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MEMBRANES AND MEMBRANE SURFACES

Dynamics of cytoplasmic membranes in pancreatic acinar cells

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[Plates 15 and 16]

In pancreatic acinar cells the intracellular transport of secretory proteins occurs through the interconnexion of distinct membrane-bounded compartments: in series, the rough surfaced endoplasmic reticulum (r.e.r.), the Golgi complex (g.c.) and the secretory (zymogen) granules (z.g.). The latter organelles are able to fuse their membrane with the plasmalemma (pm.) and discharge their content by exocytosis.

In order to investigate the mechanisms by which the intracellular transport occurs we have investigated the composition as well as the rate of synthesis and turnover of the various membranes involved in the process.

We found that these membranes have distinct differences in chemical composition and in the distribution of enzyme activities and that their rate of turnover is much slower than that of secretory proteins. Furthermore, SDS polyacrylamide gel electrophoresis of doubly labelled membrane proteins revealed that in each of these membranes there is a marked heterogeneity of turnover rates.

These data indicate (*a*) that the membranes participating in the intracellular transport interact with one another in a non-random fashion, (*b*) that they are not synthesized concomitantly with the secretion products, (*c*) that membranes are synthesized independently from one another, and (*d*) that they are re-utilized in several secretory cycles.

Consistent with these results, a model explaining the role of cellular membranes in protein secretion is described. It is proposed that the intracellular transport is effected through the specific non-random interaction (fusion–fission) and recycling of the various participating membranes.

The possible relevance of these findings to other secretory systems is discussed.

Introduction

It is well known that in all living cells specific processes, often of great complexity, are localized in individual membranes. Other cell functions, however, are not confined to one single membrane type but require the direct, functional cooperation of a number of different membranes. in general, in these cases, macromolecules or even entire cell structures are first segregated by membranes of one type, then channelled to other cell compartments and sometimes discharged to the extracellular space. Such processes include pinocytosis phagocytosis, autophagy, synthesis of discrete organelles such as lysosomes and peroxisomes, and protein secretion. The latter process will be dealt with in detail in the present report.

Studies in relation to the synthesis, intracellular transport and discharge of secretory proteins, originally carried out on the pancreatic acinar cell by Palade and his associates (Caro & Palade 1964; Jamieson & Palade 1967*a*, *b*) and later extended to other protein-secreting cell systems (Tixier-Vidal & Picart 1967; Howell, Kostianovsky & Lacy 1969; Peters, Fleischer & Fleischer 1971; Winkler, Schöpf, Hörtnagl & Hörtnagl 1972; Hopkins 1972; Castle, Jamieson & Palade 1972; Howell & Whitfield 1973) have led to the general conclusion that newly synthesized secretion products are initially released within the cisternae of the rough-surfaced endoplasmic

reticulum (r.e.r.) and subsequently transported through a series of distinct, yet functionally interconnected membrane-bounded compartments.

This series involves, in sequence, the cisternae of the r.e.r., the Golgi complex (G.c.) and finally a class of membrane-bounded storage organelles which, depending on size, are usually referred to as granules or vesicles and which are specifically capable of discharging their content by exocytosis (fusion of the granule or vesicle membrane with the plasmalemma (pm.) followed by the rupture of the point of fusion) (Palade 1959).

It is clear therefore that, during their whole intracellular life, secretory proteins are segregated by membranes and that transport along the discontinuous secretory pathway depends on the specific interactions of the different membranes involved in the process. However, the mechanisms by which such interactions occur are still unclear. Models suggested so far range between the following extremes. On the one hand it has been proposed that the participating membranes would flow within the cell along with secretion products. Membranes would be synthesized as r.e.r. membranes, then transformed into G.c. and secretion granule membranes and finally degraded at the end of each secretory cycle (Claude 1970; Morré, Keenan & Mollenhauer 1971). On the other hand, according to an alternative interpretation, the movement of secretion products would not imply the concomitant movement of the delimiting membranes, which could therefore be synthesized and turn over independently from one another. The vectorial transport of secretion products between cell compartments would be effected through non-random fusion and fission of their limiting membranes, which could be reutilized in multiple secretory cycles (Jamieson & Palade 1967*a*, *b*, 1971).

The approach that we have followed to investigate the dynamic processes by which a functionally continuous intracellular pathway is established in secretory cells includes: (*a*) the isolation in reasonably pure fractions of the different membranes involved (Meldolesi, Jamieson & Palade 1971*a*; Meldolesi & Cova 1971); (*b*) the study of their composition (lipids, proteins, enzyme activity) (Meldolesi, Jamieson & Palade 1971*b*, *c*; Meldolesi & Cova 1972) as well as of their biogenesis and turnover (Meldolesi 1974). The rationale for such an approach is that if the participating membranes are able to flow within secretory cells together with the secretion products their composition is expected to change progressively, step by step, along the secretory pathway. Moreover, their half-life, especially that of secretory granule membranes, should be comparable to that of the secretion products. If, on the contrary, the cytoplasmic membranes do not accompany the secretion products during their transport, no strict restrictions regarding membrane composition and turnover rates are expected.

The study was carried out on the acinar cell of the guinea-pig pancreas. This cell type was chosen because fractions containing the different organelles involved in secretion as well as membrane subfractions can be isolated by relatively mild procedures (Meldolesi *et al.* 1971*a*; Meldolesi & Cova 1971). Furthermore, in this system knowledge on the secretory process is very detailed and includes the description of not only the route of the intracellular transport but also its timetable and metabolic requirements, both at rest and after pharmacological stimulation (Jamieson & Palade 1967*a*, *b*, 1968, 1971). Therefore the study on the dynamics of cytoplasmic membranes could be adequately correlated with previous data on the transport of secretion products.

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Isolation of membrane fractions and correction of artefacts

There were at least two necessary prerequisites to the work reported in this paper. First of all we had to develop procedures for the isolation of membrane fractions since a large proportion or even the bulk of the proteins recovered in the fractions containing the organelles involved in secretion (rough surfaced microsomes (r.m.) = r.e.r.; smooth microsomes (s.m.) = primarily the G.c.; zymogen granules (z.g.)) and in the pm. are accounted for by non-membrane components. Thus, r.m., s.m. and z.g. contain, although in different proportion, large amounts of segregated secretory proteins; r.m. have ribosomes attached to the outer surface of their membranes and the pm. fraction includes adherent fibrillar material, arising from the basement membrane and the terminal web. Finally, varying amounts of soluble proteins of the cell sap are adsorbed on to all these structures upon homogenization (Siekevitz & Palade 1960; Meldolesi *et al.* 1971*a*; Jamieson & Palade 1967*a*, *b*; Meldolesi & Cova 1971; Tartakoff, Greene, Jamieson & Palade 1974).

Furthermore, the pancreas contains a great deal of digestive (pro)-enzymes, and a certain proportion of them is known to be released from the storage organelles during homogenization and cell fractionation. Since it has been reported that pancreatic lipase is active also in the cold (Bloor 1943) and that at least some protease may become activated during cell fractionation (Siekevitz & Palade 1958) it was necessary to establish whether and to what extent membrane components had been altered or degraded.

The approach that we used for the purification of the different membranes was to isolate first the fractions containing the complete organelles involved in the process and the pm. and to separate then the non-membrane components from the membranes, while leaving the structure of the latter apparently unaffected.

The procedures for the isolation of r.m., s.m., z.g. and pm. by differential and density-gradient centrifugation are described in detail elsewhere (Meldolesi *et al.* 1971*a*). In all cases, our interest was to concentrate on purity at the expense of recovery. The fractions were characterized by electron microscopy, by analytical determinations and by assaying the activity of marker enzymes. The r.m. (figure 1, plate 15) and s.m. were found essentially free of mitochondrial contamination; s.m. contain $< 15\%$ rough contaminants. The z.g. (figure 3, plate 15) are free of microsomes and contain $< 3\%$ contaminant mitochondria. The pm. fraction is heterogeneous as to origin since it contains sheets of membranes deriving from basal, lateral and apical poles of the cell.

In order to separate membranes from non-membrane components we took advantage on the following findings: (*a*) in r.m. isolated from several different tissues the combined treatment with puromycin and KCl at high concentration results in the detachment of most of the attached ribosomes (Adelman, Sabatini & Blobel 1973); (*b*) pancreatic microsomes and z.g. release most of the segregated secretory proteins upon suspension in isotonic buffers at pH > 7.2 (Hokin 1955; Greene, Hirs & Palade 1963; Meldolesi *et al.* 1971*a*); (*c*) washing of membranes with concentrated salt solutions releases the bulk of the proteins adsorbed from the cytoplasm upon homogeneization (Dallner, Siekevitz & Palade 1966; Meldolesi & Cova 1971, 1972); and (*d*) the filamentous material contaminating the pm. fraction can be removed by digestion with collagenase (Spiro 1967; Meldolesi & Cova 1972). The procedures for the isolation of membrane fractions which were developed accordingly are described in detail elsewhere (Meldolesi *et al.* 1971*a*; Meldolesi & Cova 1971, 1972).

Both the morphological and biochemical analysis indicate that most of the contaminants were effectively removed, leaving behind a large proportion of the membranes. Thus, under the electron microscope, both microsome membrane fractions were found to contain virtually exclusively empty vesicles, bounded by a unit membrane, which appears usually swollen and distorted and sometimes ruptured (figure 2, plate 15). The z.g. membrane fraction consists of membranes both in the form of empty vacuoles of the size expected for z.g. ghosts and of smaller empty vesicles (figure 4, plate 15). In the purified p.m. fraction most of the contaminating fibrillar material has disappeared. The sheets of membranes often joined together by tight junctions and desmosomes and the large vacuoles present before collagenase digestion appear fragmented into small vesicles and membrane pieces with free edges.

TABLE 1. RECOVERY OF PROTEIN, P.L.P., RNA, SECRETORY ENZYMES, AND ADSORBED SOLUBLE PROTEIN IN R.M., S.M., AND Z.G. MEMBRANES ISOLATED FROM THE PANCREAS OF THE GUINEA PIG

	protein %	p.l.p. %	RNA %	α-amylase %	chymotrypsinogen %	RNase %	lipase %	adsorbed protein† %
r.m. membranes	29.8	70.8	4.6	1.3	0.5	0.5	—	2.5
s.m. membranes	34.6	71.0	3.4	0.6	0.4	0.7	—	2
z.g. membranes	1.1	52.3	—	0	0	—	0.1	0

† Adsorbed protein is defined as the trichloroacetic acid-insoluble radioactivity recovered in the fractions isolated from nonradioactive pancreas tissue homogenated in radioactive postmicrosomal supernatant. The latter was obtained by centrifuging, at high speed, homogenates of pancreas slices labelled *in vitro* with [^{14}C]L-leucine. Values are expressed as percentages. 100% = the level found in r.m., s.m. and z.g., respectively. Data from Meldolesi & Cova (1972).

On the other hand the biochemical analysis revealed that a large proportion of the p.l.p. originally present in r.m. and s.m. is recovered in the membrane fractions (table 1). The lower recovery in z.g. membranes is not dependent on solubilization but on the removal of contaminating mitochondria. The concentration of ribosomes (as indicated by the level of RNA), of secretory enzymes and zymogens and of adsorbed proteins is drastically reduced.

In conclusion, the procedures used permit the isolation of membrane fractions which appear (*a*) to be representative of the membranes originally present in the organelles involved in secretion and (*b*) to contain only small amounts of non-membrane contaminants.

DESCRIPTION OF PLATE 15

FIGURE 1. Rough microsome fraction. The fraction consists of rough-surfaced vesicles of various size and content density. Most have a homogeneous content (v_1), some appear partially extracted (v_2) and some are swollen and completely extracted (v_3). (Magn. × 28 200.)

FIGURE 2. Rough microsome membrane fraction. The fraction contains primarily small smooth vesicles (v_1) devoid of any visible content. Some of them are seen in grazing section (v_2). (Magn. × 66 000.)

FIGURE 3. Zymogen granule fraction. This representative field is primarily occupied by round, dense z.g. In many granules the limiting membrane is apparently unchanged (z_1); in some cases it has formed tubular (z_2) or rounded (z_3) myelin figures. A few granules appear damaged. Most of the membrane pieces interdispersed with the organelles are probably continuous with z.g. membranes outside the plane of the section. c.v., condensing vacuoles; i.c., intercisternal granules. (Magn. × 10 600.)

FIGURE 4. Zymogen granule membrane fraction. The field contains several spherical or flattened z.g. ghosts (g), which are usually open. The small vesicles (c) and pieces of membranes (f) probably represent ghost membrane fragments. (Magn. × 55 000.)

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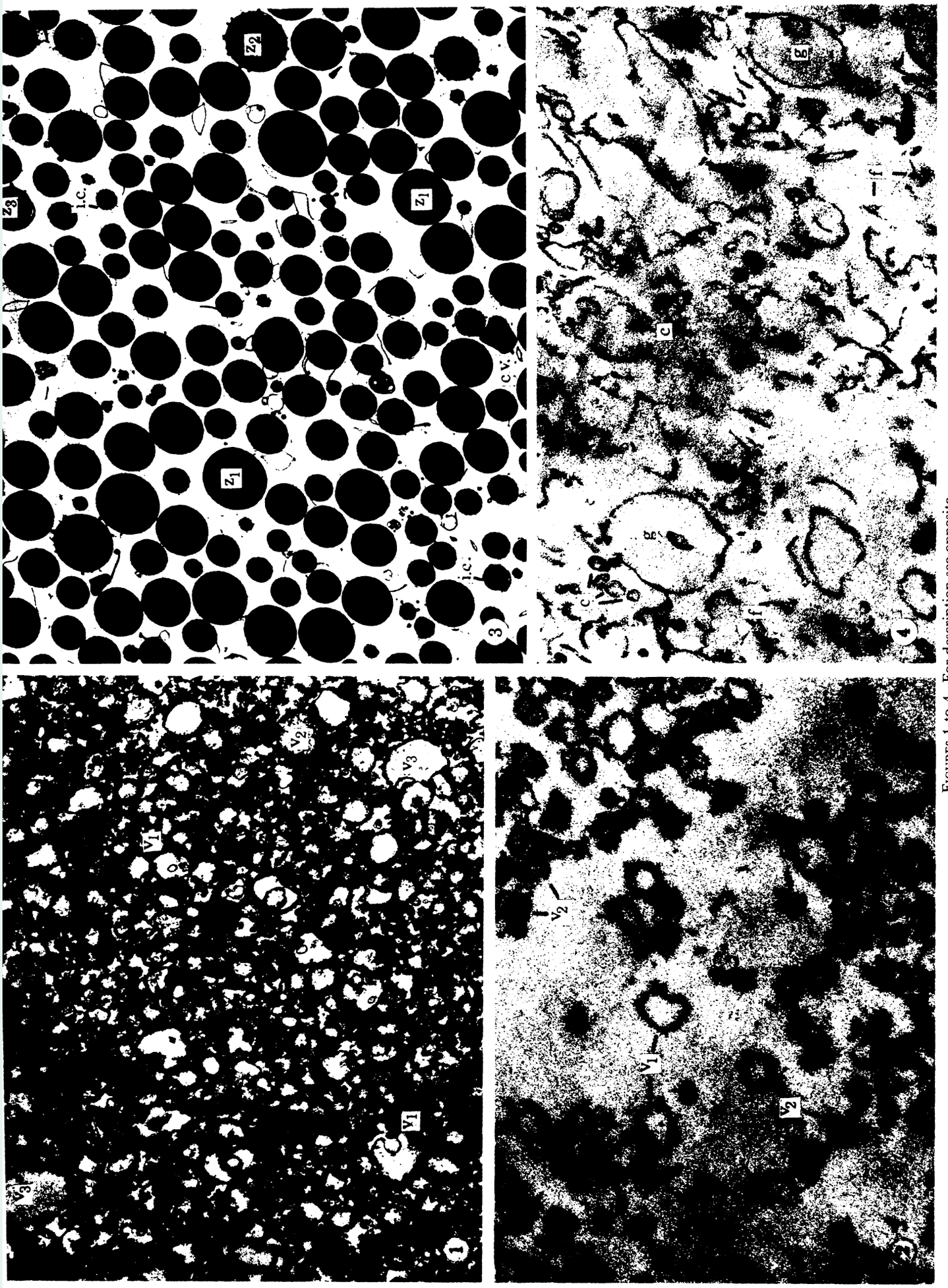

FIGURES 1 to 4. For description see opposite

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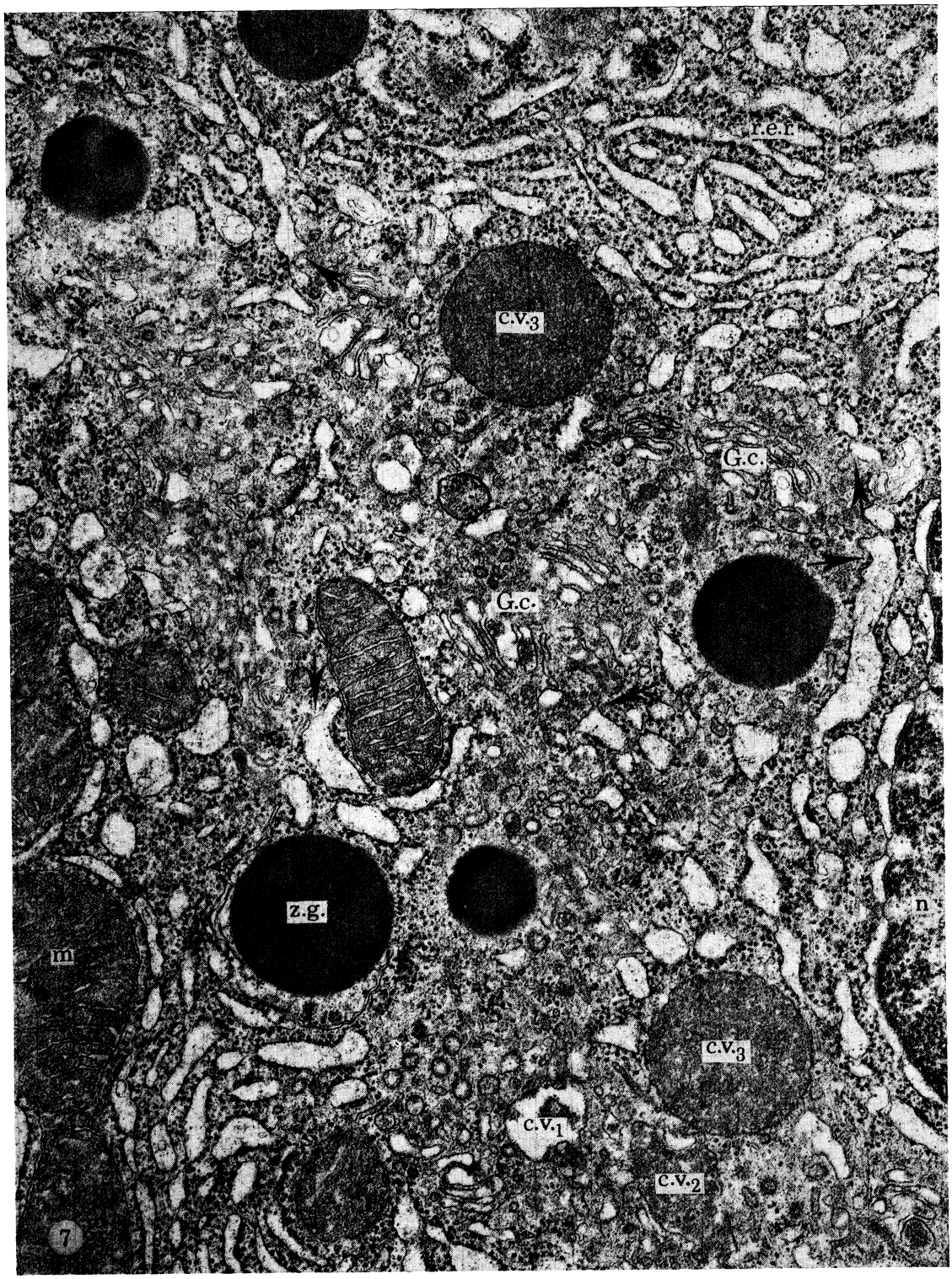

FIGURE 7. Guinea-pig pancreas acinar cell. General view of the Golgi complex. The r.e.r. cisternae immediately adjacent to the complex are part rough-, part smooth-surfaced (transitional elements). Small vesicles apparently fissioning from or fusing with the transitional elements are indicated by arrows. The Golgi complex consists of numerous vesicles of various size, usually located peripherally to the small stacks of flattened cisternae which are labelled G.c. The field includes several condensing vacuoles (c.v.) corresponding to progressive stages of maturation from 1 to 3. z.g., mature zymogen granule; n, nucleus; m, mitochondrion. (Magn. ×25000.)

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The evaluation of the possible artefacts arising during cell fractionation was carried out by a number of different experiments, including: (*a*) ageing of pancreatic homogenates and cell fractions under conditions analogous to those used for cell fractionation; (*b*) homogenization and cell fractionation in the presence of specific inhibitors of proteolytic and lipolytic enzymes; (*c*) short incubation of exogenous radioactive substrates with pancreas homogenates and cell fractions; and (*d*) mixing of liver microsomes with pancreatic post microsomal supernatant (Meldolesi *et al.* 1971 *b*, *c*; Meldolesi & Cova 1972).

These studies showed that under our experimental conditions proteolytic zymogens are not activated and therefore do not affect the protein composition of isolated membranes. However, the lipid composition of membranes is extensively affected by released lipolytic enzymes, which are probably responsible also for the solubilization of some membrane-bound enzyme molecules. These alterations have been carefully evaluated and the data on lipids and enzymes have been corrected accordingly.

Composition of cytoplasmic membranes; lipids, protein and enzymic activities

An extensive characterization of the membranes involved in the intracellular transport of secretory proteins was carried out by determining their lipid and protein composition and their spectrum of enzyme activities (Meldolesi *et al.* 1971 *b*, *c*; Meldolesi & Cova 1972).

Table 2. Lipid composition of subcellular fractions isolated from guinea pig pancreas

(Data from Meldolesi *et al.* (1971 *a*, *b*) and Meldolesi & Cova (1971).)

	r.m. membranes	s.m. membranes	z.g. membranes	pm.
p.l.p.: protein	0.32	0.57	0.46	0.44
sphingomyelin†	3.40	14.20	23.50	19.20
lecithin lysolecithin†	47.80	39.40	30.50	32.00
ph.-ethanolamine†	35.80	36.50	31.50	34.40
ph.-serine + ph.-inositol†	5.60	4.70	5.00	4.60
total cholesterol: p.l.p.‡	0.12	0.47	0.55	0.51

† % total p.l.p. ‡ Molar ratio.

As shown in table 2, membranes of the smooth variety (s.m. and z.g. membranes and the pm.) are similar in their content of phospholipid and cholesterol and in their ratio of total lipids to membrane proteins. Furthermore, their concentration of lecithin is relatively low whereas that of sphingomyelin is high. In contrast r.m. membranes contain much less sphingomyelin and cholesterol and possess a smaller lipid:protein ratio.

The protein composition was studied by two different polyacrylamide gel electrophoresis systems (the acid system of Eytan & Ohad (1970) and the sodium dodecylsulphate (SDS) disk gel method of Maizel (1971). In both systems r.m. and s.m. membranes appear to contain a large number of different polypeptide chains, with molecular masses varying between ~ 150000 and ~ 15000. Z.g. membranes have a much simpler protein composition: only two bands, one large and one small, were separated in the acid and 23 in the SDS system, with a predominance of low molecular mass chains. Pm. gives a rather complex pattern in both systems. Again, low molecular mass polypeptide chains predominate (figure 5).

Finally, further differences were found by determining the distribution of enzyme activities

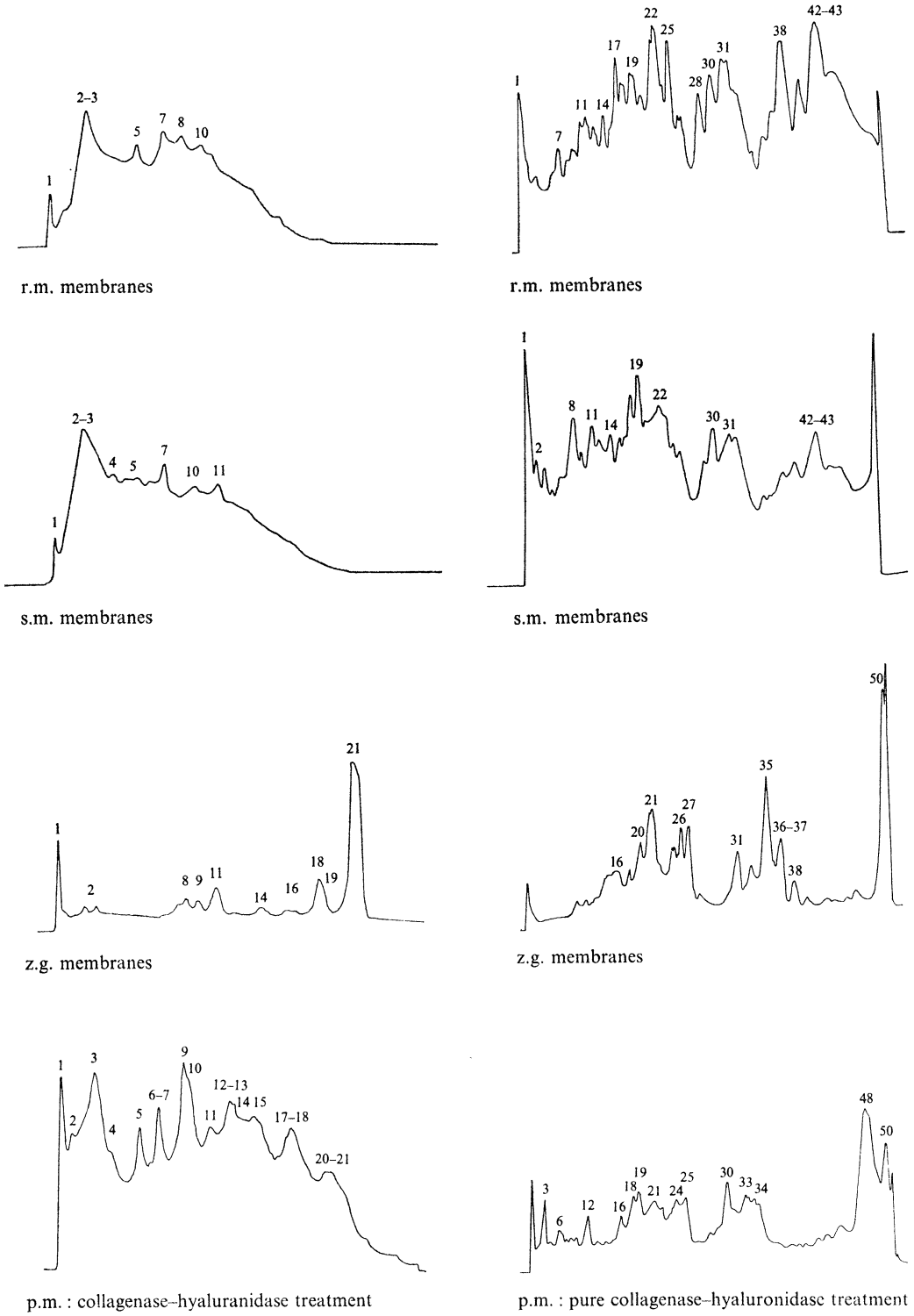

FIGURE 5. Typical densitometric tracings of polyacrylamide gels of cytoplasmic membrane proteins. Electrophoresis was performed according to the acid method of Eytan & Ohad (left) and to the SDS method of Maizel (right). Data from Meldolesi & Cova (1972).

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(table 3). R.m. membranes have the reductase activities of the two microsomal electron transport systems, but lack the enzymes of G.c.-type (TPPase and galactosyl-transferase) and pm.-type (5′ nucleotidase, β-leucyl-naphtylamidase and Mg^{2+} ATPase). S.m. membranes share with r.m. and pm. the microsomal reductases and the pm.-type enzymes, respectively. In addition, they possess TPPase and galactosyl-transferase activity. In contrast, z.g. membranes appear poor in enzyme activity. They lack both the microsomal reductases and the G.c.-type enzymes. Their activity in pm.-type enzymes is very low, except for Mg^{2+} ATPase.

TABLE 3. DISTRIBUTION OF ENZYME ACTIVITIES IN SUBCELLULAR FRACTIONS ISOLATED FROM GUINEA-PIG PANCREAS

(Data from Meldolesi *et al.* (1971*c*, and unpublished).)

enzymes	r.m.	s.m.	z.g.	pm.
NADH-cytochrome *c*† reductase (rotenone ins.)	181.0	130.0	32.8	47.2
NADPH-cytochrome *c*† reductase	27.7	17.8	0	3.5
TPPase‡	0	1.49	0	0
gal.-transferase§	1.3	22.8	0.8	1.1
5′-nucleotidase‡	0	13.2	4.2	18.3
β-leucyl naphtylaminidase‡	1.0	41.2	6.7	44.3
Mg^{2+} ATPase‡	0	11.0	38.0	46.0

† nmoles of cytochrome *c* reduced at 25 °C per min per mg phospholipid.
‡ μmoles of P_i or naphthylamine released at 37 °C for 60 min per mg phospholipid.
§ nmoles of galactose transferred at 37 °C per 60 min per mg phospholipid.

SYNTHESIS AND TURNOVER OF MEMBRANE PROTEINS

The synthesis and the turnover of membrane proteins were investigated by means of *in vitro* pulse-chase experiments and *in vivo* double label experiments using radioactive L-leucine as the tracer (Meldolesi 1974).

In vitro *experiments*

In pancreas tissue slices pulse-labelled *in vitro* for 5 min and then incubated in chase medium for 150 min the specific activity of the proteins of r.m., s.m. and z.g. membrane fractions was low relative to secretion proteins (table 4). Furthermore, a large proportion of the radioactivity recovered in membrane fractions was accounted for by the small amount of highly labelled secretory proteins present in the preparations, as revealed by the distribution of counts in SDS gels (not shown). The specific activity of true membrane proteins remained essentially unchanged during chase incubation. When the contribution of contaminants was subtracted according to the criteria described elsewhere (Meldolesi 1974) the specific activities of the membrane proteins were found to be about $\frac{1}{15}-\frac{1}{30}$ of those found in secretory proteins (table 4). Since the concentration of leucine is approximately the same in secretory and membrane proteins (Meldolesi 1974) the data clearly indicate that the rate of synthesis of membrane proteins is very slow relative to secretory proteins.

In vivo *experiments*

Because of the low rate of labelling of membrane proteins by comparison with secretory proteins, *in vitro* experiments proved inadequate to yield detailed information about the turnover rates of individual proteins of the different cytoplasmic membranes. We switched therefore to *in vivo* experiments, which can be conducted on a much longer time-scale.

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Since the work of Schimke and his associates (Arias, Doyle & Schimke 1969; Glass & Doyle 1972) it is known that relative turnover rates of proteins can be adequately determined *in vivo* by a simple double label technique, in which animals sequentially receive different isotopes of the same amino acid. As employed here, [^{3}H]L-leucine was injected either 4½ or 6½ days and [^{14}C]L-leucine 13 h before the sacrifice. Thus, the ^{14}C and ^{3}H radioactivities represent 13 h and

TABLE 4. SPECIFIC RADIOACTIVITY IN ROUGH AND SMOOTH MICROSOMES AND ZYMOGEN GRANULES AND SUBFRACTIONS ISOLATED THEREFROM OBTAINED FROM GUINEA-PIG PANCREAS SLICES PULSE LABELLED FOR 5 min WITH [^{3}H]L-LEUCINE AND INCUBATED IN CHASE MEDIUM FOR 0 AND 150 min†

(Data from Meldolesi (1974).)

cell fraction	d min^{-1} mg^{-1} protein: 0 min in chase	150 min in chase	cell fraction	d min^{-1} mg^{-1} protein: 0 min in chase	150 min in chase	cell fraction	d min^{-1} mg^{-1} protein: 0 min in chase	150 min in chase
r.m.	157143	45679	s.m.	105000	50617	z.g.	7160	298074
r.m.; secretory protein	355185	62395	s.m.; secretory protein	241975	84593	z.g.; secretory protein	4642	395062
r.m. membranes	48062	23284	s.m. membranes	42002	30025	z.g. membranes	12637	37037
corrected‡ r.m. membranes	18000		corrected‡ s.m. membranes	23000		corrected‡ z.g. membranes	13000	

† Values given are the averages of three consistent experiments.
‡ Criteria for the correction of the specific activity in membrane subfractions are described in detail elsewhere (Meldolesi 1974).

TABLE 5. ^{14}C:^{3}H RATIOS OF GUINEA-PIG PANCREAS CELL FRACTIONS: 6-DAY INTERVAL BETWEEN INJECTIONS†

(Data from Meldolesi (1974).)

	^{14}C d min^{-1} mg^{-1} protein	^{3}H d min^{-1} mg^{-1} protein	^{14}C/^{3}H		^{14}C d min^{-1} mg^{-1} protein	^{3}H d min^{-1} mg^{-1} protein	^{14}C/^{3}H
T homogenate	12300	5700	2.16	r.m. soluble protein	6120	3221	1.90
mitochondria	7814	6915	1.13	r.m. membranes	8790	11416	0.77
T microsomes	6904	4512	1.53	ribosomes	4902	6248	0.78
R microsomes	7290	5027	1.45	s.m. soluble protein	6300	3224	1.93
S microsomes	6870	4215	1.63	s.m. membranes	9623	8828	1.09
zymogen granules	8366	2140	3.91	z.g. soluble protein	8300	2000	4.15
				z.g. membranes	6247	7349	0.85

† Each of two guinea pigs was injected i.p. with 1 mCi of [^{3}H]L-leucine. Six days later each guinea pig received 300 μCi of [^{14}C]L-leucine. 13 h after the last injection the animals were killed, pancreases were pooled and the cell fractions isolated.

4½- or 6½-day timepoints, respectively, and decay kinetics can be deduced from these measurements. Proteins of relatively high turnover will have high ^{14}C:^{3}H ratios and vice versa. Isotope injections were separated by rather long intervals (either 4 or 6 days) in order to obtain a sizeable decrease of the ^{3}H radioactivity associated with the slowly turning over membrane proteins.

The results of the *in vivo* experiments are reported in table 5 and figure 6. The relative turnover rate of all membranes involved in the intracellular transport is very slow (= low double isotope ratio), not only in comparison with secretory proteins, but also with the homogenate

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and mitochondria. This is quite clear for r.m. and z.g. membranes, while the turnover rate of s.m. membranes is slightly faster.

When doubly labelled membrane fractions were studied by SDS-polyacrylamide disk gel electrophoresis, a marked heterogeneity of the turnover rates of their proteins was found. In agreement with the previous results by Schimke and his associates (Arias *et al.* 1969; Dehlinger & Schimke 1971, 1972; Glass & Doyle 1972) on liver microsomes and plasma membranes, a general correlation was found in each membrane under study between the molecular mass of a protein

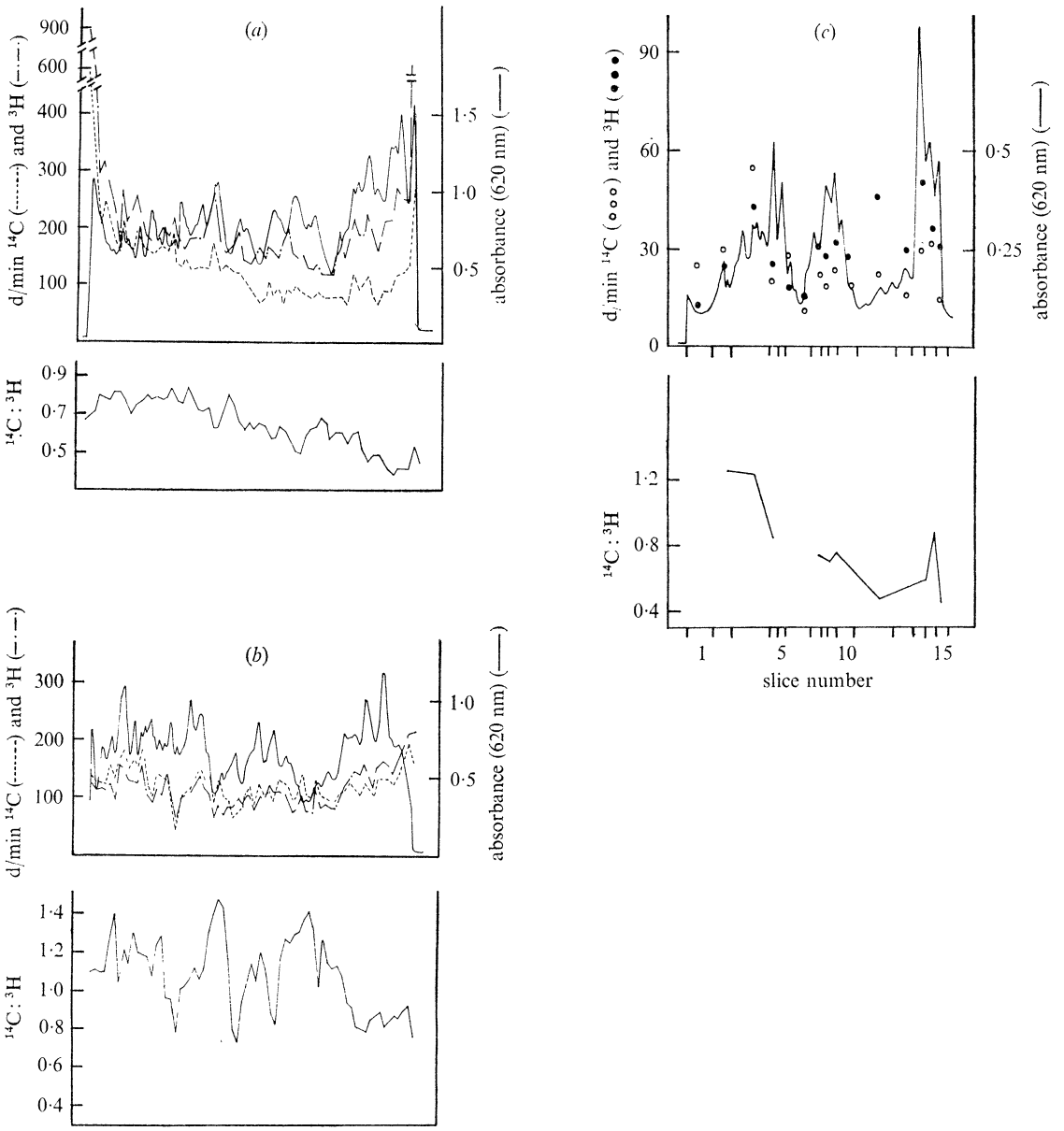

FIGURE 6. Relative turnover rates of the proteins of pancreas r.m. membranes (*a*), s.m. membranes (*b*), and z.g. membranes (*c*). *In vivo* doubly labelled membrane proteins were separated in 10.5% polyacrylamide SDS gels. The upper panels include the densitometric tracings of stained gels and the distribution of ^{3}H and ^{14}C counts in gel slices. The lower panel the ^{14}C:^{3}H ratios. r.m. and s.m. membrane gels were cut into about 70 2.8 mm thick slices; the z.g. membrane gel into 15 *unequal* slices, as indicated in abscissa. Data from Meldolesi (1974).

or protein subunit and its relative turnover rate: large proteins are turning over faster than small proteins. This correlation is quite clear in r.m. and z.g. membranes. In the experiment with the 6-day interval between injections the ratios ranged between 0.85 and 0.38 (r.m.) and between 1.25 and 0.45 (z.g.) (figure 6). In s.m. membranes the correlation is still evident (ratios between 1.47 and 0.76). However, three groups of proteins with anomalously high turnover rates are present. Their molecular masses are around 50000, 24000 and 19000 (figure 6).

The double-isotope ratios found in r.m. membrane proteins corresponds to an average half-life of ~ 5 days, with a minimum of ~ 4.5 days for high molecular mass and a maximum of ~ 28 days for low molecular mass proteins. The corresponding values for s.m. membranes are ~ 3.5, ~ 2.75 and ~ 5 days; for z.g. membranes ~ 4.5, ~ 3 and ~ 13 days.

Discussion

Pancreatic acinar cells are highly specialized towards protein secretion: it can be calculated that, in the starved guinea pig, exocrine secretion products account for about a third of the protein of the organ (Meldolesi *et al.* 1971*a*) and for 90% of its protein-synthesizing capacity (Tartakoff *et al.* 1974). Thus, in each secretory cycle a huge amount of protein (~ 40 mg/g of tissue) is synthesized, transported throughout the acinar cells, stored and finally discharged in the glandular lumen. However, since the secretory pathway is not continuous but is established through the functional interconnexion of a series of discrete membrane-bounded compartments, exocrine pancreatic secretion should be regarded not only in terms of synthesis and discharge of secretion products but also in terms of controlled movement and specific interactions of the different cytoplasmic membranes (Jamieson & Palade 1967*a*, *b*).

Knowledge on the composition of the various cell compartments involved permits a rough quantitative estimate of such a membrane circulation. In rough and smooth microsomes the ratio between secretory and membrane proteins is about 1. In z.g., due to the progressive concentration of secretion products during transport, the ratio is about 50 (Meldolesi *et al.* 1971*a*). These data suggest that the quantity of membrane required for the transport of secretory proteins from the r.e.r. to the G.c. is comparable to the amount of transported secretory proteins. On the other hand, also the assembly of z.g. involves the movement and the coalescence of large amounts of membranes, most likely contributed by the small vesicles and vacuoles which are commonly observed in the Golgi area (figure 7, plate 16) (Caro & Palade 1964; Jamieson & Palade 1967*a*). However, due to the low surface-to-volume ratio in mature z.g., most of this membrane is in excess and is therefore removed during the maturation of the granules (Jamieson & Palade 1967*b*). Finally, during the exocytosis the membrane, of z.g. (about $\frac{1}{50}$ of z.g. content) fuses with the apical portion of the pm. and is incorporated into the latter (Palade 1959). This brings about an enlargement of the acinar lumen. However, over a short time interval the enlarged lumen is reverted to its previous size. Hence, here again the excess membrane is removed, thus permitting the cellular surface to remain constant.

It is clear therefore that, in order to have secretion products transported from one cell compartment to the next, a large number of loaded vesicles have to bud off the membranes of the first compartment, cross the boundary and fuse with and be incorporated into the membranes of the latter. At each boundary the movement of membrane is most likely very large. Therefore, were the interactions between the different membranes occurring at random (in the sense that after fusion the molecular components of the incoming membrane patches are free to diffuse

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laterally in the membranes of the 'acceptor' cell compartment) one would expect a complete intermixing of the different membranes participating in the intracellular transport within a matter of a few secretory cycles. However, in all cell systems so far investigated, at least some of the various membranes involved have been found to differ markedly in their composition (Winkler, Hörtnagl, Hörtnagl & Smith 1970; Amsterdam *et al.* 1971; Morré *et al.* 1971; Zahler, Fleischer & Fleischer 1970; Schneitman 1969; Benedetti & Emmelot 1968). There is, therefore, a wide consent as to the non-random nature of these interactions. However, the mechanisms by which the identity of the participating membranes is preserved are still debated.

The membrane flow hypothesis predicts that membranes move undirectionally from the r.e.r. (the site of membrane synthesis) to the pm. and that this flow is coupled with a step by step, irreversible transformation of the membranes involved occurring concomitantly with their transfer from one cell compartment to another (Claude 1970; Morré *et al.* 1971). The excess membrane originated in the Golgi area as well as at the apical pm. as a consequence of both the concentration of secretion products and the exocytosis, would be degraded into soluble components which could be reutilized in the synthesis of new membranes.

Not one of our results appears compatible with this interpretation. Composition studies revealed that the differences between the various membranes participating in the intracellular transport are marked and do not fit the constraints of the hypothesis. In particular, z.g. membranes appear unique in their protein composition and much different from the membranes bounding the two adjacent cell compartment: the G.c. and the pm.

On the other hand, all membrane proteins are very little labelled in comparison with secretory proteins after a short pulse *in vitro* with radioactive leucine, and their specific activity does not change appreciably during chase incubation. This suggests that the different membranes are synthesized independently from one another, even if we cannot exclude the possibility that some single proteins could be synthesized in the r.e.r. and later appear in the other membrane types, as has been suggested in the liver (Dallner *et al.* 1966; Omura & Kuriyama 1971). These conclusions are strengthened by the *in vivo* turnover experiments which revealed that all membranes under study have average half-lives of the order of days, whereas the half-life of secretory proteins is of the order of hours. Furthermore, within each membrane the different proteins have greatly heterogeneous turnover rates, i.e. each individual membrane protein appears to be replaced at its own rate, independently from the others. This finding is incompatible with the idea that transfer and degradation of pieces of complete membranes is a major mechanism of membrane turnover, as requested by the membrane flow hypothesis, since this would imply synchronous loss and replacement of all membrane components.

On the contrary, our results clearly indicate that the transport of secretory proteins and the biogenesis of the membranes involved in that process are regulated according to greatly different time scales. As a consequence, participating membranes most probably are not destroyed in the course of each secretory cycle, but are extensively re-utilized.

Figure 8 shows a model which is compatible with the results reported in this paper. The model is based on the idea, originally developed by Palade and his associates (Jamieson & Palade, 1967*a*; Bergeron *et al.* 1973) that the intracellular transport occurs through specific fusion–fission and recycling of the various membranes involved. As an example, the transport of secretory proteins from the r.e.r. to the G.c. would be carried out by loaded smooth vesicles which would detach from the so-called transitional elements (part rough, part smooth cisternae located all

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around the G.c. (figure 7)), move to the G.c., discharge their content and then shuttle back to the r.e.r. for further loading.

In the model, the secretory pathway appears delimited by four types of membranes, each bounding one cell compartment. Membranes are drawn in different kinds of line to indicate their differences in chemical composition: r.e.r. membranes (----), G.c. (–·–·–), Z.g. (,,,,,,,,,), pm. (——). The boundaries between cell compartments are indicated by mosaic arrays, made up by fusion of membrane patches of different composition. It is assumed that at the point of fusion the lateral movement of membrane molecular components is restricted. This would be the mechanism by which the identity of the individual membrane types is preserved through-

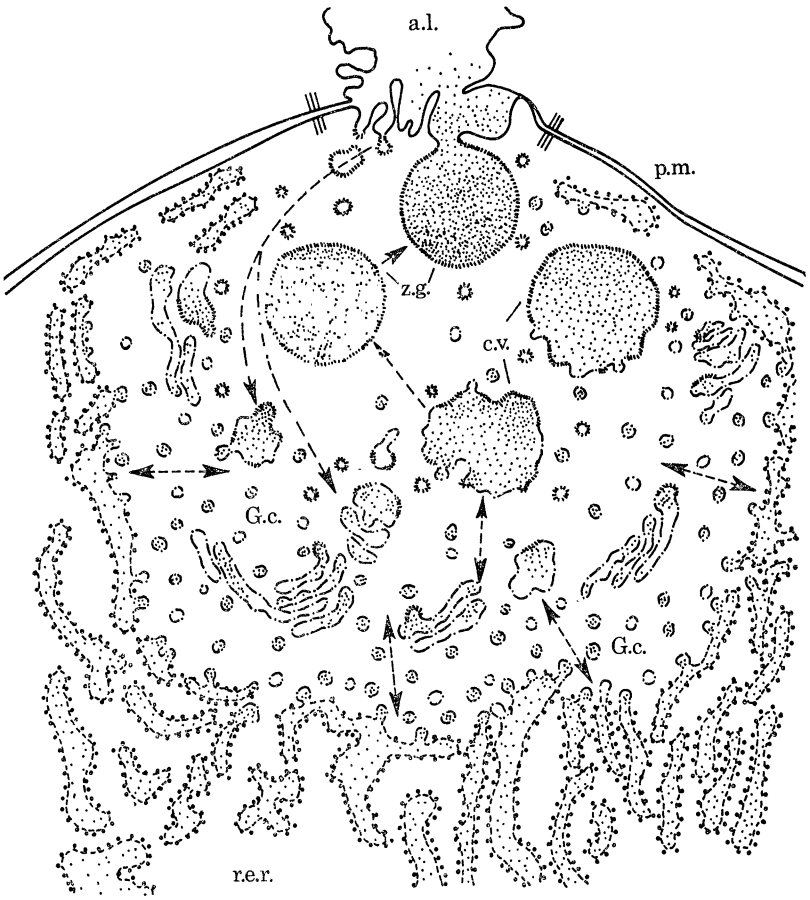

FIGURE 8. Schematic representation of a model of membrane circulation in pancreatic acinar cells r.e.r., rough-surfaced endoplasmic reticulum; G.g., Golgi complex; c.v., condensing vacuole; z.g., zymogen granule; pm., plasma membrane; a.l., acinar lumen. The explanation of the model is given in the text.

out the secretory cycle. The evolution from the mosaic arrays towards one individual membrane type is expected to occur either by means of the specific insertion of membrane patches of that type, or by the specific removal of the patches of the other type or by both these processes. The arrows pointing to opposite directions suggest that membrane-bounded organelles are able to shuttle between cell compartments. In general, it is assumed that loaded vesicles would move down, empty vesicles up the secretory pathway.

Some features of the model are highly hypothetical and need experimental confirmation. First of all, it is not known whether the membrane patches, once incorporated into cell

compartments of different composition, are retrieved as discrete membranes (as suggested by the model) or as specific macromolecules, which are able to reassemble into complete membranes at other sites of the cell. Furthermore, the membrane type responsible for the transport from the r.e.r. to the G.c. has been assigned to the latter compartment. However, it could belong equally well to the r.e.r. or even to a specific class of vesicles, so far unidentified, different from both the r.e.r. and the bulk of the G.c. Finally, z.g.-type membranes or macromolecules, retrieved from the pm., are suggested to be able to fuse with both G.c. vesicles and cisternae, thus initiating the assembly of new immature z.g. (condensing vacuoles, c.v.), whereas they could be transferred to only one of these G.c. elements or even directly added to the c.v.

The model that we propose is by no means complete. It has the advantage of clearly differentiating the long-term processes of membrane biogenesis from their short-term interrelationships connected with transport of secretory products, but fails to provide any information about very important processes such as the site of synthesis of membrane proteins (whether it occurs on bound or on free polysomes or on both, as has been suggested recently for liver microsomes (Omura 1973)) or on the mechanism of membrane assembly. Furthermore, the model does not say anything about the role of cytoplasmic membranes in the process of chemical modification of secretory proteins occurring in the course of the intracellular transport (for instance, the glycosylation of glycoproteins) as well as on their progressive concentration. And, finally, it does not provide any explanation for the startling phenomena of membrane recognition, fusion and fission which specifically enable different membranes to interact with one another in such a precise and controlled manner.

Finally, the question might be asked as to whether in other cell systems secretion is carried out by cellular mechanisms analogous to those that we have described in pancreatic acinar cells. This is likely to be the case in liver cells, since the various membranes participating in the intracellular transport are known to have distinct differences (Benedetti & Emmelot 1968; Schneitman 1969; Zahler *et al.* 1970; Morré *et al.* 1971). Moreover, the microsomal membranes and the plasmalemma have average half-lives approximately two orders of magnitude longer than the time needed for secretion products to be cleared from the cells (Arias *et al.* 1969; Dehliger & Schimke 1971; Kuriyama, Omura, Siekevitz & Palade 1969; Bock, Siekevitz & Palade 1971) and their proteins exhibit a marked heterogeneity in turnover rates. However, the turnover of the liver Golgi and secretory granule membranes has not been estimated yet.

In other cell systems, such as the chromaffin adrenal cells (Winkler *et al.* 1972; Abrahams & Holtzman 1973; Viveros, Arqueros & Kirschner 1971; Douglas & Nagasawa 1971), acinar cells of the parotid gland (Amsterdam *et al.* 1971), cells of the adenohypophysis (Douglas & Nagasawa 1971; Pellettier 1973), adrenergic nerve (De Potter & Chubb 1971) neurohypophysis terminals (Douglas & Nagasawa 1971), and cholinergic neuromuscular junctions (Ceccarelli, Hurlbut & Mauro 1972, 1973), attention has been given only to the sequence of events occurring after incorporation of the membrane of secretory granules into the plasmalemma. While most of the authors agree that excess membrane is removed from the plasmalemma by endocytosis, there have been various conjectures as to the final fate of endocytozed membrane: based on morphological, cytochemical and biochemical evidence both reutilization and degradation have been suggested.

We have recently suggested that at least some of the inconsistencies could depend on either artefacts or unphysiological experimental conditions (Meldolesi 1974). It would therefore be highly desirable to study other cell systems in a carefully controlled manner, in order to establish the generality of our findings in pancreatic acinar cells.

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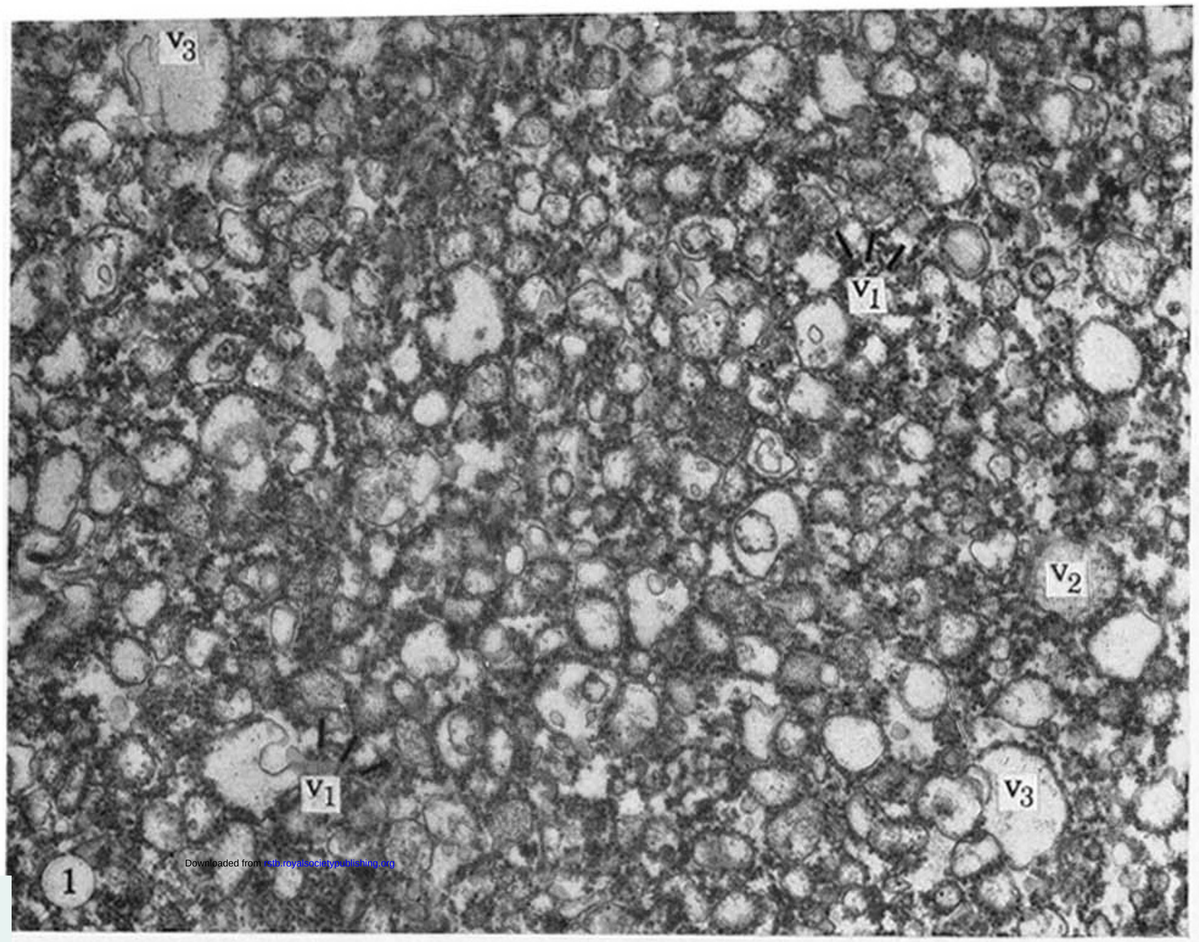

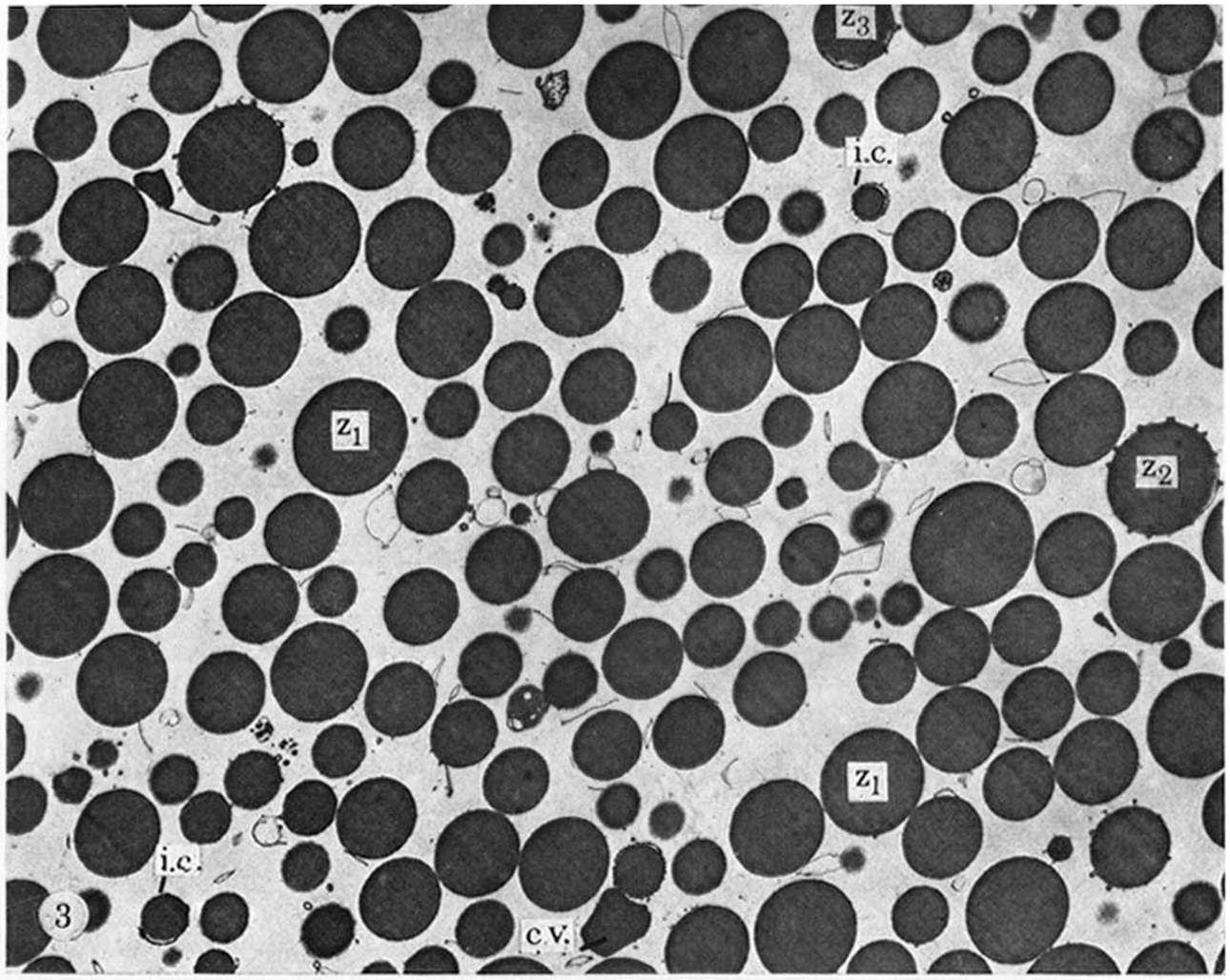

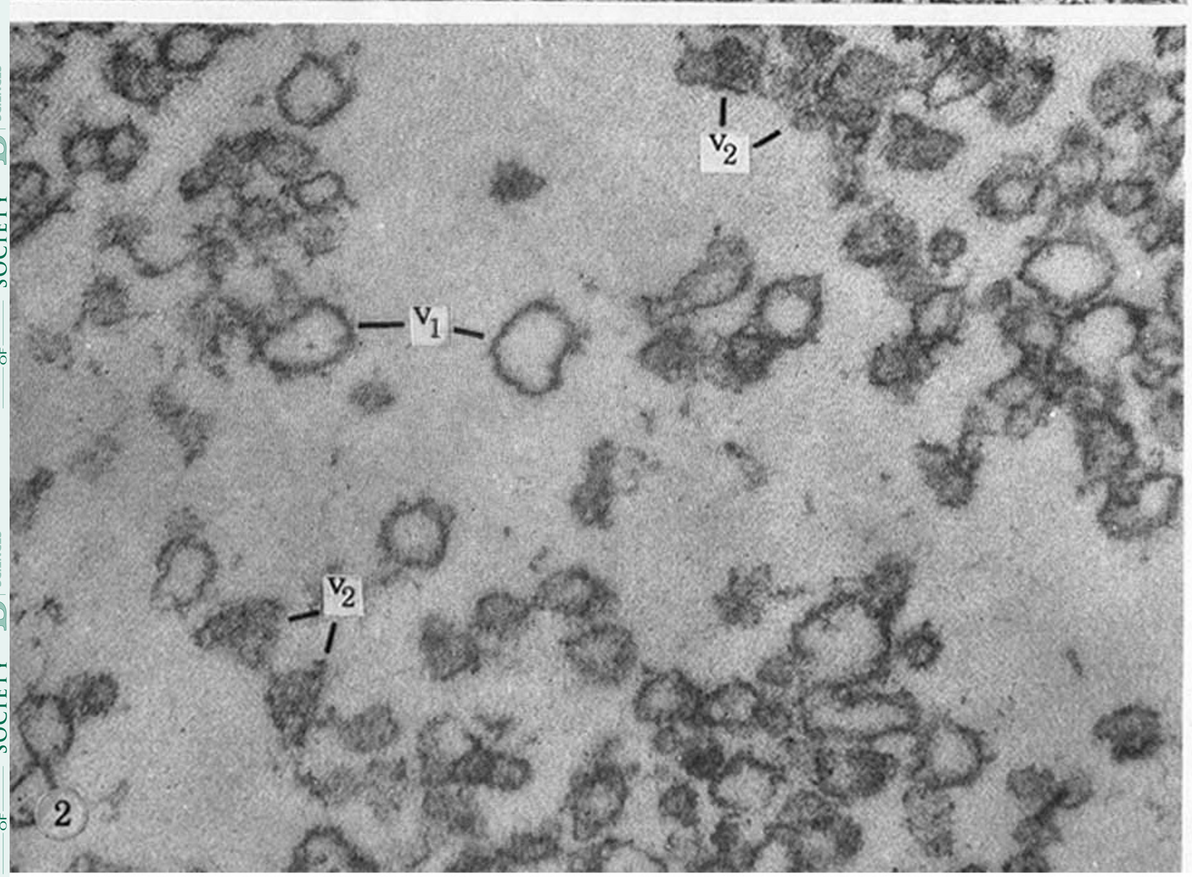

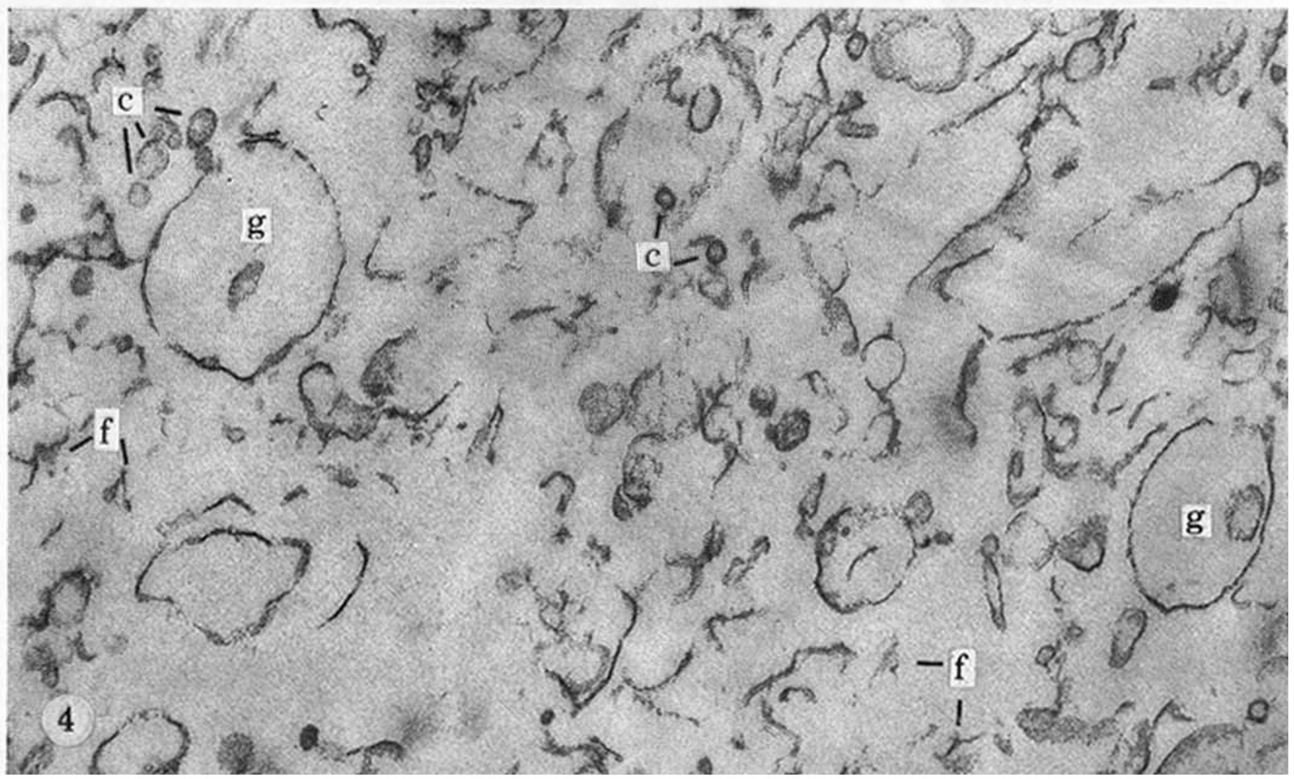

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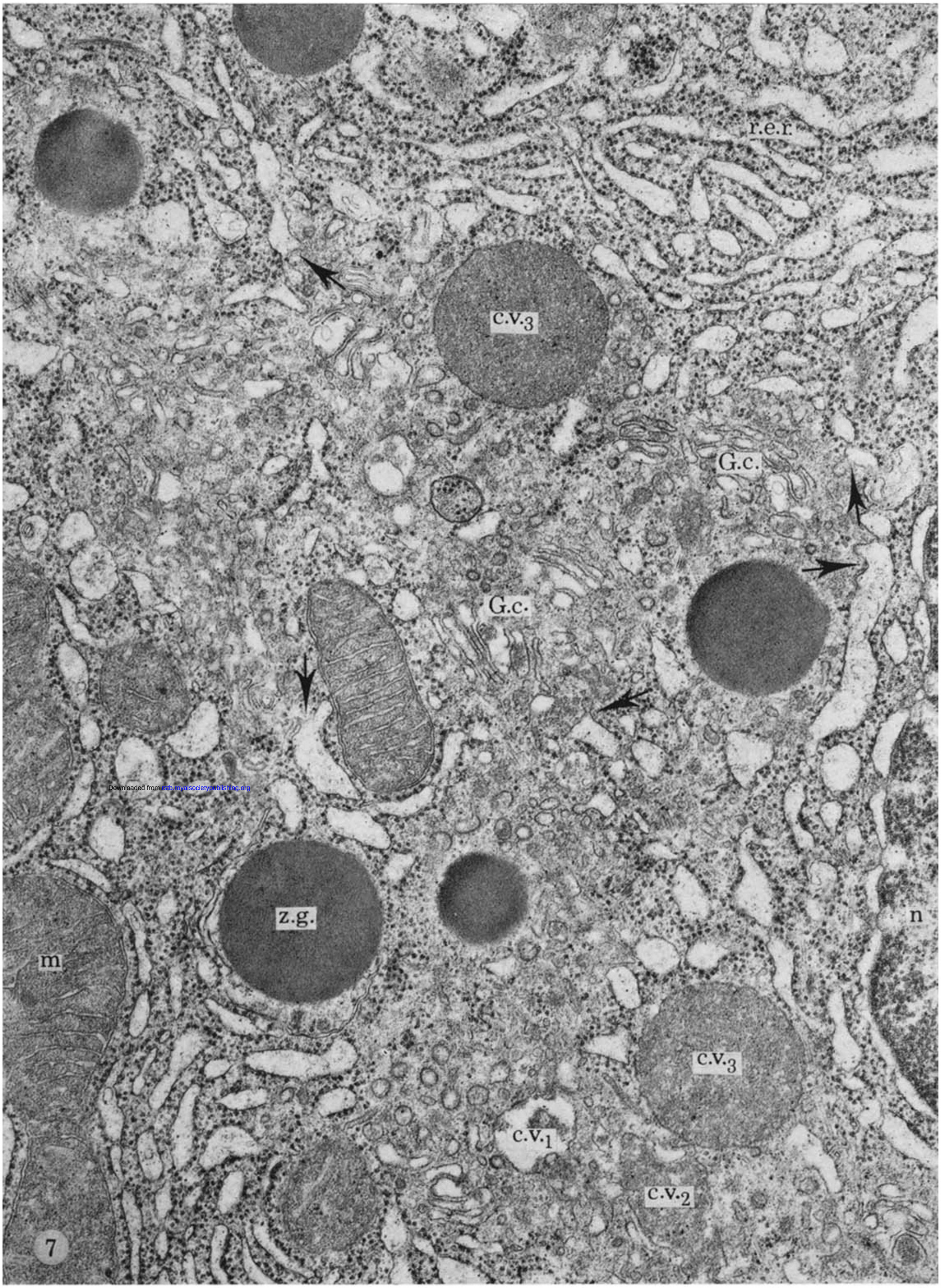

FIGURE 7. Guinea-pig pancreas acinar cell. General view of the Golgi complex. The r.e.r. cisternae immediately adjacent to the complex are part rough-, part smooth-surfaced (transitional elements). Small vesicles apparently fissioning from or fusing with the transitional elements are indicated by arrows. The Golgi complex consists of numerous vesicles of various size, usually located peripherally to the small stacks of flattened cisternae which are labelled G.c. The field includes several condensing vacuoles (c.v.) corresponding to progressive stages of maturation from 1 to 3. z.g., mature zymogen granule; n, nucleus; m, mitochondrion. (Magn. $\times 25\,000$.)