

From the livers of cycloheximide-treated rats it is possible to prepare Golgi membranes with a well-defined protein and lipid composition, greatly depleted of contents and showing a 400-fold increase in the specific activity of galactosyltransferase over the starting liver homogenate. Since we recover about 20% of the transferase and 0.05% of the protein of the homogenate, it may be calculated (if the enzyme was uniformly distributed) that the Golgi apparatus might constitute 0.25% of the total protein. This figure is in sharp contrast to estimates that the Golgi constitutes from 1.4% [11] to 3% [8] of the liver protein. The discrepancy could be due to the inclusion of contents protein and/or impurities in these latter figures, but it could also be consistent with the widely-accepted proposal that the transferase is unevenly distributed in the Golgi apparatus with low activity in the *cis*-Golgi and high in the *trans*-Golgi [23–29]. We suggest that our most purified preparation By-1CF represents a fraction of the *trans*-Golgi.

Our results with thiamine pyrophosphatase support this view. Although in rat liver, thiamine pyrophosphatase is distributed widely [16,21,30], in the Golgi apparatus it is restricted to the *trans*-Golgi [30]. However, in view of the fact that there is no convenient marker for the *cis*-Golgi membranes (but see Ref. 25) we cannot assess the *cis*-Golgi content of our fractions.

The advantage of the cycloheximide procedure is that it is relatively mild and yields a product with membranes and enzymes intact. It appears to offer substantial advantages over the recently described method for isolation of Golgi membrane by extraction of contents with Na_2CO_3 which de-

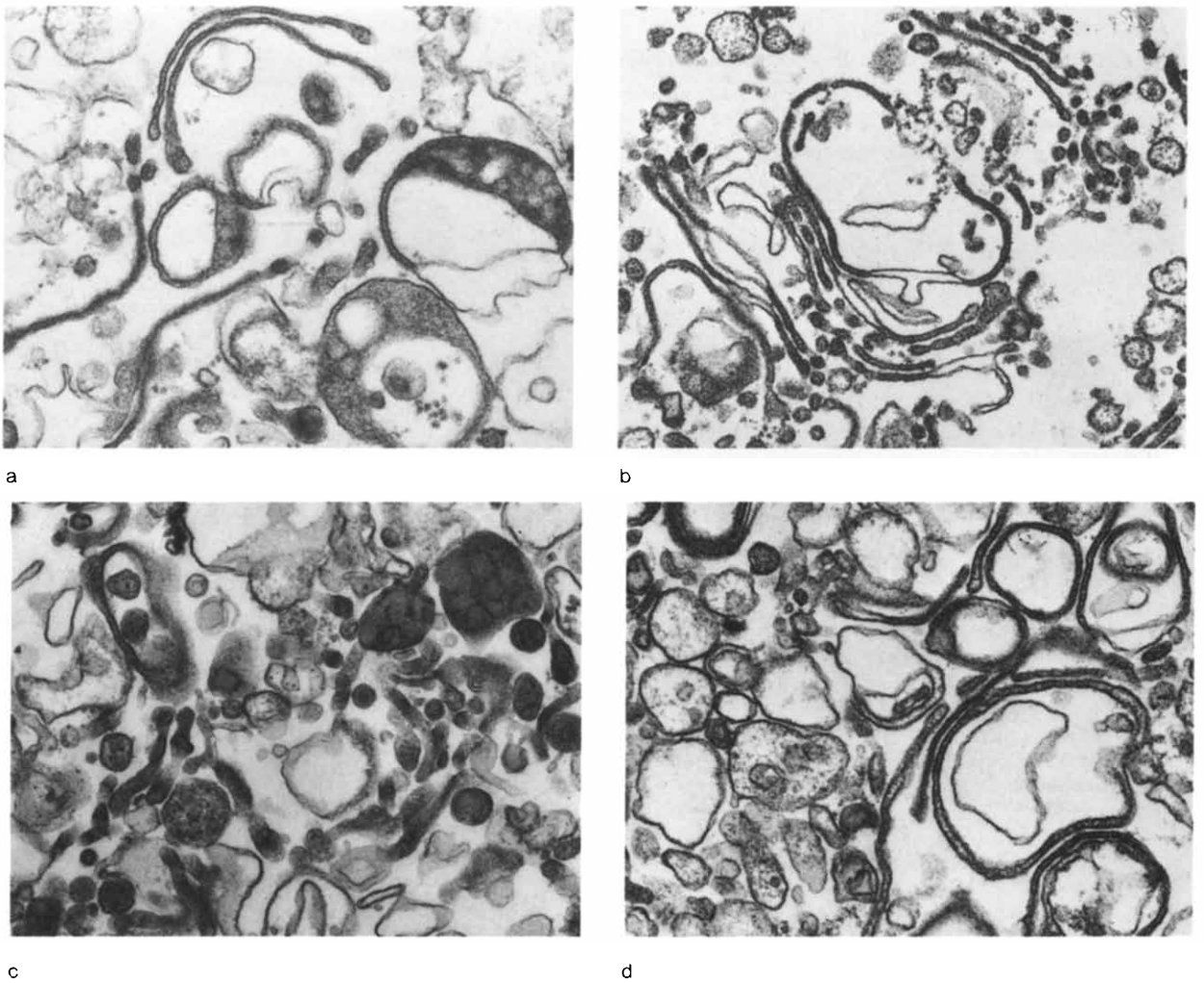

Fig. 2. Electron micrographs of Golgi fractions from control and cycloheximide-treated rats. (a) Golgi fraction BY-1 (from control rats); (b) Golgi fraction BY-1C, derived from rats after cycloheximide treatment. (c) Control after purification on a Ficoll gradient (BY-1F) and (d) the BY-1C fraction after purification on a Ficoll gradient (BY-1CF). Magnification ×34200×.

stroys galactosyltransferase activity, does not extract lipid contents effectively and which may cause enhanced deacylation of lipids leading to an increase in lysolipid (Ref. 7 and Table IV). The product of the cycloheximide treatment was very markedly depleted not only in triacylglycerol (which is undoubtedly a marker for lipoprotein content) but also in cholesterol which was generally present in very low amounts in BY-1C and BY-1CF (Table IV). Indeed our data suggest that the *trans*-Golgi membrane is little different from endoplasmic reticulum in terms of lipid composition and that free cholesterol may be a component of the contents rather than the membrane of the Golgi apparatus as isolated by the procedure of Hino et al. [11].

This interpretation contrasts with the claims of other workers that the *trans*-Golgi fraction represented by GF1 + 2 has a membrane which is rich in cholesterol [26,31]. If these claims are true, it would infer that there is a real difference between the membranes of GF1 + 2 and BY-1, which is perhaps related to a somewhat different intracellular origin for these two fractions.

It is not clear why cycloheximide treatment leads to an increase in recovery of galactosyl transferase activity in BY-1C but to a drastic decrease in the recovery of galactosyltransferase in GF1 + 2.

It seems very likely that a decrease in content of secretory material (particularly lipoprotein) would result in an increase in the density of Golgi membrane fractions. Possibly the procedure of Hino et al. which selects a higher density fraction of the Golgi allows recovery of the denser material which is lost from the GF1 + 2 fraction isolated by other procedures [8]. This explanation is compatible with the results shown in Table III.

Acknowledgements

This work was supported by grants from the Medical Research Council, the Vandervell Foundation and the Cancer Research Campaign.

References

1 Jamieson, J.D. and Palade, G.E. (1968) J. Cell Biol. 39, 580–588
2 Jamieson, J.D. and Palade, G.E. (1968) J. Cell Biol. 39, 589–603
3 Glaumann, H. (1970) Biochim. Biophys. Acta 224, 206–218
4 Judah, J.D. and Nicholls, M.R. (1970) Biochem. J. 116, 663–669
5 Geller, D.M., Judah, J.D. and Nicholls, M.R. (1972) Biochem. J. 127, 865–874
6 Dorling, P.R., Quinn, P.S. and Judah, J.D. (1975) Biochem. J. 152, 341–348
7 Howell, K.E. and Palade, G.E. (1982) J. Cell Biol. 92, 822–832
8 Bergeron, J.J.M. (1979) Biochim. Biophys. Acta 55, 493–503
9 Quinn, P.S. and Judah, J.D. (1978) Biochem. J. 172, 301–309
10 Ehrenreich, J.H., Bergeron, J.J.M., Siekevitz, P. and Palade, G.E. (1973) J. Cell Biol. 59, 45–72
11 Hino, Y., Asano, A., Sato, R. and Shimizo, S. (1978) J. Biochem. 83, 909–923
12 Dorling, P.R. and Le Page, R.N. (1973) Biochim. Biophys. Acta 318, 33–44
13 Weeke, B (1973) in A Manual of Quantitative Immunoelectrophoresis (Axelsen, N.H., Kroll, J. and Weeke, B., eds.), pp. 15–90, Universitetsforlaget Oslo, Bergen, Tromsö
14 Wisher, M.H. and Evans, W.H. (1975) Biochem. J. 146, 375–388
15 Barrett, A.J. and Heath, M.F. (1977) in Lysosomes: A Laboratory Handbook (Dingle, J.T., ed.), pp. 19–146, North Holland, Amsterdam, New York, Oxford
16 Cheetham, R.D., Moore, D.J., Pannek, C. and Friend, D.S. (1971) J. Cell Biol. 49, 899–905
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
18 Laemmli, U.K. (1970) Nature 227, 680–685
19 Allan, D. and Cockcroft, S. (1972) J. Lipid Res. 23, 1373–1374
20 Allan, D., Thomas, P. and Limbrick, A.R. (1980) Biochem. J. 188, 881–887
21 Leelavathi, D.E., Estes, L.W., Feingold, D.S. and Lombardi, B. (1970) Biochim. Biophys. Acta 211, 124–138
22 Zambrano, F., Fleischer, S and Fleischer, B. (1975) Biochim. Biophys. Acta 380, 357–369
23 Hino, Y., Asano, A. and Sato, R. (1978) J. Biochem. 83, 925–934
24 Hino, Y., Asano, A. and Sato, R. (1978) J. Biochem. 83, 935–942
25 Tartakoff, A.M. (1982) Trends Biochem. Sci. 7, 174–177
26 Rothman, J. (1981) Science 213, 1212–1219
27 Roth, J. and Berger, E.G. (1982) J. Cell Biol. 93, 223–229
28 Griffiths, G., Quinn, P.S. and Warren, G. (1983) J. Cell Biol. 96, 835–850
29 Quinn, P.S., Griffiths, G. and Warren, G. (1983) J. Cell Biol. 96, 851–856
30 Farquhar, M.G., Bergeron, J.J.M. and Palade, G.E. (1974) J. Cell Biol. 60, 8–25
31 Orci, L., Montesano, R., Meda, P., Malaisse-Lagae, F., Brown, D., Perrelet, A. and Vassalli, P. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 293–297