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SIMILARITIES OF THE GOLGI APPARATUS MEMBRANE AND THE PLASMA MEMBRANE IN RAT LIVER CELLS

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SUMMARY

A Golgi apparatus-rich fraction and a plasma membrane-rich fraction were isolated from a common homogenate of rat liver. Their respective buoyant densities, appearances in the electron microscope and 5′-nucleotidase and UDP-galactose ovalbumin galactosyltransferase activities were in accord with published data on separately isolated Golgi apparatus-rich and plasma membrane-rich fractions. Contamination by endoplasmic reticulum and mitochondria was low.

Gel electrophoresis of the membrane proteins of the Golgi apparatus-rich and plasma membrane-rich fractions (separately and mixed) showed a close similarity. After Neville's demonstration that electrophoretic patterns of membrane protein subunits from different subcellular fractions are easily distinguishable, the present work demonstrates an unusually close relationship between the Golgi apparatus membrane and the cell membrane. It is possible that membrane similarity may be mediated by the transfer of membrane-bound vesicles from the Golgi apparatus to the cell membrane.

INTRODUCTION

The Golgi apparatus plays a common role in many cell types. It is the site where sugar chains are attached to a variety of macromolecules (Northcote & Pickett-Heaps, 1966; Ronzio, 1973; Chabaud, Bouchilloux, Ronin & Ferrand, 1974). Vesicles, filled with the product of the particular cell type, bud off from the Golgi apparatus and migrate across the cell until they contact the cell membrane (Northcote & Pickett-Heaps, 1966; Neutra & Leblond, 1966; Cuminge & Dubois, 1972; Coulomb & Coulomb, 1973; Moussel & Moussel, 1973). The Golgi vesicular membrane usually fuses with the cell membrane (but see Neutra & Leblond, 1966) and the contents of the Golgi vesicle are deposited into the extracellular space. Examination of this widespread process, which seems an important route for the export of macromolecules out of the cell, suggests that as the Golgi vesicle membrane originates in the Golgi apparatus and terminates in the plasma membrane, there should be features common to the membranes which delineate the Golgi apparatus and the cell itself.

Neville & Glossmann (1971) have shown that the protein subunit composition of any membrane is unique. Although erythrocyte ghosts, kidney brush border

membranes and liver plasma membranes of rat may have a few common protein subunits – 'the differences are so striking that a single glance at the gel pattern is sufficient to identify the source of the membrane'. Although the protein subunit compositions of kidney and liver mitochondrial membranes are similar, they show few or no bands in common with the plasma membranes. The protein subunit composition of endoplasmic reticulum is also different from either plasma membrane or mitochondrial membrane.

This paper compares the protein subunit compositions of a Golgi apparatus-rich fraction and a plasma membrane-rich fraction prepared from a common homogenate of rat liver.

METHODS

Fractionation

Male Wistar rats, weighing about 200 g and fed *ad libidum* were used. All sucrose solutions were prepared in 89·5 mM K_2HPO_4, 10·5 mM citric acid, pH 7·0. The rat was killed by neck-breaking and its liver was dissected. Seven grammes of liver were quickly minced with a razor blade and transferred to 15 ml ice-cold buffered, 14·6 % (w/w), sucrose (d = 1·067 g cm^{-3}). After 10 min of gentle agitation, the sucrose solution was decanted and the diced liver was transferred to 10·5 ml ice-cold buffered, 14·6 % (w/w), sucrose in a stout tube of diameter 2·5 cm.

Tissue was homogenized with a Polytron PT20 (Camlab, Cambridge) modified by turning down the removable rotating head to give a radial clearance of 1 mm. The liver was homogenized for 90 s to 2 min at tap 0·6. Homogenization was so gentle that the tube had to be manipulated around the Polytron head. Homogenization was completed when the last distinguishable particle of liver had disappeared. Further homogenization, up to a total time of 3 min, did not cause any detectable differences in the collected fractions. The homogenate was made up to 24 ml with 14·6 % (w/w) buffered sucrose, and then centrifuged for 10 min at 600 **g**. The supernatant designated fraction 1S was recovered in 17 ± 0·5 ml. The precipitate, mainly red coloured with a thin top layer of white material was designated fraction 1 PPT. The supernatant (1S) was applied to step sucrose gradients in 3 centrifuge tubes. The gradient of buffered sucrose was made of: 4 ml 23·7 % w/w (d = 1·108 g cm^{-3}); 4 ml 30·8 % w/w (d = 1·137 g cm^{-3}); 4 ml 33·8 % w/w (d = 1·154 g cm^{-3}); and 4 ml 38·1 % w/w (d = 1·175 g cm^{-3}). The tubes were spun at 27000 rev/min (105000 **g** max.) in a 3 × 25 ml swing out head of an MSE Superspeed 50 for 2 h. Visible bands appeared at the d = 1·108/1·137 g cm^{-3} interface (designated the Golgi apparatus-rich fraction, G), and the d = 1·154/1·175 g cm^{-3} interface (designated a plasma membrane-rich fraction, PM). A much fainter band appeared at the intermediate d = 1·137/1·154 g cm^{-3} interface (designated the intermediate fraction I).

Additionally in each tube there was a precipitate designated fraction 2PPT.

Enzymic activities

The homogenate and all fractions were assayed for enzymic activity.

Prior to assay, the fractions were washed in 7 mM Tris pH 7·2, although except for determination of glucose-6-phosphate phosphohydrolase activity, where background phosphate of the buffer masked enzyme activity, the washing was not essential. The fractions were made up to 1 mg protein/ml. 5′-Nucleotidase was assayed by the method of Newby, Luzio & Hales (1975). Samples (5 μl) of homogenate or subcellular fractions were incubated for 1 h at 37 °C with 500 μl assay mixture containing 50 mM Tris (adjusted to pH 8·0 with HCl), 2 mM magnesium chloride, 0·2 mg adenosine, 200 μM AMP and tracer ^{3}H-AMP (20000–30000 cpm). The incubation was terminated by the addition of 100 μl 0·15 M zinc sulphate. Unhydrolysed AMP was precipitated by addition of 100 μl 0·15 M barium hydroxide; 500 μl of supernatant were added to 5 ml of scintillant prepared by adding 8 g PPO, 200 mg POPOP and 1 l. Triton

X-100 to 2 l. toluene. Glucose-6-phosphate phosphohydrolase was measured by the method of de Lamirande, Morais & Blackstein (1967). Cytochrome-c oxidase was measured by the method of Cooperstein & Lazarow (1951). UDP-galactose ovalbumin galactosyltransferase was assayed by a modification of the method of Freilich, Richmond, Reppucci & Silbert (1975). To 100 μl of reagent (25 mM Tris pH 6·4, 2·0 mg ovalbumin, 10 mM $MnCl_2$, 0·1 % Triton X-100, UDP-[^{14}C]galactose, 30000 dpm sp.act. 297 mCi/mmol) was added 10 μl enzyme. After incubation at 37 °C for 1 h, the reaction was terminated with 3 ml ice-cold 10 % trichloroacetic acid (TCA). The precipitate was washed in 10 % TCA (×5) at the centrifuge and then resuspended in 1 ml of 2 % sodium dodecyl sulphate (SDS) in 8 M urea at 50 °C for 3 h. Radioactivity was counted in 10 ml of scintillant consisting of 5 g PPO + 0·2 g POPOP in 1 l. toluene plus 500 ml Triton X-100. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) against bovine serum albumin as standard.

Gel electrophoresis

For gel electrophoresis, the fractions were centrifuged in 150 mM NaCl at 105000 **g** for 1 h (×2), and then resuspended in 50 mM Na_2CO_3 to a final protein concentration of 2 mg/ml. The fractions were then treated in a similar fashion to that described by Neville (1971). Fractions were exposed to 8 mg SDS per mg of protein and 10 % by volume of β-mercaptoethanol, and heated on a boiling water bath for 1 min, when the suspension clarified. They were then dialysed overnight in the cold against 0·04 M boric acid, 0·041 M Tris, 0·1 % SDS, 0·05 % dithiothreitol, 2 % sucrose. Three buffer systems were used for discontinuous electrophoresis.

(*a*) Upper reservoir buffer: 0·04 M boric acid, 0·041 M Tris, plus 0·1 % SDS.

(*b*) Spacer gel buffer: 50 ml 0·2164 M Tris titrated against 0·1 N H_2SO_4 to pH 6·1 and then made up to 200 ml. 3·2 g Acrylamide, plus 0·2 g *N*,*N*′-methylenebisacrylamide (Bis) dissolved in 100 ml spacer gel buffer make the spacer gel solution. To polymerize the spacer gel 20 μl TEMED, plus 100 μl 5 % ammonium persulphate were added to 10 ml spacer gel solution.

(*c*) Lower reservoir buffer: 0·0308 N HCl, plus 0·4244 M Tris. 11·1 g Acrylamide, plus 0·1 g Bis in 100 ml of lower reservoir buffer make the separation gel solution. To polymerize the separation gel 30 μl TEMED, plus 200 μl 5 % ammonium persulphate were added to 20 ml of separation gel solution.

Gels were run in glass tubes 8 cm long, i.d. 5 mm scrupulously cleaned with chromic acid followed by immersion in detergent in an ultrasonic bath. After applying and gelling the separation and spacer gels and filling the upper and lower reservoirs, current was applied at 0·5 mA/tube for 10 min, then 1·5 mA/tube to complete the run in about 2 h. During the run, the Kohlrausch fronts between the separation gel buffer/spacer gel buffer and spacer gel buffer/upper reservoir buffer, were clearly visible. At the end of the run, the gels were taken out of the tubes and stained with Coomassie blue (Weber & Osborn, 1969). Gels were stored in 7·5 % acetic acid. Every run was regulated against 20 μl of a mixture of marker proteins each in an amount of about 2 μg. The mixture had been treated identically to the cell fractions and consisted of: lysozyme (14300), carbonic anhydrase (29000), lactate dehydrogenase (36000), ovalbumin (43000), pyruvate kinase (57000), phosphorylase a (94000), β-galactosidase (130000) (polypeptide chain molecular weight quoted by Weber & Osborn, 1969).

Electron microscopy

Most fractions in most trials (70) were examined in the electron microscope. The fractions were fixed in 20 vol. of 2·5 % glutaraldehyde in 67 mM potassium phosphate (pH 7·4), containing 0·1 mM $CaCl_2$ and were then pelleted at 1200 **g** max. for 10 min. They were postfixed in 1 % OsO_4 in 0·1 M sodium cacodylate adjusted to pH 7·0 and dehydrated through an ethanol series which was more gradual than is commonly used (10 %, then in 10 % increments to absolute alcohol). The purpose of this gradual dehydration was to prevent breaking up of the delicate pellet. The pellets were taken, gradually, into 100 % epoxypropane at which stage they were quite firm, and then transferred into Araldite. Sections were cut on a Cambridge

Huxley Mk. II ultramicrotome. It was particularly noticeable that the designated Golgi apparatus-rich fraction was very difficult to section. The other fractions were not. Sections were stained in lead citrate (Reynolds, 1963) at 30 °C for 25 min in the staining pot (Hodson, 1974). They were examined in a Philips EM 300.

Chemicals

Lactate dehydrogenase and pyruvate kinase were purchased from the Boerhinger Corporation. Carbonic anhydrase, lysozyme, ovalbumin, phosphorylase a, β-galactosidase, bovine serum albumin and sodium dodecyl sulphate (SDS) were purchased from Sigma. UDP-[^{14}C]galactose and ^{3}H-AMP were purchased from the Radiochemical Centre, Amersham. PPO, POPOP, toluene and Triton X-100 were purchased from Koch-Light. Acrylamide, *N,N'*-methylene-bisacrylamide (Bis) were purchased from B.D.H. All reagents used for electron microscopy were purchased from Taab. All other chemicals used in this work were purchased from Hopkin and Williams.

RESULTS

Electron microscopy

The Golgi apparatus-rich fraction (*G*). Examination of representative sections through 32 preparations revealed only smooth membrane structures. Mitochondria and rough endoplasmic reticulum were not recognized. The pellet was always loosely packed (Fig. 1) but most elements of the pellet showed the 3 or 4 stacked cysternae which are characteristic of the liver Golgi apparatus. The 2 characteristic Golgi vesicles of the hepatocyte (the 0·22–0·30 μm diameter vesicle filled with very low density lipoproteins, arrowed Fig. 2, and the 50-nm diameter vesicle, arrowed Fig. 4) were present in the isolated Golgi apparatus-rich fractions (arrowed, Figs. 3, 5). The flattened cysternae of isolated Golgi bodies were less regular (double arrows, Fig. 5) than when they were *in vivo*. Smooth-membraned vesicles in the Golgi apparatus-rich fraction (arrow, Fig. 1) contributed less than 5 % of total membrane.

The plasma membrane-rich fraction (*PM*). The fraction consisted mainly of smooth-membraned vesicles (Figs. 6, 7). An occasional mitochondrion or fragment of rough endoplasmic reticulum contaminated the fraction. The smooth-membraned vesicle ranged in diameter from 0·14 to 5·0 μm, but the medial vesicle was about 0·7 μm in diameter. Most of the vesicles contained no obvious inclusions, other than small 'pegs' of material which have been widely interpreted as microvilli, and their membranes seemed flaccid. About 10 % of the vesicles were filled with what might be cytoplasm (arrow, Fig. 7). After washing with 0·9 % (w/v) NaCl solution only empty vesicles were seen in the fraction.

The intermediate fraction (*I*). This fraction showed no unique features. About 10 % of the profiles were of Golgi bodies and the rest were empty vesicles (medial diameter about 1·0 μm). The intermediate fraction looked like an overlap of the heavy tail of fraction G and the light tail of fraction PM. Nuclei, glycogen, mitochondria and rough endoplasmic reticulum were seen in the first precipitate. Rough endoplasmic reticulum and mitochondria with some glycogen were seen in the second precipitate.

Enzymic activities

The results are presented in Table 1 as the specific activity of each fraction together with the total protein. The cytochrome oxidase and glucose-6-phosphatase were markers for mitochondria and endoplasmic reticulum. In the Golgi, intermediate and plasma membrane fractions the marker enzymes showed considerably lower specific activities than either the tissue homogenate or the supernatant from which they were prepared.

UDP-galactose ovalbumin galactosyltransferase activity was almost completely recovered in the Golgi apparatus-rich fraction. Of the total activity in the homogenate, about 70% was recovered in the supernatant of the first spin. The remaining activity was located in the precipitate. Nearly all the galactosyltransferase activity could be eluted from the precipitate by a second wash and this second wash activity banded at the same density as the Golgi apparatus-rich fraction (G). The galactosyltransferase activity in the first precipitate seems to represent simple entrapment of a small part of the Golgi apparatus-rich fraction. Nearly all the galactosyltransferase activity in the supernatant ($\triangleq$ 110%) banded in the Golgi apparatus-rich fraction (G). The specific activity of this fraction showed greater than 50-fold enrichment over the homogenate. In contrast, the plasma membrane-rich fraction showed a 3-fold depression of specific activity over homogenate. Some activity (3%) was present in the intermediate density band (I) of specific activity 8-fold greater than the homogenate.

Specific activity of 5′-nucleotidase was highest in the plasma membrane-rich fraction (PM). The specific activity was 10-fold enriched over the homogenate and 22-fold enriched over the supernatant from which it was prepared. Golgi apparatus-rich fraction (G) also showed a persistent enrichment of its 5′-nucleotidase specific activity over the homogenate, but it was much less than that of fraction PM. In spite of the enrichment only 7% of total 5′-nucleotidase activity was recovered in fraction PM. Most of the activity was found in the first (68%) and second (37%) precipitates.

Gel electrophoresis

Protein patterns of the saline-washed membranes of the Golgi apparatus-rich and plasma membrane-rich fractions were examined. Aliquots were run singly and in equal weight mixtures (total weight in the range 5–100 μg protein) from fractions isolated from 12 separate rats. The Golgi apparatus-rich fraction patterns isolated from the 12 separate rats were not distinguishable. There was some slight variation in the plasma membrane-rich fraction. In all cases, the patterns of the Golgi apparatus-rich fraction and the plasma membrane-rich fractions were similar. Figs. 8–10 show patterns from the 2 fractions isolated from the rat livers which showed the greatest difference. Each figure shows the patterns of 3 gels after application of a fixed quantity of membrane protein, coded G (membrane proteins of the Golgi apparatus-rich fraction), PM (membrane proteins of the plasma membrane-rich fraction), or G+PM (mixed half weights of G and PM). Fig. 8 shows gels after 12 μg protein application. The 7 major bands run and stain identically. Fig. 9 shows

Table 1. *Enzyme activities and total protein of rat liver tissue fractions*

	First spin			Second spin			
	Homogenate	Supernatant	1st p.p.t.	G	Intermediate	PM	2nd p.p.t.
Protein/g liver, mg	121 ± 4	70 ± 6	57 ± 5	1·76 ± 0·41	0·31 ± 0·11	0·66 ± 0·20	7·2 ± 2·1
5′-nucleotidase (4)*	2·1 ± 0·3	1·0 ± 0·2	2·6 ± 0·3	5·1 ± 0·7	8·7 ± 0·2	22·1 ± 4·0	11·5 ± 1·2
Galactosyltransferase (5)†	197 ± 23	235 ± 43	82 ± 12	10266 ± 881	1684 ± 430	69 ± 45	64 ± 15
Cytochrome oxidase (3)‡	43·2 ± 12·1	58·8 ± 6·3	37·4 ± 11·2	4·0 ± 0·3	11·7 ± 2·8	16·2 ± 4·7	315·6 ± 84·2
Glucose-6-phosphatase (4)§	2·8 ± 0·2	3·2 ± 0·2	2·7 ± 0·3	1·1 ± 0·1	1·4 ± 0·1	2·3 ± 0·4	6·8 ± 0·3

Activity: * μmol adenosine liberated/mg protein/h.
† dpm/mg protein.
‡ $\Delta \log_{10}$ ferrocytochrome c/mg protein/min.
§ μmol phosphate liberated/mg protein/h.
Numbers in parentheses represent the numbers of trials (each in triplicate). Values are given ± standard deviation.

gels after 20 μg protein application. The extra band in gel PM is arrowed. This band is only faintly visible in gel G and is visible in the mixture (G+PM). Fig. 10 shows gels after 70 μg protein application. The major band difference is again arrowed. All 12 isolations were treated by this method of increasing protein application. In the fractions illustrated in Figs. 8–10 the major difference appears in band 8 (protein bands numbered in terms of decreasing visibility). In two isolations the major difference appeared in band 12 (again at the same R_f position in the gel). In one gel there was no distinguishable difference in the first 26 bands. Up to 36 bands could be distinguished in each fraction. The major 7 bands of all gels had the following molecular weights (in decreasing order of their staining capacity with Coomassie brilliant blue): 1, 53000; 2 and 3 (nearly equal), 63000 and 85000; 4 and 5 (nearly equal), 59000 and 103000; 6, 70000; and 7, 98000.

DISCUSSION

The purity of the Golgi apparatus-rich fraction and plasma membrane-rich fractions

The Golgi apparatus-rich fraction and the plasma membrane-rich fraction of rat liver float at different buoyant densities. Golgi apparatus-rich fractions are normally prepared from rat livers homogenized in hypertonic sucrose (0·5 M). Plasma membrane-rich fractions are prepared from rat livers homogenized in either hypotonic solutions (1 mM $NaHCO_3$) or isotonic sucrose (0·25 M). The buoyant density of the plasma membrane fraction seems to be independent of the osmotic pressure of the homogenization medium but the same is not true for the Golgi apparatus-rich fraction. The method described here, which isolates the 2 fractions from a common homogenate, is based on these considerations. The tissue was homogenized in 0·45 M sucrose.

The Golgi apparatus-rich fraction after homogenization in hypertonic sucrose is reported to collect in the density range 1·064–1·162 g cm^{-3} (Mahley, Hamilton & LeQuire, 1969; Leelavathi, Estes, Feingold & Lombardi, 1970; Morré, Cheetham & Nyquist, 1972; Gang, Lieber & Rubin, 1973; Ovtracht, Morré, Cheetham & Mollenhauer, 1973). More exactly, van Golde, Fleischer & Fleischer (1971) report the Golgi apparatus-rich fraction to have a density of 1·12–1·14 g cm^{-3}. In this work, the Golgi apparatus-rich fraction was collected in the density range 1·108–1·137 g cm^{-3}. The plasma membrane-rich fraction whether prepared from livers homogenized in hypotonic solution (Emmelot & Bos, 1969; Dorling & le Page, 1973) or isotonic solution (Berman, Gram & Spirtes, 1969; Nigam, Morais & Karasaki, 1971) collects in the density range 1·16–1·18 g cm^{-3}. In this work, the plasma membrane-rich fractions were collected in the density range 1·154–1·175 g cm^{-3}.

The Golgi apparatus-rich fraction had the characteristics of high yield and high purity. Galactosyltransferase activity (Table 1) was mainly confined to the Golgi apparatus-rich fraction. The 20% of total activity found in the first precipitate represents simple entrapment of Golgi particles as it could be readily eluted from the pellet by a second wash which subsequently banded in the Golgi apparatus-rich fraction. Therefore, more than 95% of the galactosyltransferase activity was found in

the Golgi apparatus-rich fraction and most of the remaining activity was found in the intermediate fraction. The intermediate fraction was designed into the preparation to provide a clear barrier between the Golgi apparatus-rich and plasma membrane-rich fractions. The Golgi apparatus of rat liver has previously been shown to be the sole locus of galactosyltransferase activity (Fleischer, Fleischer & Ozawa, 1969; Fleischer & Fleischer, 1970). The yield of membrane protein in the Golgi apparatus-rich fraction (correcting for galactosyltransferase activity) is 2·26 mg protein/g liver. The figure is in agreement with other published results. The variation in the yield from different male Wistar rats recorded in this series of experiments was far greater than the measured error. This strongly suggests that in rats fed *ad libidum* there is significant variation (up to 50%) in the concentration (or possibly size, but this was not detected in trials) of Golgi apparatus in liver. Electron-microscopic examination of many sections confirmed the purity of the Golgi apparatus-rich fraction.

The plasma membrane-rich fraction showed no visual or enzyme activity parameters similar to the Golgi apparatus-rich fraction. Galactosyltransferase activity was nearly undetectable and less than 1% of the specific activity of the Golgi apparatus-rich fraction. The profiles seen in the electron microscope were similar to those reported for plasma membrane-rich fractions isolated from tissue homogenized in isotonic sucrose (Touster, Aronson, Dulaney & Hendrickson, 1970; Nigam *et al.* 1971) although cell-cell junctions reported in fractions isolated from tissue homogenized in hypotonic solutions (Emmelot, Bos, Benedetti & Rümke, 1964; Dorling & le Page, 1973) were not observed. 5′-Nucleotidase specific activity (×10 over the homogenate; ×22 over the supernatant from which it was prepared) was consistent with the values given in the literature. Published enrichments include: homogenate activity ×12 (Nigam *et al.* 1971), homogenate activity ×17 (Coleman, Michell, Finean & Hawthorne, 1967), homogenate activity ×12 (Berman *et al.* 1969), homogenate activity ×15 (Stein, Widnell & Stein, 1968), post nuclear supernatant activity ×25 (Dorling & le Page, 1973). The yield of protein in the plasma membrane-rich fraction seemed relatively low. Protein yield and 5′-nucleotidase total activity could both be increased by increasing the higher density range of the band from d = 1·175 to d = 1·185 g cm^{-3}. Contamination from endoplasmic reticulum (marked by glucose-6-phosphatase) rose steeply. The plasma membrane-rich fraction designated in this paper contains about 7% of the total 5′-nucleotidase activity. By other procedures, up to 17% of total 5′-nucleotidase activity has been recovered in a plasma membrane-rich fraction of rat liver (Dorling & le Page, 1973). In rat fat cells more than 80% of total 5′-nucleotidase activity is located on the outside of the plasma membrane (Newby *et al.* 1975). From such observations, it is clear that the designated plasma membrane-rich fraction of this paper does not include all the plasma membrane of rat cells.

Similarities of the Golgi apparatus-rich and plasma membrane-rich fractions

Gel electrophoresis of the 2 fractions showed a close similarity and in one case (out of 12) complete identity of the protein subunit patterns. The identity consisted

not only of the R_f values but also of each band's staining capacity with Coomassie brilliant blue. This dye is not a quantitative marker for total protein. For instance, ovalbumin stains considerably less than an equal weight of pyruvate kinase. Nevertheless, the relative staining capacity of each band in the electrophoretic pattern may be taken as correlative evidence of similarity.

The close similarity of the 2 common patterns should be set in the following context of reports of rat liver fractions. The patterns have no bands in common with rough endoplasmic reticulum membrane stripped of its ribosomes and isolated from the same homogenate (Hodson & Miller, unpublished observation). The Golgi pattern has no bands in common with microsomal membrane proteins (Fleischer & Fleischer, 1970). The plasma membrane pattern has no major bands in common with mitochondrial membrane or endoplasmic reticulum including the ribosomes (Neville & Glossman, 1971). Smooth microsomal membranes, rough microsomal membranes, inner mitochondrial membranes and outer mitochondrial membranes all have readily distinguishable electrophoretic protein patterns (Schnaitman, 1969).

The similarity of the Golgi apparatus-rich fraction membrane subunits and the plasma membrane-rich fraction subunits suggests an intimate relationship between the 2 membranes. An identity could never be established by the present method for two reasons: I. Gel electrophoresis can provide only correlations in band comparison and the many minor bands of the fraction cannot easily be visualized. Presumably this is why the galactosyltransferase, certainly present in G and certainly absent in PM was never seen as there were no recognizable bands present in G and absent in PM. And II, the plasma membrane fraction reported here does not represent the whole plasma membrane. It is often claimed that the plasma membrane may be a mosaic of separate organelles (see Wallach, 1967) and that citrate (used in the buffer solution in the present preparation) promotes vesiculation of the plasma membrane (Emmelot & Bos, 1969). It may well be that the present preparation selects only an element of the total plasma membrane mosaic. The yield although slightly greater than that reported for isotonic homogenization is less than half that reported for hypotonic homogenization. It has been stated without published evidence that the protein subunit patterns of differently isolated plasma membrane fractions are distinct and different (Berman *et al.* 1969).

The Golgi apparatus membrane and at least a fraction of plasma membrane of rat liver have a common protein subunit composition. This relationship between different cellular organelles seems to be unique (although lysosomal membranes appear not to have been studied) in rat liver cells and is possibly mediated by membrane-bound vesicles which originate in the Golgi apparatus and terminate at the plasma membrane. In hepatocytes, the vesicles are filled with very low density lipoprotein (Mahley *et al.* 1969; Chapman, Mills & Taylaur, 1972, 1973). In hamster seminal vesicle there is a suggestion that the opposite process may occur, where pinocytotic vesicles bud off the plasma membrane and fuse into the Golgi apparatus (Mata & David-Ferreira, 1973). In non-growing, non-dividing cells where Golgi vesicles fuse into the plasma membrane, there has to be some mechanism for stripping the excess membrane from the plasma membrane. The dynamics of the process

with special reference to the origin and continuity of the Golgi apparatus and the plasma membrane, promise to be full of interest.

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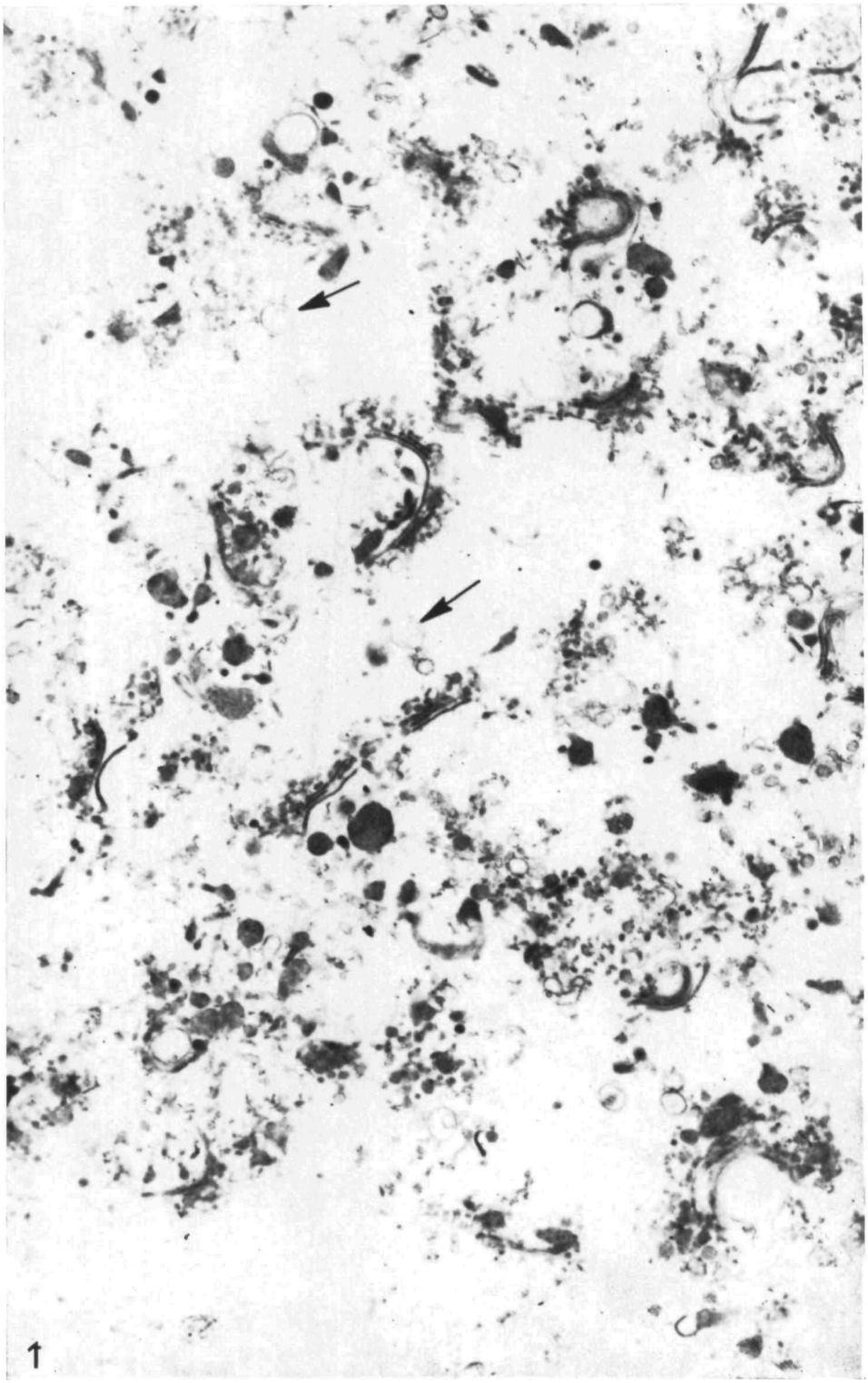
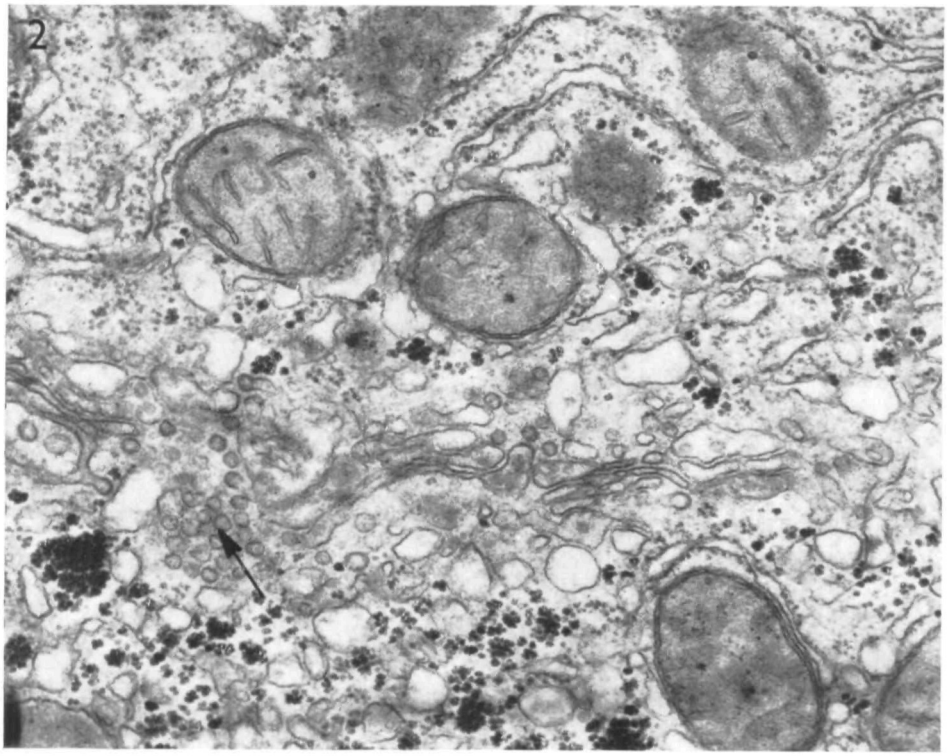


Fig. 1. Survey shot of the loosely pelleted Golgi apparatus-rich fraction (G). Note the predominance of elements with 3–4 flattened cysternae. Smooth-membraned vesicles, the main contaminant, are arrowed. × 9000.



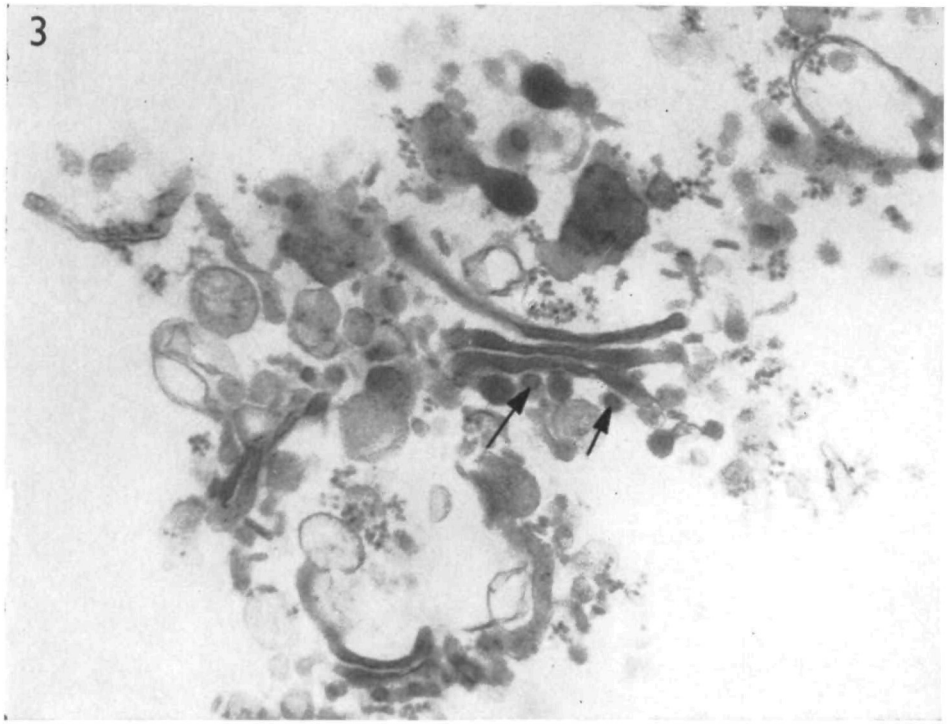


Fig. 2. Golgi apparatus in a hepatocyte. Small vesicles associated with the complex are arrowed. × 39000.

Fig. 3. An isolated Golgi apparatus with associated small vesicles (arrowed). × 49000.

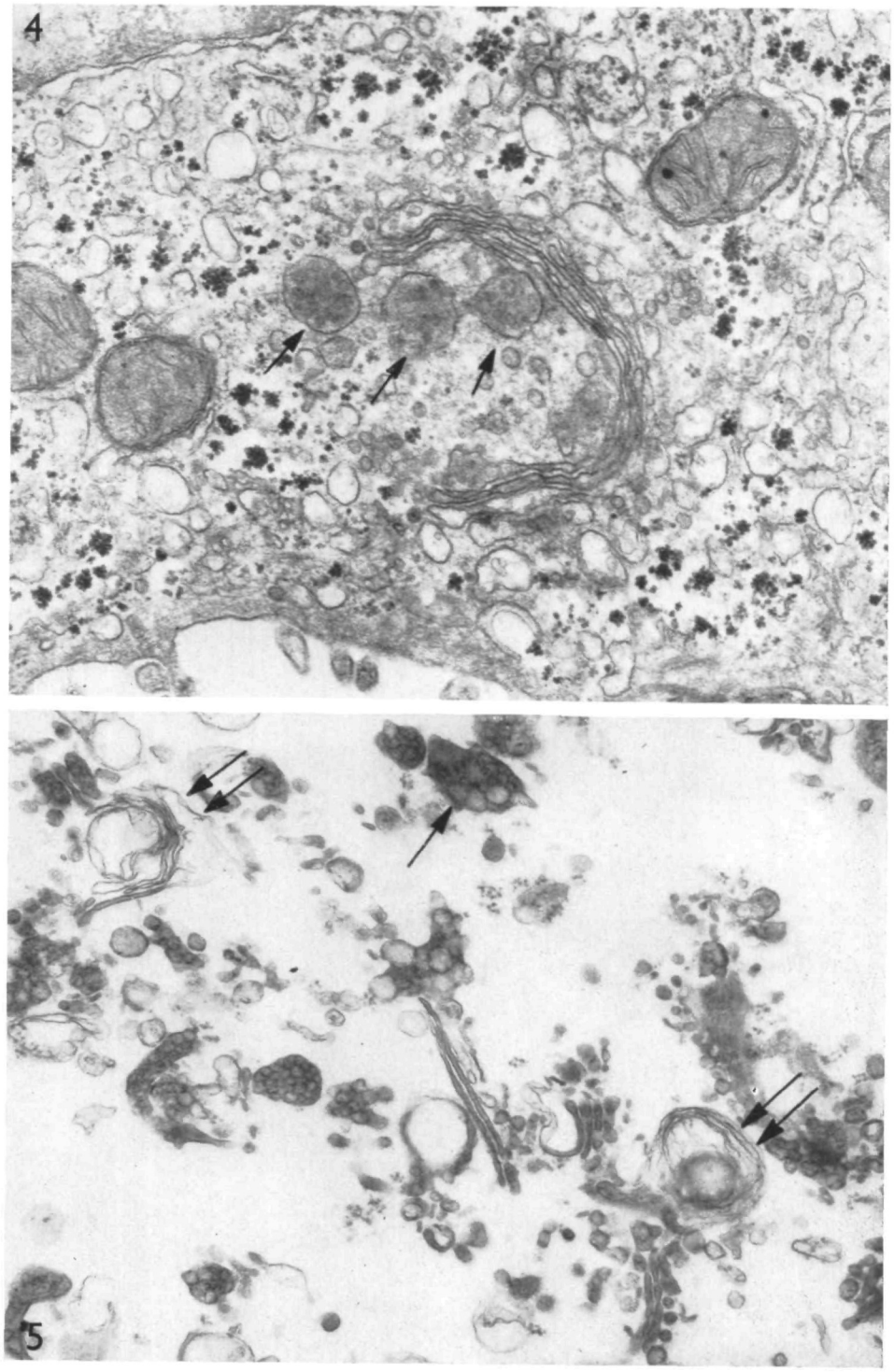
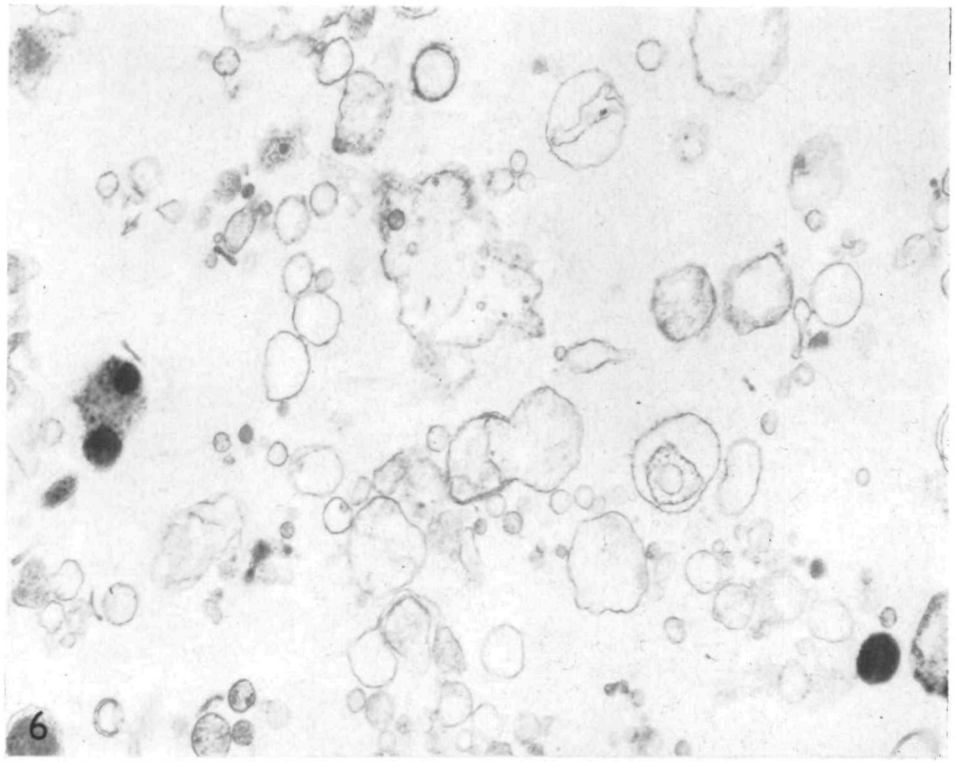


Fig. 4. Golgi apparatus in hepatocyte. Vesicles filled with lipoprotein of very low density are arrowed. × 39000.

Fig. 5. Isolated Golgi apparatus. Vesicles filled with lipoprotein of very low density are indicated by a single arrow. Golgi apparatus which show some disorganization of their cysternae are indicated by paired arrows. × 45000.



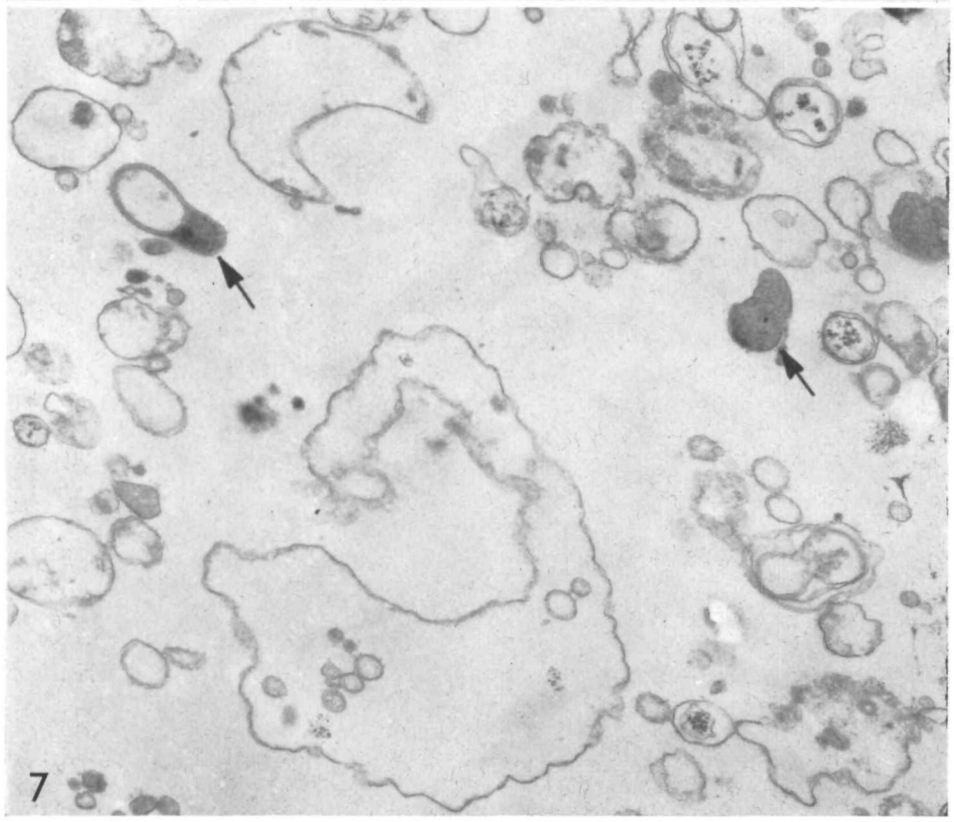
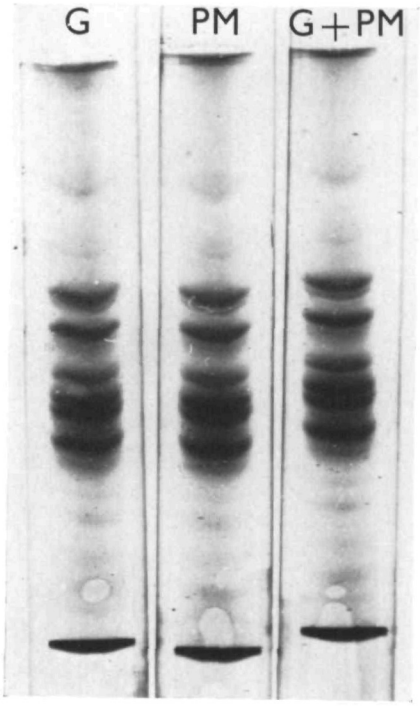


Fig. 6. The designated plasma membrane-rich fraction. × 21 000.

Fig. 7. The designated plasma membrane-rich fraction. Occasional vesicles show electron-dense contents (arrows) which are absent after washing in 0·9% (w/v) NaCl solution. × 21 000.



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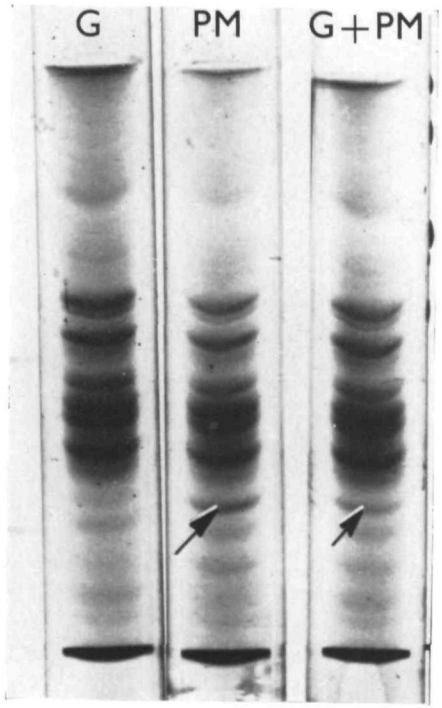

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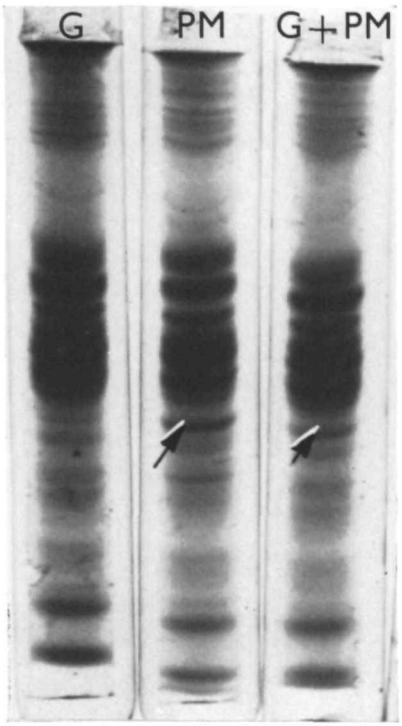

10

Figs. 8–10. Patterns of protein subunits after electrophoresis of solubilized membrane proteins of the Golgi apparatus-rich fraction (G), the plasma membrane-rich fraction (PM) and equal half weights of each fraction mixed (G+PM). All results are from the rat liver homogenate which showed the greatest difference in the 2 patterns. Fig. 8, 12 μg protein in each tube; Fig. 9, 20 μg protein in each tube; Fig. 10, 70 μg protein in each tube. The major difference is a band which appears in the PM tube, arrowed in Figs. 9 and 10.