PANCREATIC MICROSOMES

An Integrated Morphological and Biochemical Study

By G. E. PALADE, M.D., and P. SIEKEVITZ, Ph.D.

(From The Rockefeller Institute for Medical Research)

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INTRODUCTION

A previous study of the microsome fraction, separated by differential centrifugation from liver homogenates, has shown that this fraction consists primarily of fragments of the endoplasmic reticulum of hepatic cells (1). It has been found that most of these fragments are derived from the rough surfaced parts of the reticulum, whereas relatively few apparently come from its smooth surfaced portions. An additional minor component is represented by the dense, peribiliary bodies found in the parenchymal cells of the liver.

Since information of this kind was restricted to hepatic microsomes, it was considered desirable to extend it to microsomal fractions derived from other organs, such as the pancreas. In the predominant cell type of this gland, *i.e.*, the acinar or exocrine cell,[1] the endoplasmic reticulum occupies a greater proportion of the cytoplasmic volume than it does in the parenchymal cell of the liver. In addition, most of the elements of the reticulum belong to the rough surfaced variety, smooth surfaced segments being exceedingly rare.

The results of the present study indicate that pancreatic microsomes are also fragments of the endoplasmic reticulum derived almost exclusively from the rough surfaced parts of the latter. Except for a few minor differences, the response of pancreatic microsomes to various reagents and experimental procedures is similar to that of hepatic microsomes. At variance with the results obtained in the fractionation of liver, postmicrosomal fractions which consist primarily of small, dense particles of nucleoprotein were obtained from pancreas homogenates.

Experimental

The general plan of the experiments and the detailed experimental procedures have been described in reference 1. For the present work, guinea pigs (~500 gm.) rather than rats, were chosen because their pancreas was found to be more voluminous and more sharply outlined.

[1] The other cell types present in the gland are the centro-acinar cells and duct epithelia, the endocrine cells or cells of the islets of Langerhans, and the cells of connective tissue and blood vessels.

The animals were anesthesized with ether, and their pancreatic gland dissected from the surrounding fat and connective tissue and removed. Even with this precaution, the removed glands contained a noticeable amount of fat which, however, caused no difficulty in the fractionation of the tissue. The amount of contaminating fat was smaller in young animals (~300 gm.) than in adults.

Methods.—Homogenization was performed at 0° to 4°C. in 0.88 M sucrose and the 1:10 (weight:volume) homogenate thus obtained was centrifuged for 30 minutes at 20,000 *g* thus separating lipide inclusions in a thick, floating pellicle, and tissue debris, whole and fragmented cells, nuclei, zymogen granules, and mitochondria in a common sediment. The fat pellicle and the sediment were discarded, whereas the supernatant was further centrifuged for 60 minutes at 105,000 *g* thereby yielding a small, red pellet slightly paler and less translucent than the microsome pellet usually obtained in the fractionation of liver homogenates. The first postmicrosomal fraction was obtained by centrifuging the supernatant of the usual microsome pellet for 2 hours at 105,000 *g*, and the second postmicrosomal fraction by centrifuging the supernatant of the preceding fraction for 15 hours at 105,000 *g*.

The pellets of the microsomal and postmicrosomal fractions were prepared for chemical determinations as described earlier (1). Ribonucleic acid (RNA), phospholipide, and the RNA-free and fat-free protein were obtained by the method of Schneider (2). Mejbaum's orcinol method (3) was employed for the determination of RNA using as a standard a sample of yeast-RNA containing 0.090 mg. P per mg. Protein nitrogen (N) was determined by nesslerization (4), and phospholipide phosphorus (PLP-P) was measured by digesting the alcohol-ether extract of the pellet and determining the P of the digest by the Fiske-SubbaRow method (5).

Microsomes to be treated with different reagents, or incubated under various conditions (*cf.* Table II), were obtained from 1 gm. wet weight pancreas and were resuspended by homogenization in 0.88 M sucrose (final volume: 10 ml.).

Materials.—The guinea pigs used were albinos from the colony of the laboratory. The ribonuclease (RN-ase) was obtained from the Nutritional Biochemical Company, Cleveland, and the deoxycholic acid from the Wilson Laboratories, Chicago. Immediately before use, the deoxycholic acid was dissolved in a NaOH solution and the ensuing Na-deoxycholate solution was then carefully titrated back to a pH of 7.5 to 7.7.

The procedures for fixing, embedding, and sectioning tissues and pellets, as well as the procedures used for electron microscopy have been described previously (1). In this work all the microsome pellets were fixed and processed *in toto*.

OBSERVATIONS AND INTERPRETATION OF RESULTS

History

In light microscopy, the acinar cell of the pancreas has been the subject of numerous studies (*cf.* references 6 and 7) which have stressed the intense basophilia exhibited by its cytoplasm, especially in the basal region of the cell body. While the affinity of the basal cytoplasm for basic dyes was early and generally recognized, its structure remained for a long time a subject of controversy. Earlier workers, using acid- or alcohol-containing fixatives, found it organized in filaments[2], lamellae and whorls or *Nebenkerne* (8, 9), and assumed that the basophilia and the laminated structure were together characteristic features of a highly active form of cytoplasm for which Garnier

[2] In view of their high affinity for dyes in general, Matthews described these structural elements as "chromidia."

proposed the name "ergastoplasm" (9). In subsequent studies, based on material fixed with "mitochondrial fixatives," *i.e.*, OsO_4-, or formaldehyde-containing mixtures, the same cytoplasm was described as being amorphous (10, 11); consequently its staining affinities were atrributed to a diffusely distributed "basophil substance," rather than to discrete, structural entities. More recent histochemical work (12, 13) has indicated that the cytoplasmic basophilia of the exocrine cell of the pancreas is primarily due to the presence of relatively large amounts of ribonucleic acid.

In electron microscopy, the basophil cytoplasm of the acinar cell of the pancreas has already been the object of a number of observations which have pointed out its high degree of organization (14–19). A more detailed study was recently published by Sjöstrand and Hanzon (20, 21).

The Morphology of the Exocrine Cell

The pancreatic acinar cell of the guinea pig, like that of other animals thus far examined, is distinguished by its voluminous and preferentially oriented endoplasmic reticulum and by its high content of small, dense particles of a type recently described (22, 23).

Basal Region.—In sections of the basal half of the cell, almost the entire cytoplasmic space is occupied by numerous, tightly packed profiles of the endoplasmic reticulum which are frequently disposed with remarkable regularity (Figs. 1 and 2). Practically all these profiles belong to the rough surfaced variety, *i.e.*, bear small, dense particles, 10 to 15 mμ in diameter, attached to the outer aspect of their limiting membranes. The profiles measure 40 to 100 mμ in diameter and vary in shape from circular to elongated, the latter form being predominant. They are usually disposed in rows which may extend for as long as 5 to 10 μ and which are generally disposed parallel to one another at more or less regular intervals of 100 to 150 mμ thus forming relatively large arrays or packages (Figs. 1 and 2). The regularity of the arrangement is only occasionally disturbed by branching profiles, branching rows, and anastomoses that connect adjacent rows (Fig. 3). The arrays appear to be usually oriented parallel to the various surfaces provided by other cell structures, *i.e.*, the nuclear surface or the cell membrane (Fig. 2). Perfect concentric arrangements, centered on the nucleus, are rarely encountered; in most cases a number of more or less independent arrays of various sizes are present, each oriented parallel to the surface of a different cell structure such as the nucleus, the cell walls, or a group of mitochondria (Fig. 3). Occasionally concentric or whorl-like figures are formed in the cytoplasm by one or more rows of profiles. Such formations correspond in all probability to the *Nebenkerne* described in light microscope studies (7–9). Within or among these variously disposed arrays of rough surfaced elements, smooth surfaced profiles are exceedingly rare. Where present, they are of circular shape, measure 40 to 100 mμ in diameter, and occur in small clusters (Figs. 4 and 7). Occasionally the membrane and content of smooth and rough surfaced profiles are found in

continuity (Figs. 3 and 4), a finding which indicates that the two varieties of profiles do not represent two unrelated structures, but rather correspond to differentiated portions within a common structural system, *i.e.*, the endoplasmic reticulum, as already shown for other cell types (24, 25). Smooth surfaced profiles in short rows or small clusters are also found in the immediate vicinity of the cell membrane (Fig. 2).

The orderly arrangement of the rough surfaced portions of the endoplasmic reticulum, although of frequent occurrence, is not the only one encountered. Sometimes circular and oval profiles predominate and their alignment in rows as well as the preferential orientation of the latter is limited to small portions of a cell section (Fig. 7). Finally, in rare instances, all the profiles appear to be scattered at random without any preferred orientation, throughout an entire cell section.

The rough surfaced elements of the endoplasmic reticulum usually have an apparently amorphous content which varies in density from cell to cell. It is frequently less dense than the cytoplasmic matrix, but occasionally the situation is reversed. In many exocrine cells, however, the profiles of the endoplasmic reticulum contain one or more round, homogeneous bodies of high density and relatively large size (Fig. 6). In three dimensions they apparently correspond to spherical granules embedded in the light substance which fills the cavities of the endoplasmic reticulum. In such cells, the profiles of the reticulum appear to be distributed at random and assume relatively large diameters and a predominantly circular shape. In density and texture, the granules mentioned are similar to the zymogen granules of the pancreatic exocrine cell, but their size is smaller and their location different (26).

The cytoplasmic matrix is disposed in narrow, more or less regular bands around the profiles of the endoplasmic reticulum. Most of the numerous, small, dense particles present in the cytoplasm are found attached to the outer surface of these profiles. Favorably oriented sections indicate that the attached particles are exceedingly numerous and occasionally disposed in close hexagonal packing on the surface of the limiting membrane of the endoplasmic reticulum. In addition to the attached particles described, a certain number of similar particles are found apparently freely scattered about the cytoplasmic matrix.

The examination of serial sections and of numerous sections of various incidences indicates that in three dimensions most of the elongated profiles of the endoplasmic reticulum correspond to cisternae of various sizes and outlines. The rows of profiles represent a continuous spectrum of formations which can be adequately described as fenestrated cisternae, when they have few and small interruptions, and as reticular sheets, when the interruptions are numerous and relatively large. The arrays formed by the rows of profiles, represent stacks of cisternae, fenestrated cisternae, or reticular sheets. Not-

withstanding the parallelism usually prevailing within these arrays, the continuity of the system appears to be maintained by the branchings and anastomoses already described (Fig. 3).

The reticular disposition, usually masked to a greater or lesser extent by the preferential orientation of the elements of the system, becomes more apparent when the cavities of the endoplasmic reticulum are distended by the accumulation of fluid (16). The cytoplasmic matrix is disposed in a network complementary to that of the endoplasmic reticulum. This disposition, although present in all cells, is more obvious in the exocrine cell of the pancreas, because the trabeculae of the two complementary networks are of comparable dimensions.

Apical Region.—In sections, most of the apical region of the cell is occupied by the predominantly circular, relatively large (0.5 to 1.2 μ), and homogeneously dense profiles of the zymogen granules (Fig. 5). In between these granules, there are numerous profiles of the endoplasmic reticulum which belong to the rough surfaced variety, like the profiles encountered in the basal half of the cell (Figs. 1 and 5). In the apical zone, however, most of these profiles are circular or oval in shape and generally appear to be distributed at random throughout the cytoplasm of the region. In three dimensions they probably correspond to a randomly disposed reticulum composed of strings of vesicles and short tubules. This randomly oriented apical part of the network gradually merges around the nucleus with the basal, preferentially oriented bulk of the system.

As in other cell types (24, 25), the centrosphere region is occupied by arrays of smooth surfaced profiles, characterized by narrow lumina and tight packing, and corresponding in appearance to formations described as Golgi apparatus in the pancreatic exocrine cell of the mouse (21) and in many other cells (27, 28). Intermediate forms, *i.e.*, profiles partly covered and partly free of granules are found in this region as well as occasional examples of structural continuity between the usual rough surfaced profiles of the endoplasmic reticulum and the supposedly characteristic smooth surfaced elements of the centrosphere region.

Nucleus.—The nucleus has a fine granular texture in which two or three types of particules differing in size and density can be distinguished. The nuclear mass is surrounded by an envelope which appears to be a perinuclear cisterna of the endoplasmic reticulum (29). A similar interpretation has been advanced for a number of other cell types (29, 25). The envelope is fenestrated or provided with pores and its cytoplasmic surface bears small attached particles like other elements of the endoplasmic reticulum (23, 29).

Cell Membrane.—The plasma membrane of the exocrine cell sends small finger-like projections (microvilli) into the lumen of the acinus, shows adhesion plates along the upper sides of the cell body, and appears frequently infolded,

or invaginated, along the lower sides and the basal pole of the cell (Fig. 2). The invaginations are usually shallow and frequently associated with small vesicles (~40 mμ diameter) which appear either isolated, or in clusters, or in rows, and which are morphologically similar to the smooth surfaced elements of the endoplasmic reticulum. A common basement membrane (~10 mμ thick) surrounds each acinus.

Variations.—As already indicated, considerable variation is encountered in the same tissue specimen from one exocrine cell to another, in relation to the amount of preferred orientation, the shape of the elements, and the density of the content of the endoplasmic reticulum. The reasons for these variations are unknown. Some of them appear, however, to represent responses of the endoplasmic reticulum to various cell injuries. For instance predominantly circular profiles with a light content and a random distribution are encountered at the very periphery of the block in the cytoplasm of cells cut open during the trimming of the tissue. It may be assumed that damage to the cell membrane results in the swelling and breaking down of the reticulum into a collection of isolated vesicles. In cells deeply located in the block, the cavities of the system are considerably enlarged and, in the most central region, the profiles of its elements are again predominantly circular. Swelling and fragmentation appear therefore to be caused also by the acidification and anoxemia which in the central zones of the block precede the fixation of the tissue (16). It is noteworthy that in general the density of the material that fills the cavities of the endoplasmic reticulum appears to vary inversely as the density of the cytoplasmic matrix. When the content is light, the matrix is dense, and *vice versa*. It should be pointed out, that even within a satisfactorily preserved region of a given specimen there is still noticeable variation in the appearance of the endoplasmic reticulum from one cell to another. The finding suggests that the reticulum is a labile structure undergoing continuous changes which may well be connected with certain phases in the metabolism of the cell (*cf.* reference 26).

Pancreatic Homogenates

The structures described *in situ* in the exocrine cell are found in recognizable form in sections of fixed and embedded pellets of pancreas homogenates. These pellets exhibit a remarkable degree of stratification with cell fragments and nuclei in the bottom layer, followed in sequence by more or less distinct layers of zymogen granules, of mitochondria, and of fragments of the endoplasmic reticulum. These latter can be identified as such by the dense, small particles which remain attached to their outer surface in spite of the various changes introduced by the homogenization of the tissue in a foreign medium. The top layer of the pellets consists primarily of agglomerated, small, dense particles.

Microsomes

Morphology.—The microsome pellets are formed almost exclusively by the fragments of the endoplasmic reticulum already mentioned in the description of homogenates. In sections of pellets fixed and embedded *in toto*, these fragments appear as circular or oval profiles, varying in diameter from 80 to 300 mμ (Figs. 8 to 10). In three dimensions most of these profiles correspond to spherical vesicles. In this respect they differ noticeably from liver microsomes, which, after similar preparatory procedures, retain the characteristic, flattened appearance shown by the cisternal elements of the reticulum *in situ*. It appears that in the case of the pancreas, tissue homogenization causes a more extensive fragmentation of the system than in the case of the liver, and that the resulting fragments are more sensitive to changes in the surrounding medium. The microsomal vesicles are bounded by a thin, ~7 mμ, continuous, and apparently homogeneous membrane which still bears on its outside surface attached particles of small size (~15 mμ) and high density (Fig. 9). By comparison with the situation found *in situ*, the number of attached particles appears to be noticeably reduced. It is therefore assumed that a certain number of particles get detached during tissue homogenization and fractionation. Most microsomal vesicles contain material of appreciable density which appears either homogeneous or finely granular in texture (Fig. 10). In some cases the content is as high in density as the granules found *in situ* in the cavities of the endoplasmic reticulum but, at variance with the situation encountered inside the cell, the dense content rarely appears surrounded by a halo of light material in isolated microsomes. A few microsomal vesicles appear to be only partly filled or entirely "empty"—*i.e.*, their content has the same density as the embedding plastic (Figs. 8 and 9). Smooth surfaced profiles are exceedingly rare among pancreatic microsomes. In addition to the elements described, microsomal pellets contain relatively few swollen, fragmented, and extracted mitochondria and a few dense, smooth surfaced, more or less homogeneous bodies of ~150 mμ diameter (Fig. 10). The latter correspond in size and appearance to the granules found in islet cells (26) and may derive from this source. They might also represent immature[3] or fragmented zymogen granules. The contaminants described are more frequently encountered in the bottom layers of microsome pellets.

Like their hepatic counterparts, pancreatic microsomes appear to be sensitive to changes in the osmolar concentration of the medium before or during fixation. Microsomes isolated in 0.88 M sucrose swell when fixed in 1 per cent OsO_4 in an "isotonic" medium, *i.e.*, 0.15 M NaCl, but the swelling is less pronounced than in the case of hepatic microsomes.

[3] Granules which are less dense, less homogeneous, and more irregular in outline than usual zymogen granules. *In situ* such granules are encountered in the centrosphere region (Golgi zone) of the cell.

Cytologically speaking the microsomal fraction separated from pancreas homogenates is more homogeneous than the corresponding fraction obtained from liver. It is almost exclusively composed of fragments of the rough surfaced part of the endoplasmic reticulum and its contamination by other cell components is negligible.

As in the case of the liver, two major structurally different components can be distinguished by further analysis in pancreatic microsomes, namely (*a*) a membranous component represented by the limiting membrane of the microsomal vesicles and derived from the membrane of the endoplasmic reticulum, and (*b*) a particulate component represented by small, dense particles attached to the outer surface of the vesicles and corresponding to the particles associated with the endoplasmic reticulum *in situ*. The content of the microsomal vesicles, already mentioned in liver preparations as a third component, is more clearly demonstrated in pancreatic microsomes in which it appears as a relatively dense, amorphous, or finely granular material which fills most microsomal vesicles. In some cases this content is of remarkably high density and as such may correspond to the intracisternal granules found *in situ*.

Chemistry.—Data concerning the chemical composition and the biochemical activity of the microsome fraction are given in Table I. The amounts of protein N and RNA contained in pancreatic microsomes are of the same order of magnitude as those previously found in hepatic microsomes (1). The percentages of protein N and of RNA of the original pancreas homogenate recovered in the microsome fraction are also comparable with the corresponding figures reported for liver. This finding appears rather unexpected in view of the greater relative volume assumed by the endoplasmic reticulum in pancreatic exocrine cells. It may be due, however, to the usual contamination of the glandular tissue, and therefore of the whole homogenate, by adipose and connective tissues. In a series of six experiments, the RNA/protein N ratio of the usual pancreatic microsomal fraction had the following values: 1.36; 0.68; 1.41; 1.30; 0.53; 0.54. In contrast with the situation found in liver microsomes, in which all values were grouped around 1.0 (1), the figures obtained on pancreas microsomes seem to cluster around two different means; *i.e.*, 1.35 and 0.58. The fact can be explained by a higher protein content in some fractions and by a lower RNA content in others. It is possible that the variations encountered have functional significance but the point cannot yet be decided with the information at hand.

A comparison of data obtained for pancreas and liver microsomes reveals two marked differences. The first one is that pancreas microsomes have a much smaller amount and a lower concentration of phospholipides than do liver microsomes. The finding very likely reflects a similar difference between whole pancreas and liver homogenates. Values previously reported by Hokin (30) for dog pancreas were also much lower than those found in the literature

for whole rat liver and liver microsomes. The values reported here for guinea pig pancreas and microsomes derived therefrom are even lower: they represent only one-fourth to one-half of the figures published by Hokin. A second difference concerns the lack of diphosphopyridine nucleotide (DPNH)-cytochrome *c* reductase activity in pancreatic microsomes when assayed under

TABLE I

Biochemical Composition of Microsomal and Postmicrosomal Fractions of Guinea Pig Pancreas

M = sediment obtained after centrifuging a mitochondrial supernatant for one hour at 105,000 *g*; PM_1 = sediment obtained after centrifuging the supernatant of M for 2 hours at 105,000 *g*; PM_2 = sediment obtained after centrifuging the supernatant of PM_1 for 15 hours at 105,000 *g*; FS_1 or FS_2 = supernatant from PM_1 or PM_2.

Experiment	Fraction	Mg. protein N/gm.*	Mg. RNA /gm.*	Mg.RNA/ Mg. protein N	μg. PLP-P/ gm.*	μg. PLP-P/ mg. protein N
1.	Homogenate	13.1	5.37‡	0.41‡	300	22.9
	Microsomes (M)	1.56	1.06	0.68	22	14.1
	PM_1	0.65	0.65	1.00	3	4.6
	PM_2	0.88	1.23	1.40	2	2.3
	FS_2	4.58	0.52	0.11	1	0.2
2.	Homogenate	13.3	7.94‡	0.51‡	230	17.3
	M	1.78	2.42	1.36	53	29.8
	PM_1	0.73	1.50	2.05	19	26.0
	FS_1	5.72	2.13	0.37	23	4.0
3.	M	3.36	1.79	0.53	—	—
	PM_1	0.90	0.73	0.81	—	—
	PM_2	1.52	1.57	1.03	—	—
	FS_2	4.89	0.71	0.14	—	—

* Per gram wet weight pancreas pulp.

‡ These figures are corrected (9) for the DNA present which would react and give some color with the orcinol reagent.

the same experimental conditions under which high enzyme activity was found in liver microsomes (31, 1).

A hemochromogen similar to the one previously described in microsomes separated from liver (32, 1) and from intestine and mammary gland (33) was also found in much smaller amounts in pancreatic microsomes from which it can be extracted by alcohol or deoxycholate. Deoxycholate extracts of microsomes separated from the pancreas and the liver of the guinea pig were found to have similar absorption spectra. In the oxidized state, the preparations showed sharp Soret peaks at 413 mμ with much smaller and broader peaks at 540 and 575 mμ. When reduced with hydrosulfite under nitrogen, the Soret peaks shifted in both cases to 425 mμ, a sharp peak appeared at 556 mμ in

hepatic microsomal extracts, and at 557 in those obtained from pancreas microsomes. In addition liver preparations gave a small flat peak at 530 mμ, which was absent in pancreatic extracts. On the basis of the absorption peaks at 556 to 557 mμ, the concentration of the hemoprotein in pancreatic microsomes was estimated to be only 10 per cent of its concentration in hepatic microsomes. The hemochromgen concerned was found to occur together with the enzyme DPNH-cytochrome *c* reductase in microsomes separated from liver (32, 1), intestinal mucosa, and mammary gland (33). Their presence in the same cell fraction was interpreted as indicative of a functional association of the cytochrome and the enzyme in electron transfer in microsomes (32, 33). Since pancreatic microsomes do not show DPNH-cytochrome *c* reductase activity[4], although they contain a small though measurable amount of hemoprotein, the findings here reported do not support the hypothesis of functional association mentioned above.

Various Treatments of the Microsomes

The results of the various treatments to which pancreatic microsomes were subjected are summarized in Table II and illustrated in Figs. 11 to 14.

"*Aging.*"—When pancreatic microsomes were resuspended in 0.88 M sucrose, incubated for 30 minutes either at 0°C. or at 30°C., and recentrifuged thereafter for 2 hours at 105,000 *g*, only about 60 per cent of the original amounts of RNA and protein N were recovered in the pellet. The lost 40 per cent were satisfactorily accounted for in the supernatant. There was no preferential loss of the components under consideration since the ratio RNA/protein N did not markedly change during the "aging" process. It is noteworthy that the RNA lost by the microsomes was recovered mostly as acid insoluble RNA in the incubating medium, not as acid soluble nucleotides. It can be assumed therefore that the RNA losses are not due to the activity of pancreatic ribonuclease, but to other, non-specific factors. "Aging" also resulted in a 30 to 50 per cent loss in hemochromogen. Under comparable conditions, the losses incurred by microsomes separated from rat liver were appreciably smaller, namely less than 15 per cent for protein N and less than 20 per cent for RNA and hemochromogen. Electron micrographs of sectioned pellets of "aged" microsomes (Fig. 11) showed that "aging" induced a certain number of morphological changes. For instance, many vesicles appeared swollen; a few showed signs of disintegration; many particles remained attached to the limiting membrane of the microsomal vesicles, whereas numerous others appeared freely scattered throughout the pellet. No preferential loss of vesicles or particles could be detected.

It appears therefore that pancreatic microsomes are less stable than their

[4] One per cent of the activity of liver microsomes could have been detected.

hepatic counterparts so that even at low temperature their decay is appreciable within a short time. The process seens to affect equally the membranous and the particulate component of the fraction.

Ribonuclease Treatment.—After incubation in RN-ase for 30 minutes at 30°C., more than 80 per cent of the original RNA of the microsomal preparations became unsedimentable. In the pellet the RNA/protein N ratio dropped

TABLE II

Effect of Incubation and of RN-ase Treatment on Biochemical Composition of Pancreas Microsomes

The microsomes were isolated from guinea pig pancreas homogenized in 0.88 M sucrose solution (centrifugation: 60 min. at 105,000 *g*). They were resuspended in either 0.88 M sucrose solution or in 0.88 M sucrose solution containing RN-ase. The suspensions were incubated under the conditions given below and centrifuged thereafter for 120 min. at 105,000 *g*. The pellets and supernatant thus obtained were analyzed chemically.

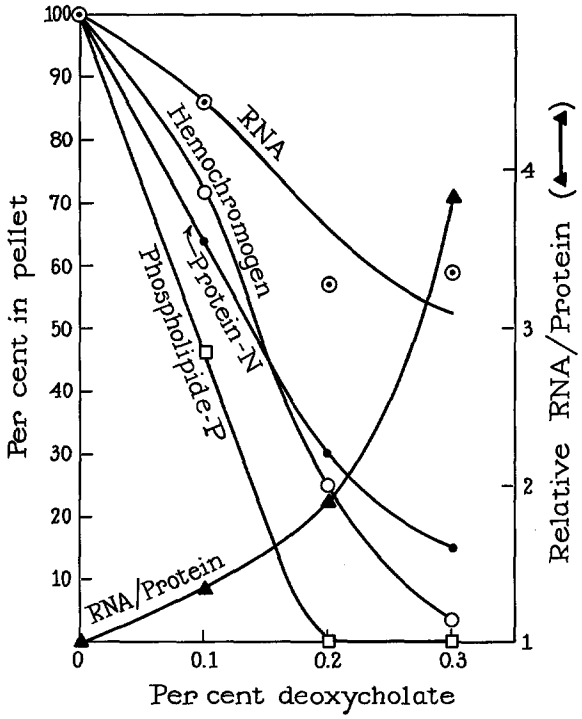
Conditions of treatment	Component	Mg. Protein N/gm.*	Mg. RNA/gm.*	mg. RNA/mg. protein N	μg. PLP-P/gm.*	μg. PLP-P/mg. protein N	Per cent hemochromogen
None	Pellet	2.40	3.38	1.41	55	23	100
Incubated at 0°C. for 30 min. in 0.88 M sucrose	Pellet	1.68	2.34	1.39	28	17	65
	Supernatant	0.72	1.02	1.43	0	—	34
Incubated at 30°C. for 30 min. in 0.88 M sucrose	Pellet	1.36	2.02	1.48	24	18	51
	Supernatant	1.26	1.24	0.99	0	—	50
Incubated at 30°C. for 30 min. in 0.88 M sucrose containing 0.5 mg. RN-ase/ml.	Pellet	2.08	0.62	0.30	46	22	47
	Supernatant	0.50	0.04	0.08	0	—	68

* Per gram wet weight pancreas pulp.

to 0.3. The RN-ase treatment induced a moderate agglutination of the microsomal vesicles, and resulted in an almost complete removal of the small particulate component of the microsomal fraction (Fig. 12). These results are comparable to those obtained in experiments with liver (1), except that in the case of pancreatic microsomes incubation in the presence of the enzyme caused less extensive agglutination so that the removal of the attached particles could be observed under better conditions.

Deoxycholate Treatment.—As in the case of the liver, deoxycholate treatment caused a rapid clarification of the microsome suspensions which, upon recentrifugation, yielded much smaller, yellowish, opalescent pellets. The percentage of microsomal protein and RNA recovered in this pellet decreased

with the rise of deoxycholate concentration as indicated in the graph of Text-fig. 1. Approximately 55 per cent RNA and 20 per cent protein N were recovered in the pellet after treatment with 0.3 per cent deoxycholate with the lost components being satisfactorily accounted for in the supernatant fluid.



TEXT-FIG. 1. Effect of deoxycholate on the biochemical composition of pancreas microsomes. The microsomes were obtained from guinea pig pancreas homogenized in 0.88 M sucrose solution (centrifugation: 60 min. at 105,000 *g*). They were suspended in 0.88 M sucrose solution containing the amounts of Na deoxycholate (pH 7.5–7.7) indicated. The treated suspensions were centrifuged immediately for 120 min. at 105,000 *g*, and the pellets and supernatant fluids thus obtained were analyzed chemically. The results indicate what per cent of the original untreated material is still sedimentable after deoxycholate treatment.

The electron micrographs of sectioned pellets of deoxycholate-treated microsomes revealed a high degree of sensitivity of the membranous component to this reagent. Even after treatment with as little as 0.1 per cent deoxycholate there were relatively few vesicles found in the pellets (Fig. 13) and after 0.3 deoxycholate, membranous elements were exceedingly scarce or absent (Fig. 14). Such pellets were made up primarily of small, dense particles some of which were sharply outlined whereas others appeared poorly defined and were frequently embedded in an amorphous material.

It can be seen that conversely to the RN-ase incubation, deoxycholate treatment brings about the "solubilization" of the membranous component of the microsome fraction so that what is left of the microsomes are small, dense particles with a high RNA/protein N ratio. These results are again similar to those obtained with liver microsomes, except that deoxycholate seems to have a more destructive effect on both components, membranous and particulate, of pancreatic microsomes. Indeed, the membranous component is destroyed at lower concentrations than the corresponding component in hepatic microsomes and the particles suffer more damage, which parallels the greater RNA losses. Pancreatic microsomes thus appear to be in general less stable than their hepatic counterparts.

Postmicrosomal Fractions

In view of the rather large number of small, dense particles which appear to be freely scattered in the cytoplasmic matrix *in situ*, as well as in view of the noticeable losses in attached particles incurred by the elements of the endoplasmic reticulum during tissue homogenization and fractionation, an attempt was made to separate one or two postmicrosomal fractions by further centrifugation of the microsomal supernatant. Table I gives gross chemical analyses of these postmicrosomal fractions and of the final supernatant, *i.e.*, the supernatant of the last fraction. Of the relatively large amount of RNA still present in the usual microsomal supernatant, almost three-fourths was recovered in the two postmicrosomal fractions, while less than 10 per cent of the amount found in the original pancreas homogenates remained in the final supernatant. The two postmicrosomal fractions also contained a certain amount of protein, which represented approximately 70 to 80 per cent of their weight[5]. Only 10 per cent of the protein N of the original homogenates was recovered in these fractions while ~35 per cent remained in the final supernatants. The RNA/protein N ratio was higher in the two postmicrosomal fractions than in the usual microsomes and dropped to a small value in the final supernatant.

The morphological analysis of the pellets (Figs. 14 to 19) showed that both postmicrosomal fractions consisted mainly of small, dense particles, ~15 mμ in diameter, which occurred either individually scattered or, more frequently, disposed in chains (Figs. 15 and 16) or clusters. In the bottom layer of the second postmicrosomal fraction, the particles were frequently found aggregated in large masses which occasionally reached 300 to 600 mμ in diameter (Fig. 18). A certain amount of order could be noticed in the arrangement of the particles in many such aggregates. The first postmicrosomal fraction also contained a limited number of vesicles of the smooth and rough surfaced

[5] Assuming that the only significant contributions to the dry weight of these particles are made by protein and RNA.

variety. In the second postmicrosomal fraction such membranous elements were exceedingly rare.

Thus, in contrast to the situation encountered in the fractionation of liver homogenates (1), postmicrosomal fractions consisting primarily of small dense particles can be separated from pancreas homogenates. These fractions contain RNA and protein but practically no phospholipides. Their RNA/protein N ratio varies from 1 to 2.

DISCUSSION

The Morphology of the Pancreatic Exocrine Cell.—The cytoplasmic organization of the pancreatic exocrine cell of the guinea pig appears to be similar to that found in other species thus far examined, such as the rat (14–16) and the mouse (18, 20, 21). As already stated it is characterized by the presence of a voluminous endoplasmic reticulum which is almost exclusively formed by rough surfaced elements and which usually shows a considerable amount of preferred orientation in the basal half of the cell.

The high degree of preferential orientation seems to be due primarily to the tendency of its cisternae and reticular sheets to maintain a more or less constant spacing between them. The present observations might be taken to suggest that the high degree of preferred orientation is the result of the packing imposed by the considerable volume of the reticulum in pancreatic exocrine cells, but observations of other cell types (25, 26, 1) indicate that such an arrangement also occurs in the absence of packing. The finding suggests that the arrangement may result from an interplay of attraction and repulsion forces between the elements concerned and that the usual spacing of $\sim$100 mμ may represent the minimum possible distance allowed by this interplay.

As in other cell types examined, the system appears to be rather labile and to react promptly by swelling, fragmentation, and loss of preferential orientation to a number of diverse conditions, such as damage to the cell membrane, and acidification or anoxemia before fixation. Noticeable variations are commonly encountered in the density of the diffuse material that fills the cavities of the system. These variations are apparently converse to those shown by the density of the cytoplasmic matrix and as such they suggest the existence of active fluid exchanges between the two cytoplasmic phases separated by the membrane of the endoplasmic reticulum. In the pancreatic exocrine cell of the guinea pig, the system is distinguished by the presence of relatively large intracisternal granules which were described and discussed in a separate paper (26).

The cytoplasmic matrix contains a considerable number of small, dense particles of a type recently described (22, 23) which appear to have a distinctive affinity for the membrane of the endoplasmic reticulum since the outer surface of this membrane is dotted with attached particles whereas other

cytoplasmic membranes are free of them. Numerous particles of similar morphology are found freely scattered in the cytoplasmic matrix.

The other components of the acinar cell, *i.e.*, the nucleus, the perinuclear cisterna, the mitochondria, and the membranous system of the centrosphere region were not studied in detail in the preceding observations. In their general organization they are similar to corresponding structures described in other cell types.

The present interpretation of the structures found in the cytoplasm of the basal half of the pancreatic exocrine cell differs, in many respects, from that given by previous authors. A regular disposition of structural elements was first noticed in electron micrographs by Dalton (14) who ascribed it to a system of "concentrically arranged lamellae." Bernhard *et al.* (15), in a study covering not only the exocrine cell of the pancreas but other glandular cells as well, assumed that the structural units involved were "ergastoplasmic filaments" or "chromidia" and noticed that these "filaments" or "fibrils" were frequently paired or showed double contours. After the introduction of better methods for fixation and embedding, the structural elements concerned were described as vesicles and tubules (canaliculi) and homologized with the endoplasmic reticulum (16, 34). Sjöstrand (17) interpreted them as "double membranes," a type of structure which he found prevalent in many cell components (mitochondria, cell membranes). He assumed that "double membranes" in general are solid lamellae formed by central lipide layers covered on each side by protein films but mentioned that the cytoplasmic double membranes of the pancreatic acinar cell may be more complex in structure. The cavitary nature of these structural elements was recognized by Weiss who described them as a collection of independent "ergastoplasmic sacs" (18) and by Watanabe (19) who named them "intracytoplasmic sacs" and adequately described their polymorphism. Finally Sjöstrand and Hanzon (20) identified the same structure as a special type of "intracellular cytoplasmic membrane" characterized by its association with dense particles, 140 A in diameter. These authors noted in addition that these membranes usually occurred in pairs joined at their ends except for the membrane close to the nucleus, which was always found to be single. The continuity of this membrane with the innermost membrane of the nucleus and the formation of a perinuclear cisterna was missed. In the present study the structures under consideration are interpreted as a characteristic differentiation of a cytoplasmic system of general occurrence, the endoplasmic reticulum, which is represented as a network of continuous, membrane-bound cytoplasmic spaces. The differentiation encountered in the exocrine cells of the pancreas consists in the extreme development of two common features of the system, *i.e.*, its association with small particles and its tendency to show preferred orientation. A more or less similar type of reticulum is encountered in many other cell types actively engaged in protein synthesis for secretion purposes.

Microsomes, Morphology.—As in the case of the liver, the microsome fraction separated from pancreas homogenates consists of fragments of the endoplasmic reticulum, especially of fragments of its rough surfaced elements.

Most of these fragments are, however, of smaller dimensions and assume the appearance of spherical vesicles, tubular and cisternal elements[6] being absent or exceedingly rare. Despite this partial change in morphology, the derivation mentioned is clearly indicated by the small, dense particles that "label" both microsomes and rough surfaced parts of the endoplasmic reticulum. It is assumed that the breaking down of the reticulum into microsomes is not due to mechanical tearing but rather to a generalized pinching-off process taking place upon cell injury. This assumption is supported by the following findings: (*a*) the limiting membrane of the microsomal vesicles is usually continuous, —fragments with broken, torn, or open ends are not encountered; (*b*) a fragmentation of the endoplasmic reticulum into apparently independent vesicles is frequently encountered in cells undergoing cytolysis and in cells damaged by trimming at the periphery of tissue blocks. If the mechanical factors involved in tissue homogenization are directly responsible for the fragmentation of the network, then it follows that the broken fragments "heal" rapidly and thus form closed vesicles.

The pancreatic microsomal fraction is more homogeneous than the corresponding liver preparation. It contains only occasionally smooth surfaced vesicles and includes no formations comparable to the dense peribiliary bodies found among liver microsomes. The greater homogeneity of the microsome population is evidently a reflection of the organization of the pancreatic exocrine cell which has an endoplasmic reticulum almost exclusively comprised of rough surfaced elements and which does not contain other structures comparable in size and density to the usual microsomes. At the present time, the only recognizable contamination of any significance is represented by damaged mitochondria and by dense granules which may be derived from the specific granules of islet cells or from the immature zymogen granules of acinar cells.

By more detailed analysis, three structurally different components can be distinguished in the microsome fraction. The first is of membranous nature and corresponds to the limiting membrane of the endoplasmic reticulum; the second component is particulate in character and is represented by the small, dense particles attached to the surface of the membrane mentioned; the third component, of variable morphology, corresponds to the content of the microsomes. Although different from one microsomal vesicle to another, it is in general of appreciable density and appears to be amorphous or finely granular in texture. In some instances the content is very dense and occasionally formed dense granules are found, surrounded by a halo of light material, inside the microsomal vesicles. Such cases are highly reminiscent of the intracisternal

[6] Such elements do not occur more frequently among the microsomes which contaminate heavier fractions, *e.g.*, mitochondrial and zymogen fractions of homogenates (43). Even when isolated in 1.46 M sucrose (50 per cent), pancreatic microsomes do not retain the shape of intracellular cisternae.

granules described *in situ*. The first two components are present both in pancreatic and hepatic microsomes; the same obtains probably for the third, which, however, is more clearly demonstrated in pancreatic preparations. Formed bodies were found only within pancreatic microsomal vesicles.

Microsomes, Chemistry.—Pancreatic microsomes like their hepatic counterparts contain a relatively large amount of protein. They have also in common a relatively high content of RNA, but are distinguished by their small content of phospholipides and hemoprotein and their lack of DPNH-cytochrome *c* reductase activity. In the light of the present information, it would appear therefore that the phospholipides and the enzyme, although widely considered as characteristic microsomal components (*cf.* reference 35), are not present in large or detectable amounts in all microsomes. Further work will show whether the pancreatic microsomes are unique in their chemistry, or whether the composition of the microsomal fraction varies noticeably from tissue to tissue. It remains also to be established by future work whether pancreatic microsomes are able to incorporate labelled amino acids into proteins, or whether this activity repeatedly demonstrated *in vitro* and *in vivo* for liver preparations is an isolated attribute of hepatic microsomes.

The results of the various treatments to which microsomes were subjected showed that the pancreatic fraction is more labile than the corresponding hepatic preparation. It loses more protein and RNA by "aging," its membranous component is "solubilized" at lower deoxycholate concentrations, and its particulate component is more easily removed by incubation in RN-ase. These experiments also indicate that, as in hepatic microsomes, most of the RNA of the pancreatic microsomal fraction is associated with the small particles described, whereas most of the protein and of the small amount of phospholipides present are probably located either in the membranes or in the content of the microsomal vesicles.

Postmicrosomal Fractions.—The postmicrosomal fractions isolated from pancreas homogenates are characterized by their high content of protein and RNA. They appear to consist primarily of nucleoproteins with a RNA/protein N ratio of 1 to 2. Morphologically these fractions consist of small, dense particles of $\sim$150 A in diameter similar to the particles which appear in large numbers in the cytoplasm of the exocrine cell either free or attached to the membrane of the endoplasmic reticulum. It is noteworthy that in the case of the pancreas more or less "pure" fractions of small particles can be obtained by relatively mild means, entirely comparable to those by which mitochondrial and microsomal fractions are obtained at present. The fact may be of significance for future cytochemical work.

The frequent association of the small particles in chains and relatively large, more or less orderly organized masses suggests that the individual particles are not different from one another, but represent either a homogeneous popu-

lation, or a population composed of a limited number of particle types. At the present level of resolution, the individual particles found in the two post-microsomal fractions are morphologically similar. The particles of the second fraction were found to differ thus far only by a more extensive association and a higher RNA/protein N ratio. The higher RNA content may be significant, whereas the higher degree of association is probably the result of the longer centrifugation used for separating this fraction.

It is evident that the present division of the free nucleoprotein particles found in pancreatic homogenates into two fractions is entirely arbitrary. More refined techniques are needed to find out whether these particles represent a homogeneous population or belong to a number of different classes as demonstrated by the work of Petermann *et al.* (36, 37) for the corresponding particles of spleen and liver homogenates.

Literature on Pancreas Fractionation.—There are a few reports in the literature dealing with the fractionation of pancreatic tissue. For example, Claude isolated microsomes and secretion granules from pancreas homogenized in saline and found them similar in many respects to those isolated from liver (38). Lang and Siebert (39) isolated nuclei from pancreatic homogenates (hog) prepared in 40 per cent sucrose solutions and, in collaboration with Fisher, tested nuclear and cytoplasmic fractions for proteolytic activity (40). Using the same technique, Siebert *et al.* (41) fractionated pancreas homogenates into nuclei, mitrochondria, and "microsomes plus cytoplasm" and tested these fractions for their effect on blood pressure. Hokin (30) separated zymogen granules and microsomes from dog pancreas and investigated the RNA content and the proteolytic activity of these fractions. Petermann mentioned that "macromolecular nucleoprotein particles," similar to those found in liver and spleen homogenates (36, 37), have been noted in ultracentrifugal analyses of pancreas preparations (42).

SUMMARY

The pancreatic exocrine cell of the guinea pig has a voluminous endoplasmic reticulum distinguished by extensive association with small, dense particles, and by its orderly disposition in the basal region of the cell.

In addition to the small, (~15 mμ), dense particles attached to the limiting membrane of the endoplasmic reticulum, numerous particles of similar appearance are found freely scattered in the cytoplasmic matrix.

The various cell structures of pancreatic exocrine cells can be satisfactorily identified in pancreatic homogenates.

The microsome fraction consists primarily of spherical vesicles (80 to 300 mμ), limited by a thin membrane (7 mμ) which bears small (~15 mμ) dense particles attached on its outer surface. The content of the microsomal vesicles is usually of high density. Pancreatic microsomes derive by extensive fragmentation mainly from the rough surfaced parts of the endoplasmic reticula

of exocrine cells. A few damaged mitochondria and certain dense granules (~150 mμ) originating probably from islet cells, contaminate the microsome fraction.

Pancreatic microsomes contain RNA, protein, and a relatively small amount of phospholipide and hemochromogen. They do not have DPNH-cytochrome *c* reductase activity. In six experiments the RNA/protein N ratios were found grouped around two different means, namely 0.6 and 1.3.

Pancreatic microsomes are more labile than liver microsomes but react in a similar way to RN-ase-(loss of the particulate component and RNA), and deoxycholate treatment (loss of the membranous component and of phospholipide, hemochromogen, and most of the protein).

Postmicrosomal fractions consisting primarly of small (~15 mμ), dense particles of ribonucleoprotein (RNA/protein N ratio = 1 to 2) were obtained by further centrifugation of the microsomal supernatant.

The small nucleoprotein particles of these fractions are frequently found associated in chains or clusters.

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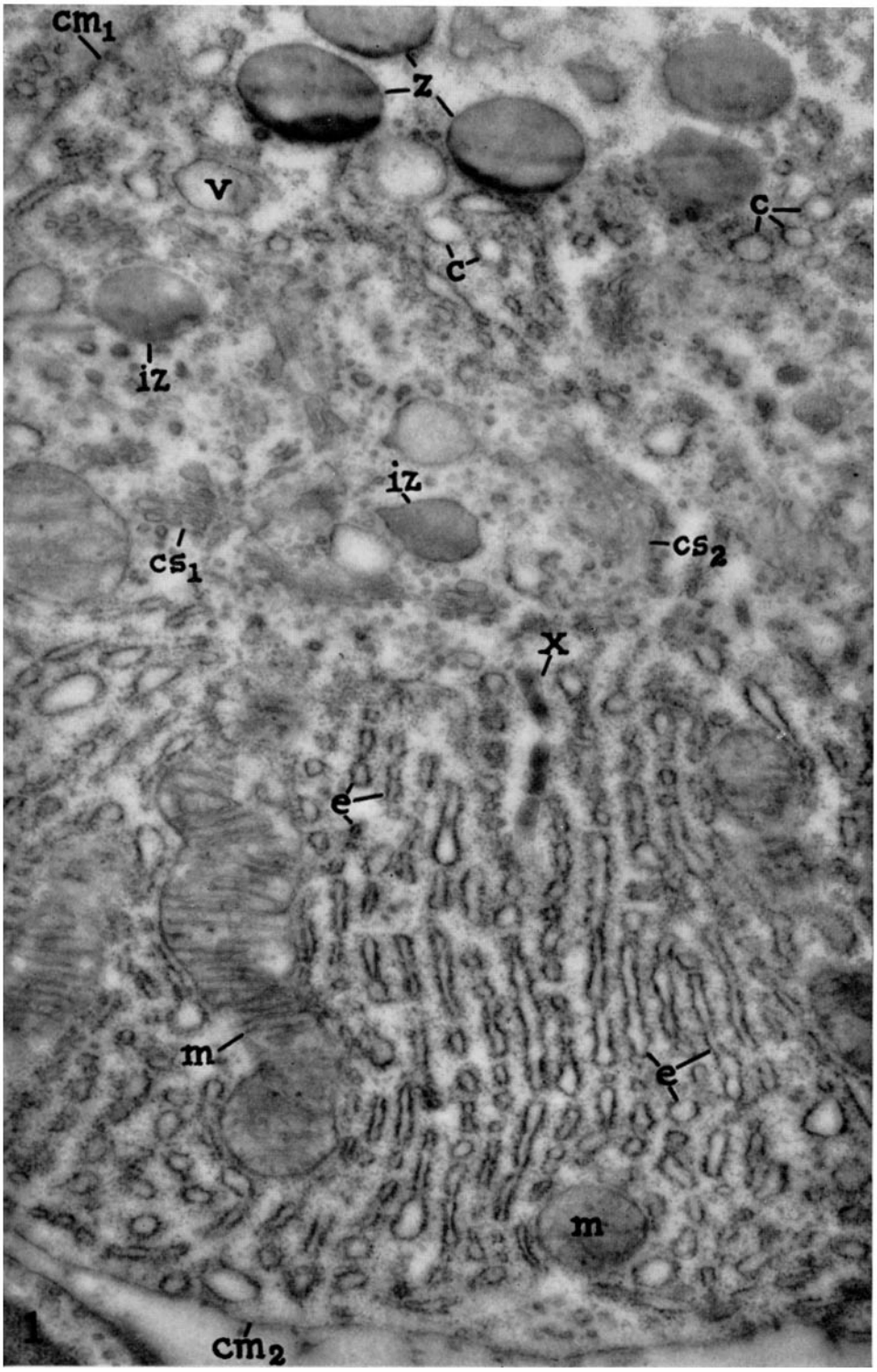
EXPLANATION OF PLATES

PLATE 170

FIG. 1. Acinar cell of the pancreas (guinea pig). The section, which bypasses the nucleus, cuts parallel to the basal-apical axis of the cell. The cell membrane appears at cm_1 and cm_2. The basal region of the cytoplasm (lower half of the figure) is occupied by a number of mitochondria (*m*) and by numerous elements of the endoplasmic reticulum (*e*) which belong to the rough surfaced variety and are disposed in preferentially oriented rows.

The apical region of the cell contains a few zymogen granules (*z*) and vacuoles (*v*) and a relatively small number of rough surfaced profiles that belong to the endoplasmic reticulum. Note that the latter are of predominantly circular shape (*c*) and seem to be distributed at random.

The centrosphere zone (Golgi zone) occupies an intermediate position between the basal and the apical regions. Its membranous elements appear either normally (cs_1) or obliquely (cs_2) sectioned. Vacuoles partly filled with dense material represent immature zymogen granules (*iz*). The structure marked *x* is still unidentified. × 26,000.



(Palade and Siekevitz: Pancreatic microsomes)

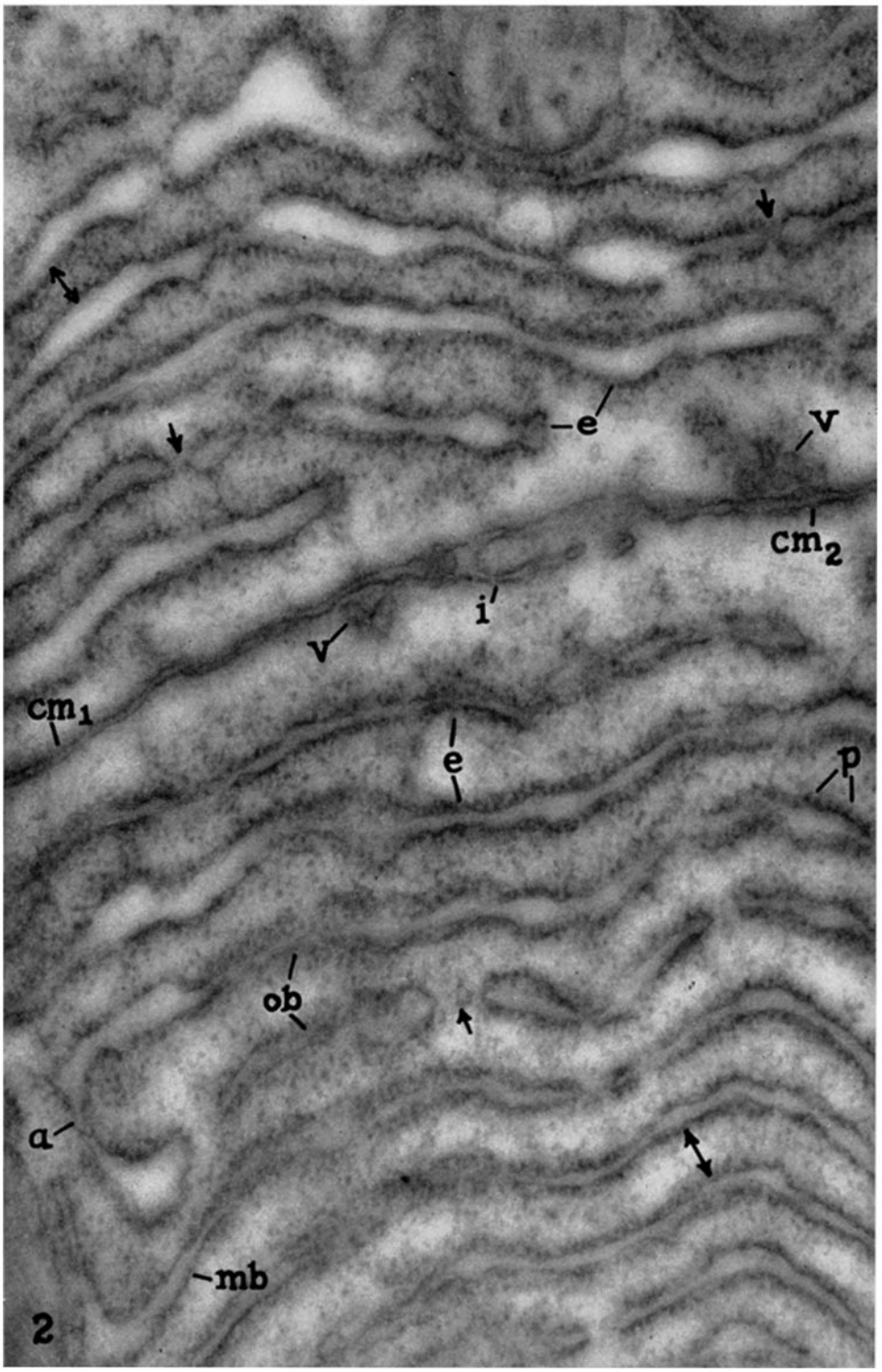
PLATE 171

FIG. 2. Small field in the basal cytoplasm of two adjacent acinar cells (guinea pig, pancreas).

The respective cell membranes (cm_1, cm_2) run parallel to one another except for a shallow invagination (*i*) formed by cm_2. Small clusters of smooth surfaced vesicles (*v*) are present in the cytoplasm of both cells in the immediate vicinity of the cell membranes.

The rest of the field is occupied by rough surfaced profiles of the endoplasmic reticulum. They are of predominantly elongated shape (*e*) and appear disposed in rows which run parallel to one another at more or less regular intervals. Simple arrows mark spaces separating profiles within the same row; double arrows indicate spaces in between adjacent rows. In this case, the ER (endoplasmic reticulum) profiles are generally disposed parallel to the cell membranes. Most of the ER profiles are normally sectioned and show clearly their lumina, homogeneous content, and limiting membranes (*mb*) with attached particles (*p*) on the surface that faces the matrix. The lumen and the limiting membrane of obliquely sectioned ER elements (*ob*) are blurred to a varied extent. In three dimensions, the ER profiles correspond to simple and fenestrated cisternae disposed in stacks. An anastomosis between two adjacent cisternae can be seen at *a*.

Note the variation in width shown by the cisternal lumina and the correlated variation in density shown by the luminar content. Small dense particles, similar to those attached to the membrane limiting most of the ER elements, are found freely scattered in the cytoplasmic matrix. Note that the inner surface of the cell membrane is free of attached particles. × 64,000.



(Palade and Siekevitz: Pancreatic microsomes)

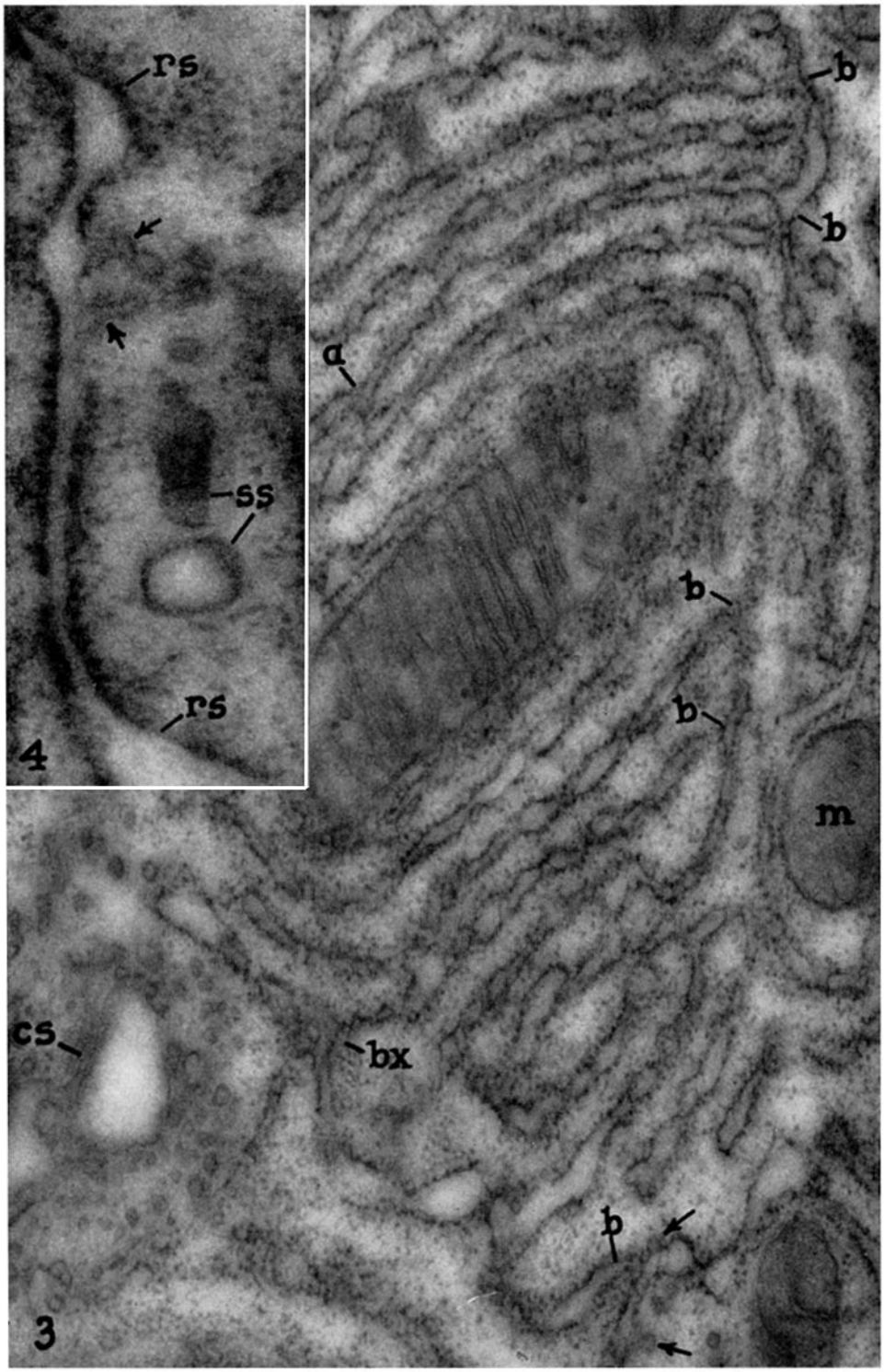
PLATE 172

FIG. 3. Relatively large field in the basal region of an exocrine cell of the pancreas (guinea pig).

A few mitochondrial profiles (*m*), and an extension of the membranous system (*cs*) of the centrosphere region (Golgi apparatus) can be seen in this field which is occupied primarily by elongated profiles of the endoplasmic reticulum. These profiles belong to the rough surfaced variety and are disposed in more or less parallel rows around a mitochondrion. Numerous branchings (*b*) and an anastomosis (*a*) indicate that the endoplasmic reticulum is still a continuous system even when its elements are preferentially oriented. A four-way branching is indicated by *bx*. The arrows point to small, smooth surfaced vesicles which appear to be in continuity with the rough surfaced elements of the system. × 47,000.

FIG. 4. Small field in the basal region of an exocrine cell of the pancreas (guinea pig).

A rough surfaced, elongated profile of the endoplasmic reticulum can be seen at *rs*. Part of a similar profile appears along the right margin of the figure. A row of smooth surfaced profiles (*ss*) are present in the intervening cytoplasmic matrix. Some of these smooth surfaced vesicles are in continuity (arrows) with the rough surfaced element marked *rs*. Such appearances indicate that the two types of profiles represent local differentiations within a common, continuous system, not two different, unrelated structures. × 90,000.



(Palade and Siekevitz: Pancreatic microsomes)

PLATE 173

FIG. 5. Small field in the apical zone of an exocrine cell of the pancreas (guinea pig). The cell membrane appears at *cm* running in parallel with the membrane of a centroacinar cell (*ca*).

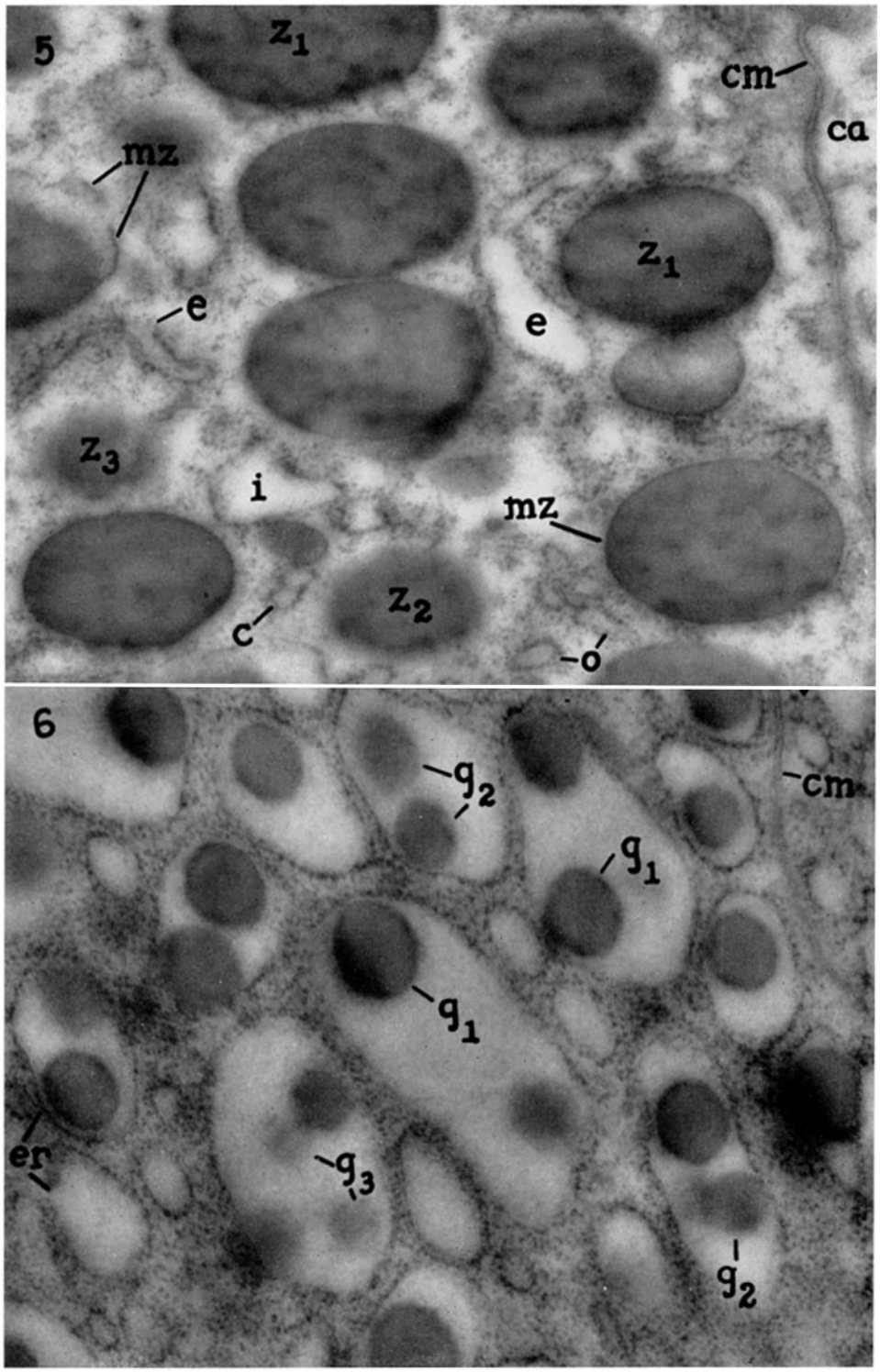
The apical zone of the exocrine cell is occupied by the relatively large, homogeneously dense profiles of zymogen granules (z_1, z_2, z_3). Their variation in size and sharpness of outline is due to sectioning. The larger, sharply outlined profiles are medial sections (z_1), the smaller ones lateral sections (z_2, z_3). As expected, the latter are less dense and poorly outlined. A thin dense membrane can be distinguished only around certain mature zymogen granules (*mz*, right); it appears more clearly around immature or damaged zymogen granules (*mz*, left).

Rough surfaced profiles of the endoplasmic reticulum appear in the cytoplasmic matrix and among the zymogen granules. These profiles are randomly distributed and of various shapes, *e.g.*, circular (*c*), oval (*o*), elongated (*e*), and irregular (*i*). × 42,000.

FIG. 6. A small field showing intracisternal granules in the basal region of an exocrine cell of the pancreas (guinea pig).

The cell margin appears at *cm*. The cytoplasm is occupied by relatively large profiles of the endoplasmic reticulum (*er*) which belong to the rough surfaced variety and contain dense granules in their cavity. The variation in size and sharpness of outline shown by the profiles of these intracisternal granules is largely due to sectioning. Small, poorly outlined profiles represent lateral sections (g_2, g_3); large, more clearly outlined profiles (g_1) correspond to medial sections. Some ER elements contain a single intracisternal granule, some others two or even four granules. The cytoplasmic matrix in between the ER profiles contains a large number of freely scattered small, dense particles.

Note the difference in size and location between the zymogen granules in Fig. 5 and the intracisternal granules in Fig. 6; note also their similarity in high density and fine texture. × 42,000.



(Palade and Siekevitz: Pancreatic microsomes)

PLATE 174

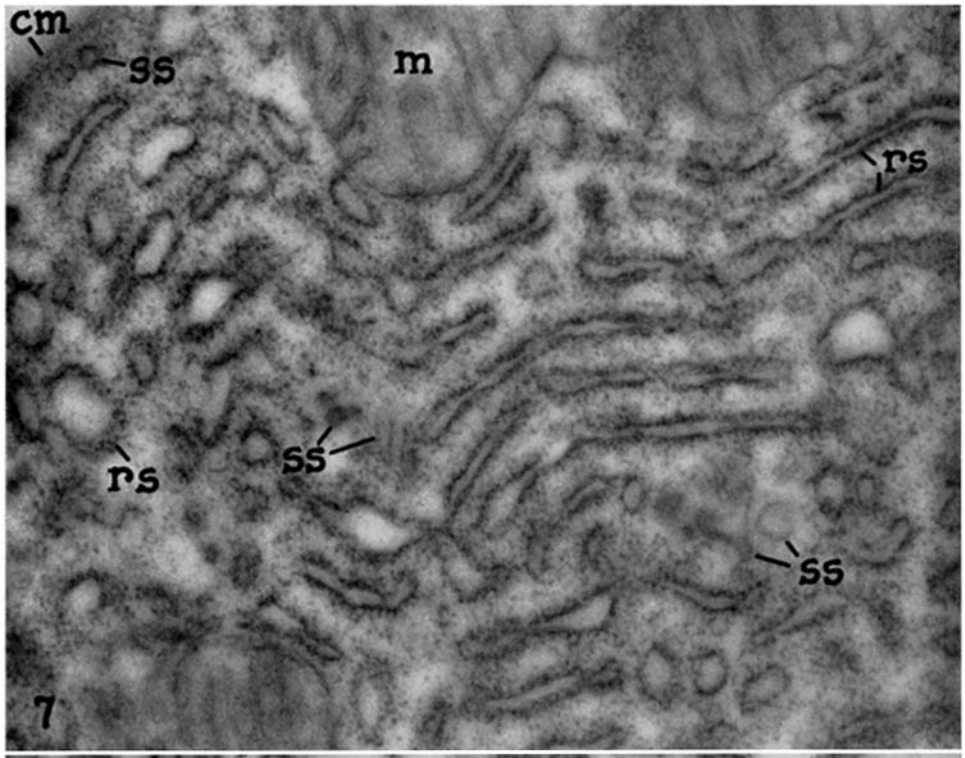
FIG. 7. Basal cytoplasm in an acinar cell of the pancreas (guinea pig). The cell membrane can be seen at *cm* and a mitochondrial profile at *m*. The rest of the field is occupied by profiles of the endoplasmic reticulum. Among the latter, those of rough surfaced variety (*rs*) are clearly predominant; only a few smooth surfaced profiles appear in clusters (*ss*), either in between the rows of rough surfaced elements or immediately below the cell membrane. Note the variation in shape and distribution shown by the rough surfaced profiles of the endoplasmic reticulum: elongated, regularly disposed elements predominate in the right half of the field, whereas circular and oval profiles with a less orderly distribution occur in the left half. × 42,000.

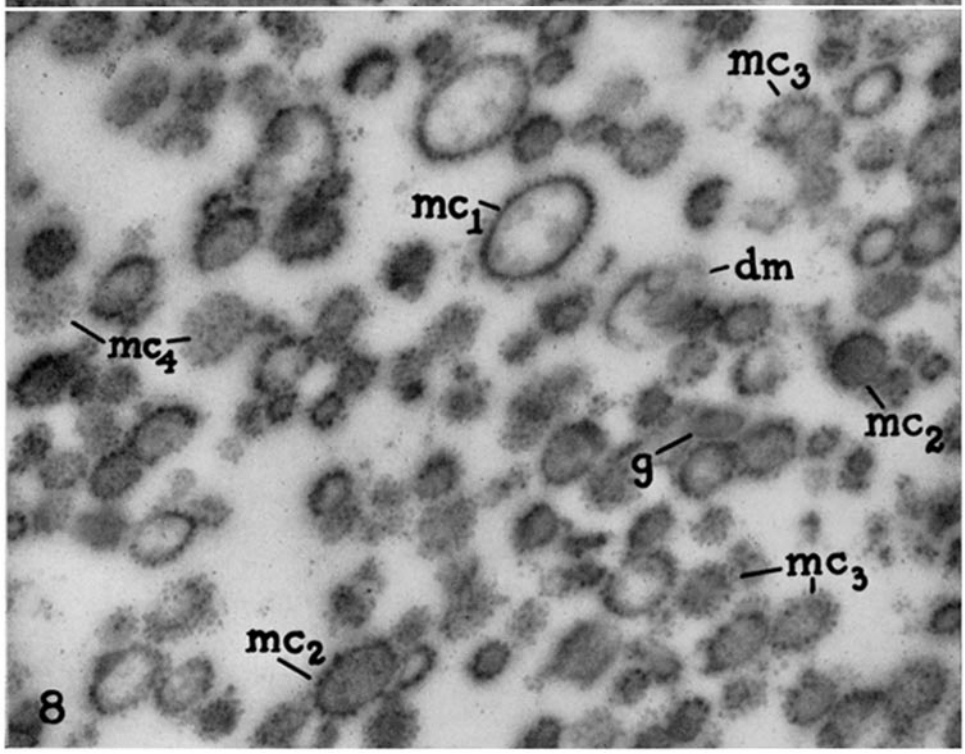
FIG. 8. Representative field in a section of a microsome pellet isolated from a pancreatic homogenate (guinea pig), prepared in 0.88 M sucrose. The pellet was fixed *in toto* in 2 per cent OsO_4 in 0.88 M sucrose, and then embedded in *n*-butyl methacrylate, and sectioned.

The micrograph shows that the microsomal pellet consists of spherical or ovoid vesicles limited by a membrane which bears small, dense particles attached to its outer surface. The microsomal vesicles have a content which varies in density from one element to another but which in general is distinguished by a relatively high density. Part of the variation in the size and appearance of microsomes is due to sectioning. A medial section (mc_1, mc_2) gives a larger profile limited by a sharply outlined membrane with a single row of attached particles on its outer surface. Lateral sections give smaller profiles, with a blurred membrane covered partially (mc_3) or entirely (mc_4) by attached particles. Part of the microsomal variation is due however to other factors such as unequal swelling and extraction. Compare for instance the medial profiles mc_1 and mc_2.

Damaged mitochondria (*dm*) and dense granules (*g*) are found contaminating in small numbers the microsomal fraction.

A comparison with Fig. 7 shows that the microsomes are identical in fine structure and similar in size to the rough surfaced elements of the endoplasmic reticulum. It is concluded that the microsomes derive by extensive fragmentation from the rough surfaced portion of the endoplasmic reticulum. × 42,000.





(Palade and Siekevitz: Pancreatic microsomes)

PLATE 175

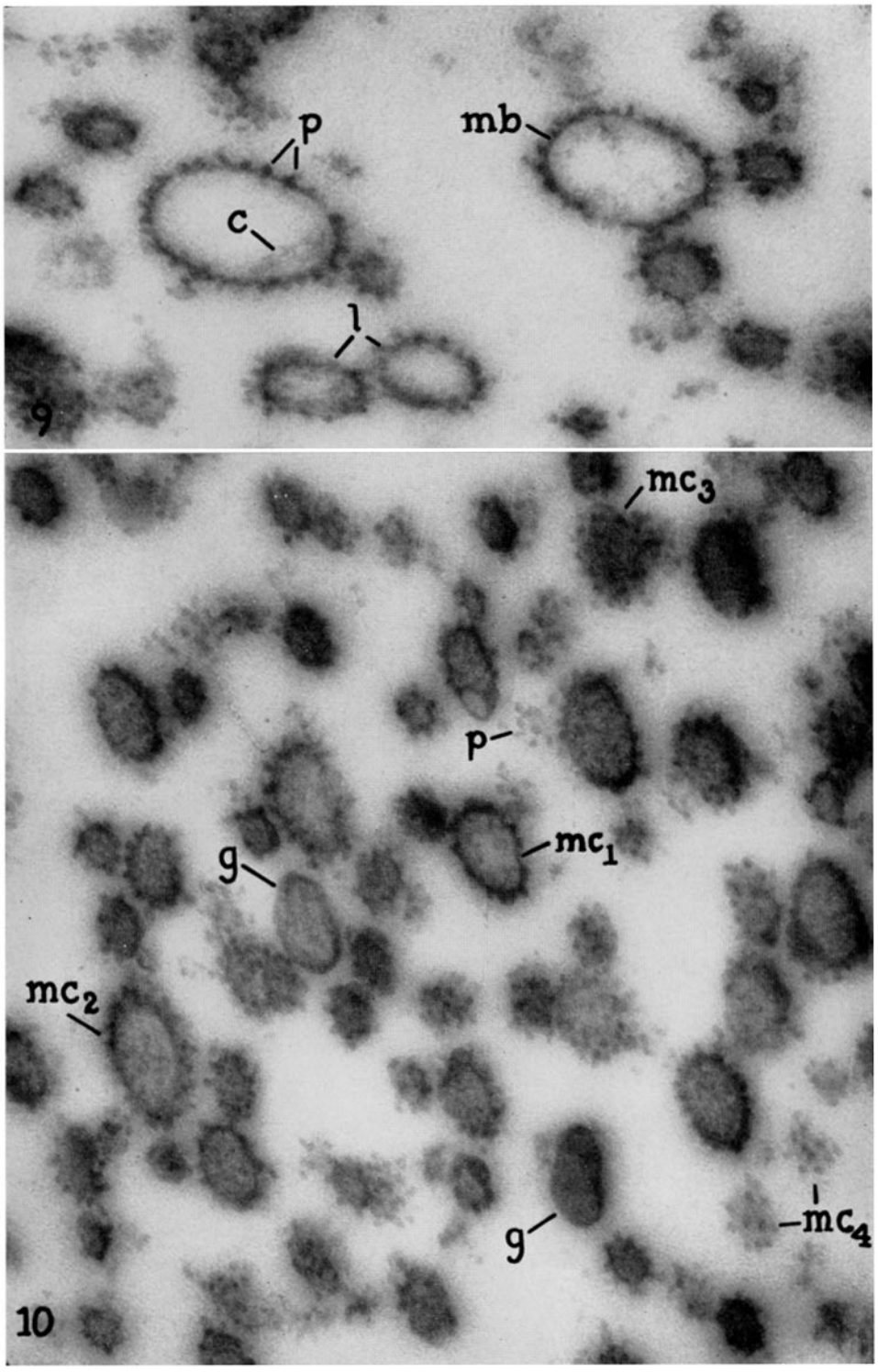
FIGS. 9 and 10. Electron micrographs of sectioned microsomal pellets. Same preparatory technique as for Fig. 8.

FIG. 9. Two swollen, medially sectioned microsomal vesicles show their limiting membrane (*mb*), their light, partially extracted content (*c*), and their attached small particles (*p*). Lateral sections (*l*) give either an oblique or a full faced view of the limiting membrane of the microsomal vesicles and of the numerous particles attached thereon. × 90,000.

FIG. 10. Numerous microsomal vesicles appear sectioned medially (mc_1) or laterally (mc_2, mc_3, mc_4). Groups of apparently free particles (*p*) may represent extremely lateral sections of microsomes.

Note that the content of the microsomal vesicles is of relatively high density and in some cases of finely granular texture. Dense lumps may be microtomy artefacts.

Dense granules (*g*) with a smooth surface contaminate this microsome fraction. Their origin is uncertain. × 80,000.



(Palade and Siekevitz: Pancreatic microsomes)

PLATE 176

FIGS. 11 and 12. Pancreatic microsomes incubated for 30 minutes at 30°C. in 0.88 M sucrose alone (Fig. 11) or in 0.88 M sucrose in the presence of 5 mg. ribonuclease per ml. (Fig. 12).

After incubation, both microsomal suspensions were centrifuged for 2 hours at 105,000 *g* and the pellets obtained were fixed in 2 per cent OsO_4 in 0.88 M sucrose.

FIG. 11. Microsomal visicles (*mc*) bearing attached particles can still be recognized in this pellet. Some vesicles appear agglutinated (*a*) or swollen (*s*). Irregular clusters of apparently free particles (*p*) are also present. Note that the microsomal content is generally of low density.

FIG. 12. Microsomal vesicles (*mc*) are recognized with difficulty. They have lost their attached particles and appear agglutinated in irregular clusters (*c*). Their content is of relatively high density.

Both figures ×60,000.

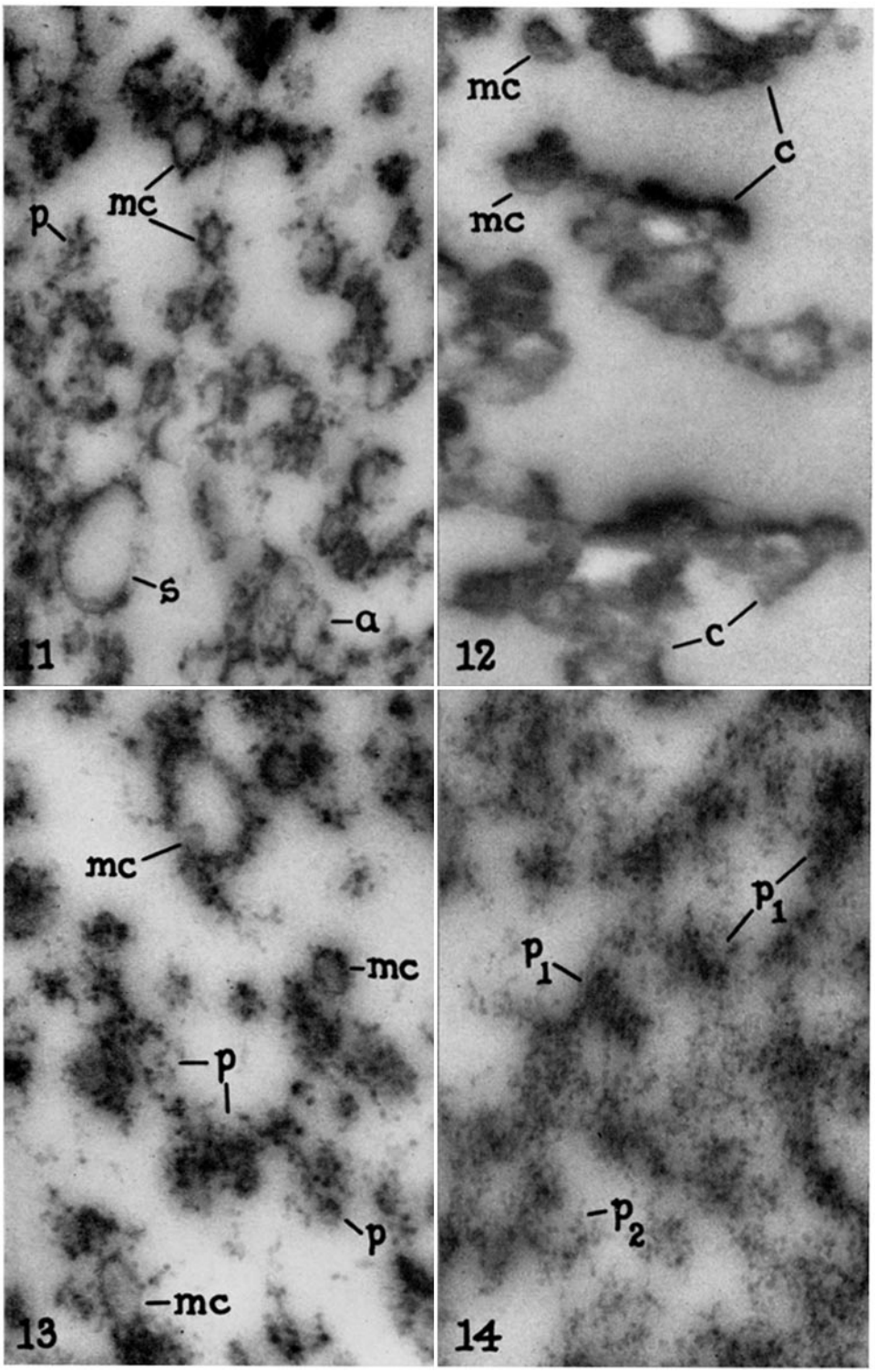
FIGS. 13 and 14. These figures illustrate an experiment in which pancreatic microsomes were treated with increasing concentrations of deoxycholate. The concentration was 0.1 per cent for the specimen in Fig. 13 and 0.3 per cent for that in Fig. 14. After treatment the microsomal suspensions were centrifuged for 2 hours at 105,000 *g* and the pellets obtained were fixed in 2 per cent OsO_4 in 0.88 M sucrose.

FIG. 13. A number of microsomal vesicles (*mc*) are still recognizable in this pellet which contains in addition irregular clusters of small particles (*p*) and masses of amorphous material.

FIG. 14. The pellet consists primarily of small dense particles disposed in clusters (p_1) and chains (p_2). No vesicles are present in this field. In general they are only occasionally encountered after 0.3 per cent deoxycholate. The experiment indicates that deoxycholate treatment results in a preferential destruction of the membranous component of the microsomal fraction.

Note that the particles in Fig. 14 show more variation in size and density, and in general appear smaller than particles in Figs. 9 and 10 and Fig. 13. The differences mentioned may reflect losses in protein incurred by the particulate component as a result of the treatment with 0.3 per cent deoxycholate. Compare also Fig. 14 with Figs. 16 and 17. In the preparation in Fig. 14 the RNA/protein N ratio was two to four times higher than in the preparations shown in Figs. 16 and 17.

Both figures × 60,000.



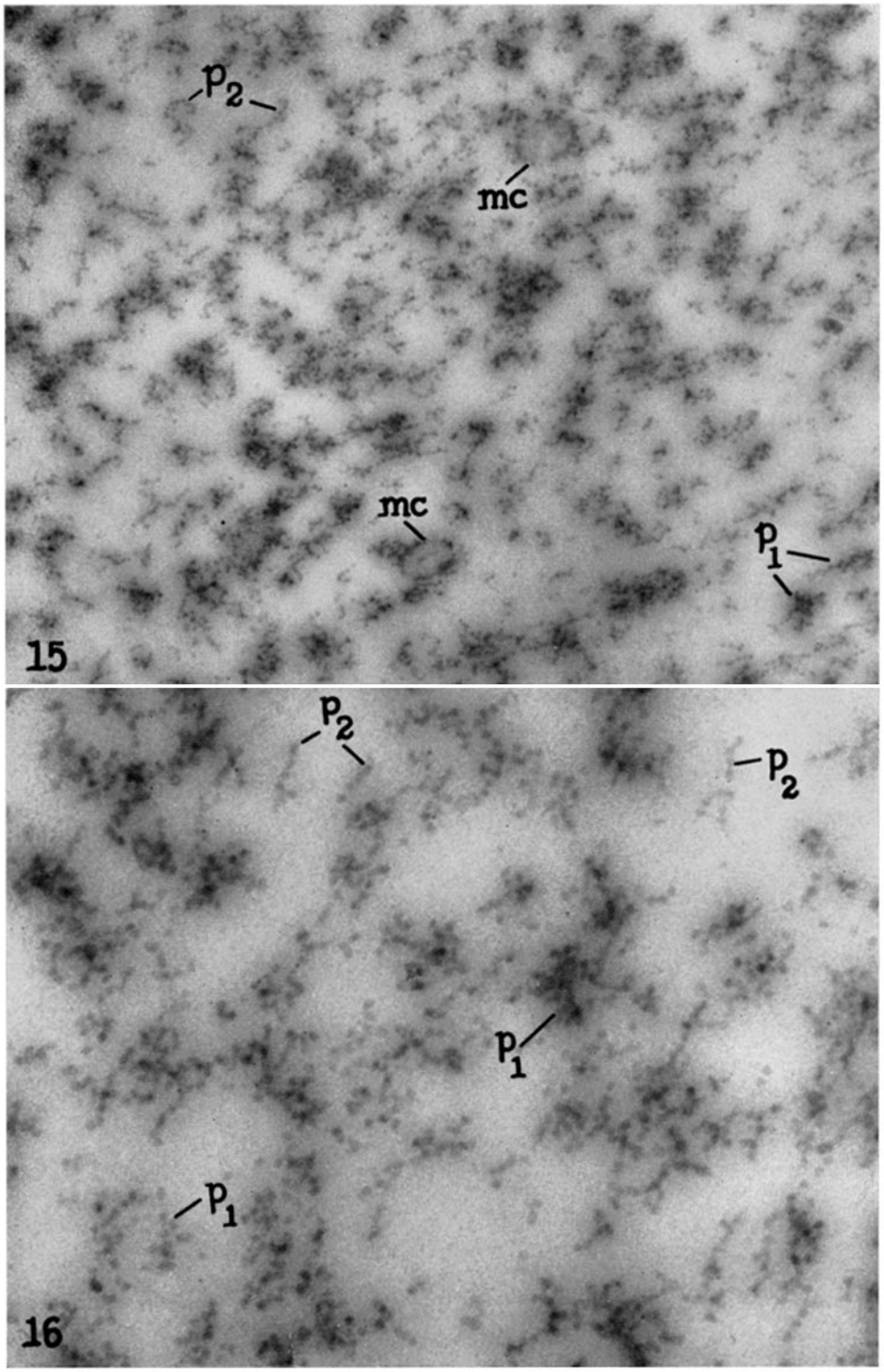
(Palade and Siekevitz: Pancreatic microsomes)

PLATE 177

FIGS. 15 and 16. First postmicrosomal fraction. The pellets obtained by centrifuging the supernatant of the microsomal fraction for 2 hours at 105,000 *g* were fixed *in toto* in 2 per cent OsO_4 in 0.88 M sucrose, then embedded in *n*-butyl methacrylate, and sectioned.

FIG. 15. Representative field in a sectioned pellet. The micrograph shows that the fraction consists of small dense particles disposed in clusters (p_1) or chains (p_2). A few swollen, rough surfaced, microsomal vesicles (*mc*) are still present among these particles. × 40,000.

FIG. 16. Small field in a postmicrosomal pellet showing, at a higher magnification, the small dense particles which are the main constituent of this fraction. They measure 100 to 200 A in diameter and appear disposed in clusters (p_1) or chains (p_2). × 80,000.



(Palade and Siekevitz: Pancreatic microsomes)

PLATE 178

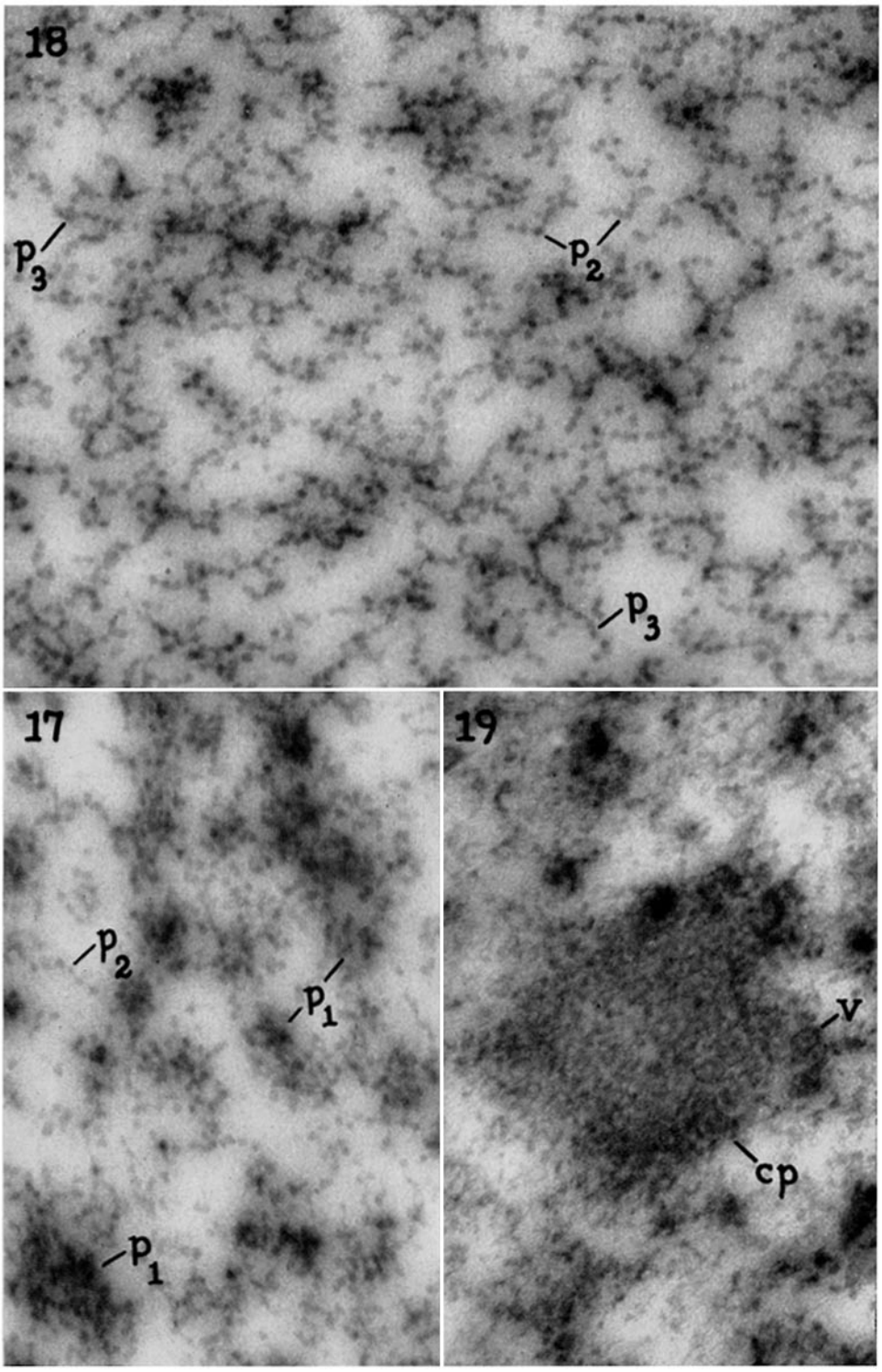
FIGS. 17 to 19. Second postmicrosomal fraction. The pellets, obtained by centrifuging the supernatant of the first postmicrosomal fraction for 15 hours at 105,000 *g*, were fixed *in toto* in 2 per cent OsO_4 in 0.88 M sucrose.

The micrograph in Fig. 17 shows a representative field in such a pellet which consists primarily of small dense particles, most of them disposed in small clusters (p_1). Chains of particles (p_2) appear in between these agglomerations.

A pellet in which practically all particles are disposed in simple (p_2) or branched (p_3) chains appears in Fig. 18.

In the deeper layers of the pellet, large clusters of particles are relatively frequently encountered. An example ($c\not{p}$) is shown in Fig. 19. Note that in this layer small vesicles (v) with a relatively dense content contaminate the fraction.

All figures × 80,000.



(Palade and Siekevitz: Pancreatic microsomes)