Freeze-fracture Cytochemistry:

Localization of Wheat-germ Agglutinin

and Concanavalin A Binding Sites

on Freeze-fractured Pancreatic Cells

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ABSTRACT   The combined application of thin-section and critical-point-drying "fracture-label" is used to determine the pattern of distribution and partition of wheat-germ agglutinin and concanavalin A binding sites on the membrane faces of freeze-fractured exocrine and endocrine rat pancreatic cells. Whereas the exoplasmic face of plasma membranes is preferentially labeled by both lectins, the endoplasmic reticulum and nuclear envelope are strongly and uniformly labeled by concanavalin A but not by wheat-germ agglutinin. The results support current views in the glycosylation of membrane proteins and do not support the backflow of sialidated glycoproteins to the endoplasmic reticulum.

Recently, we showed that cytochemical and immunochemical techniques can be combined with freeze-fracture to gain generalized access for direct labeling of the fracture faces of biological membranes as well as of exposed groups in cross-fractured cytoplasm (14–16). The results of these "fracture-label" techniques can be assessed either by observation of thin sections of freeze-fractured specimens ("thin-section fracture-label") (15, 16) or of platinum/carbon replicas of critical point dried, freeze-fractured preparations ("critical-point-drying fracture-label") (14).

Initial application of thin-section fracture-label showed that numerous concanavalin A binding sites could be labeled on the membrane faces of freeze-fractured plasma membranes, endoplasmic reticulum, and nuclear envelope membranes of leukocytes, HeLa cells, and hepatocytes (16). We report here the results of thin-section and critical-point-drying fracture-label of isolated rat exocrine and endocrine pancreatic cells. We used colloidal gold and ferritin conjugates to determine and compare the patterns of distribution of wheat-germ agglutinin (WGA) and concanavalin A (Con A) binding sites on the fracture face of their plasma and intracellular membranes. Our results show that whereas Con A binding sites are present on nuclear envelope, endoplasmic reticulum, secretory vesicle, and plasma membranes, wheat-germ agglutinin binding sites are absent from the nuclear envelope and the endoplasmic reticu-

lum. These results are consistent with current views on the pathways of glycosylation of membrane proteins (5, 12, 18, 19, 22, 23, 24) and do not support the reflux of fully glycosylated products to the endoplasmic reticulum (3, 7, 25, 29). They demonstrate the capacity of fracture-label to investigate the topochemistry of plasma and intracellular membranes *in situ*.

MATERIALS AND METHODS

Cells

Pancreas tissue was excised from 3- to 8-d-old rats (Sprague-Dawley) and digested with collagenase type IV (5 g/ml in Hanks' solution, from Worthington Biochemical Corp., Freehold, N. J.) for 6 min at 37°C, and isolated cells or small clusters of acinar and islet cells were obtained. The cells and cell clusters were harvested by centrifugation (800 rpm, 6 min), washed twice in Hanks' solution and fixed in 1% glutaraldehyde for 2 h at 4°C. After fixation, the cells were embedded in 30% bovine serum albumin and the suspension was gelled by cross-linking with 1% glutaraldehyde for 30 min at room temperature. Excised pancreas tissue from adult rats was also fixed directly in 1% glutaraldehyde for 2 h at 4°C. The gels and tissues were sliced into ~1 × 2 × 2-mm pieces, impregnated gradually in 30% glycerol, and frozen in partially solidified Freon 22 cooled by liquid nitrogen.

Freeze-fracture

For thin-section fracture-label (15, 16), frozen gel pieces and tissues were transferred to a glass container filled and immersed in liquid nitrogen and finely crushed with a glass pestle precooled in liquid nitrogen. For critical-point-drying

fracture-label (14), frozen gels and tissues were transferred to a petri dish filled with liquid nitrogen placed on top of liquid nitrogen/solid carbon dioxide slush and were fractured with a liquid nitrogen–cooled scalpel. All samples were thawed in a solution containing 30% glycerol and 1% glutaraldehyde in 310-mosM phosphate buffer, pH 7.5, gradually deglycinerated in 1 mM glycyl-glycine in the same buffer, and washed twice.

Cytochemical Labeling

After thawing and deglycerination, freeze-fractured gel and tissue fragments were incubated in a solution of WGA (0.25 mg/ml in 0.1 M Sorensen phosphate buffer, 4% polyvinylpyrrolidone, pH 7.4) for 30 min at 37°C. Control fragments were preincubated in 0.4 M N-acetyl-D-glucosamine for 15 min at 37°C followed by incubation in a WGA solution (see above) in the presence of 0.4 M N-acetyl-D-glucosamine. The fragments were then washed twice in Sorensen's buffer with 4% polyvinylpyrrolidone and incubated in the presence of colloidal gold/ovo-mucoid complex (1, 9, 10) for 60 min at room temperature. Freeze-fractured gel and tissue fragments were also labeled with ferritin-WGA conjugates (a generous gift from Dr. M. F. Maylié-Pfenninger, 200 mg/ml) in phosphate-buffered solution for 30 min at 25°C. For fracture-labeling with Con A, fragments were incubated in the lectin (1 mg/ml in 0.1 M Sorensen's phosphate buffer with polyvinylpyrrolidone, pH 7.4; 30 min at 37°C), washed twice in the same buffer, and labeled with a colloidal gold/horseradish peroxidase complex (2, 9, 10). Controls were preincubated in 0.4 M Methyl-α-D-mannopyranoside for 15 min at 37°C followed by incubation in the presence of the lectin and the sugar as described above.

Processing for Electron Microscopy

THIN SECTIONS: Labeled gel and tissue fragments were washed twice in Sorensen's phosphate buffer in polyvinylpyrrolidone, fixed in 1% osmium tetroxide in veronal acetate buffer pH 7.6 for 120 min at 4°C, stained en bloc with uranyl acetate (5 mg/ml), dehydrated in acetone, and embedded in Epon 812. Hardened blocks were thick sectioned, and appropriate areas of fracture were selected by light microscopy. Thin sections were examined unstained or post-stained with uranyl acetate and lead citrate with an electron microscope equipped with a goniometer stage.

PLATINUM/CARBON REPLICAS: Labeled gel and tissue fragments obtained by fracture with a cold scalpel were fixed in 1% osmium tetroxide (in veronal acetate buffer pH 7.4 for 30 min), dehydrated in ethanol, and critical point dried in ethanol/carbon dioxide. The dried gel fragments were then placed fracture side up onto a specimen carrier coated with double sticky tape, shadowed with a 2 nm of platinum/carbon evaporated from an electron gun, and the replicas were reinforced with carbon. The shadowed fragments were digested in sodium hypochlorite, and the replicas were washed and mounted on Formvar-coated grids.

RESULTS

Ultrastructure

THIN SECTION: Observation of thin sections of pancreatic acinar cells after freeze-fracture and thawing shows preservation of membrane and cytoplasmic components (Figs. 1 and 2). As expected, fracture frequently follows plasma and intracellular membranes; cracks provide complementary views of the fracture process (Fig. 2). As in other membranes (15, 16), thin sections of protoplasmic faces consist of a series of short segments with an interrupted unit membrane (trilaminar) profile (Figs. 5 and 13). This aspect is frequently not observed in fractured exocrine secretory vesicles where the unit membrane profile is often absent (Figs. 9 and 10) or, less frequently, appears continuous (not illustrated). Occasionally, membranous blebs or blisters are associated with fractured membranes, particularly when they are cross-fractured (Fig. 10).

PLATINUM/CARBON REPLICAS: Platinum/carbon replicas of freeze-fractured cells after thawing, deglycerination, dehydration, and critical point drying show preservation of the original freeze-fractured structures (Figs. 3 and 22). Fracture faces of plasma and intracellular membranes are evident (Figs. 3, 6, 8, and 22) and finer details such as nuclear pores (Fig. 22), as well as the typical anastomosing network of strands in tight junctions (Figs. 3 and 17) can also be resolved. In cross-fracture, the cytoplasm appears rough but compact, i.e., without displaying a system of cavities and fibrillar components (Figs. 11 and 22).

Although the initial observation of replicas of critical-point-dried, freeze-fractured cells indicates good preservation, there are important changes in the fine structure of fractured membranes relative to that of conventional freeze-fractured replicas.
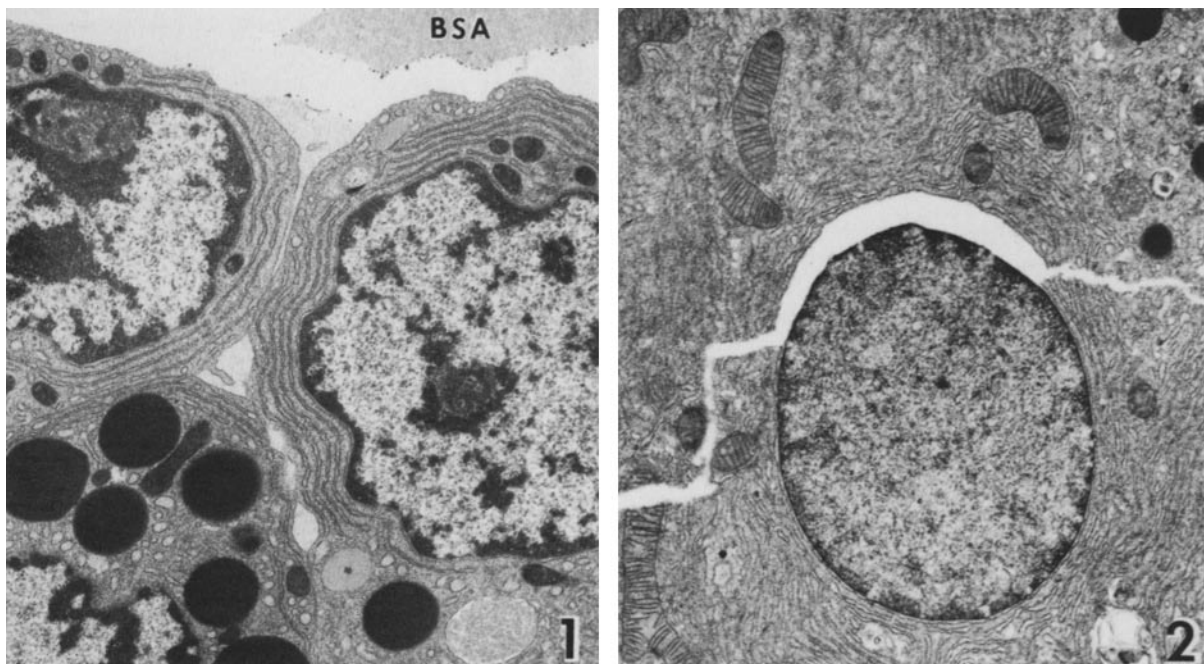
FIGURES 1 AND 2   Thin-section views of freeze-fractured rat exocrine pancreatic cells embedded in bovine serum albumin. In Fig. 1, fracture follows the plasma membrane adjoining the *BSA*; in Fig. 2, the course of the fracture crosses the cell interior and partially follows the nuclear envelope. × 8,000.

FIGURE 3 Platinum/carbon replica of the plasma membrane of an exocrine pancreatic cell (protoplasmic face, PM_p) after freeze-fracture and critical point drying. *TJ*, tight-junction band. × 47,000.

The typical appearance of freeze-fractured membranes—membrane particles and rugosities against a relatively smooth background—is no longer seen. Neither particles nor rugosities can be resolved: instead, protoplasmic faces display a relatively smooth texture with a varying complement of minuscule, erratic fissures (Figs. 3, 6, and 7). Exoplasmic faces appear as uniformly rough surfaces without any additional regular detail. In general, they are recognized because of the overall cellular context: for instance, in lateral membranes they alternate with the protoplasmic faces of adjacent membranes (Figs. 6–8). In exception to this general rule, the exoplasmic faces of zymogen granules (which presumably overlie condensed granular material) are well preserved; the protoplasmic faces are less well preserved (Fig. 11).

Cytochemistry

EXOCRINE PANCREAS

WGA: Thin sections of freeze-fractured specimens treated with WGA and labeled by ovomucoid–colloidal gold show that almost all of the label is associated with the exoplasmic halves of plasma membranes (Fig. 4), with only a few colloidal gold granules present over the protoplasmic faces (Fig. 5). No label is present on the fracture faces of endoplasmic reticulum (Fig. 4) or of nuclear envelope membranes (not illustrated). Ferritin labeling of the exoplasmic fracture faces of secretory granule membranes is variable and appears most intense over condensing vacuoles (Fig. 10), less intense over mature zymogen granules (Fig. 9). The surface of the acinar lumen is also strongly labeled (Fig. 4, *lower inset*). Presumably, the colloidal gold particles were able to penetrate the looser matrix within that region, gaining access to the luminal surface of the plasma membrane, particularly the microvilli. Nonspecific label could occasionally be found trapped within narrow fissures in the gel.

In replicas of critical-point-dried material, it is impractical to attempt the identification of isolated ferritin molecules, as these are difficult to distinguish from the background, particularly against the rough texture of exoplasmic fracture faces. Colloidal gold particles are, however, easily observed, as they are not dissolved during hypochlorite cleaning of the replicas

and do not detach from their platinum/carbon casts. They are observed as black (electron-opaque) circles (10–15 nm in diameter) with which a white cone of shadow is generally associated (Fig. 7). However, in high-contrast replicas their immediate identification can be somewhat difficult (Fig. 6). Unfortunately, it is clear that low-contrast replicas are also undesirable. A compromise must, therefore, be made between replica contrast and granule visibility, and particular care must be taken during the photographic processing of the micrographs.

Examination of platinum/carbon replicas of WGA-treated, colloidal gold–ovomucoid fracture-labeled exocrine pancreatic cells confirms thin-section observations. Dense labeling is observed over the exoplasmic faces of plasma membranes, with only a few granules present over the protoplasmic faces (Figs. 6–8). The distribution of the gold granules in each face appears random. Only a few gold particles can be observed over exoplasmic or protoplasmic faces of the endoplasmic reticulum (Fig. 8) or the nuclear envelope (not illustrated; see Fig. 22 for a similar aspect in an endocrine pancreatic cell). On exoplasmic faces of fractured secretory granules, the label is generally moderate but clearly seen (Fig. 11). This aspect co-exists with a minority of vesicles, frequently of less regular shape and with a rough texture, which are intensely labeled (Fig. 11, asterisk).

CON A: Similar to WGA, Con A labels the exoplasmic face of freeze-fractured plasma membranes as observed in thin sections (Figs. 12 and 18) or in replicas of critical-point-dried cells (Fig. 17). Protoplasmic faces are only sparsely labeled (Fig. 17), and the label is frequently absent from their thin sections (Fig. 13), presumably because of the inherently smaller area of membrane face observed. The distribution of the label is apparently random and unrelated to that of the tight-junction strands (Fig. 17). Remarkably, however, thin sections of fracture-labeled cells show that the exoplasmic faces of endoplasmic reticulum (Figs. 14 and 18) or of nuclear envelope membranes (Fig. 15) are heavily labeled. Neighboring exoplasmic fracture faces of plasma membranes and endoplasmic reticulum indicate heavier labeling of the latter (Fig. 18). Label can also be seen over cross-fractured endoplasmic reticulum or nuclear envelope membranes (Fig. 14, 15, and 18, arrowheads). In replicas of critical-point-dried, fracture-labeled cells, dense labeling by colloidal gold granules is also associated with
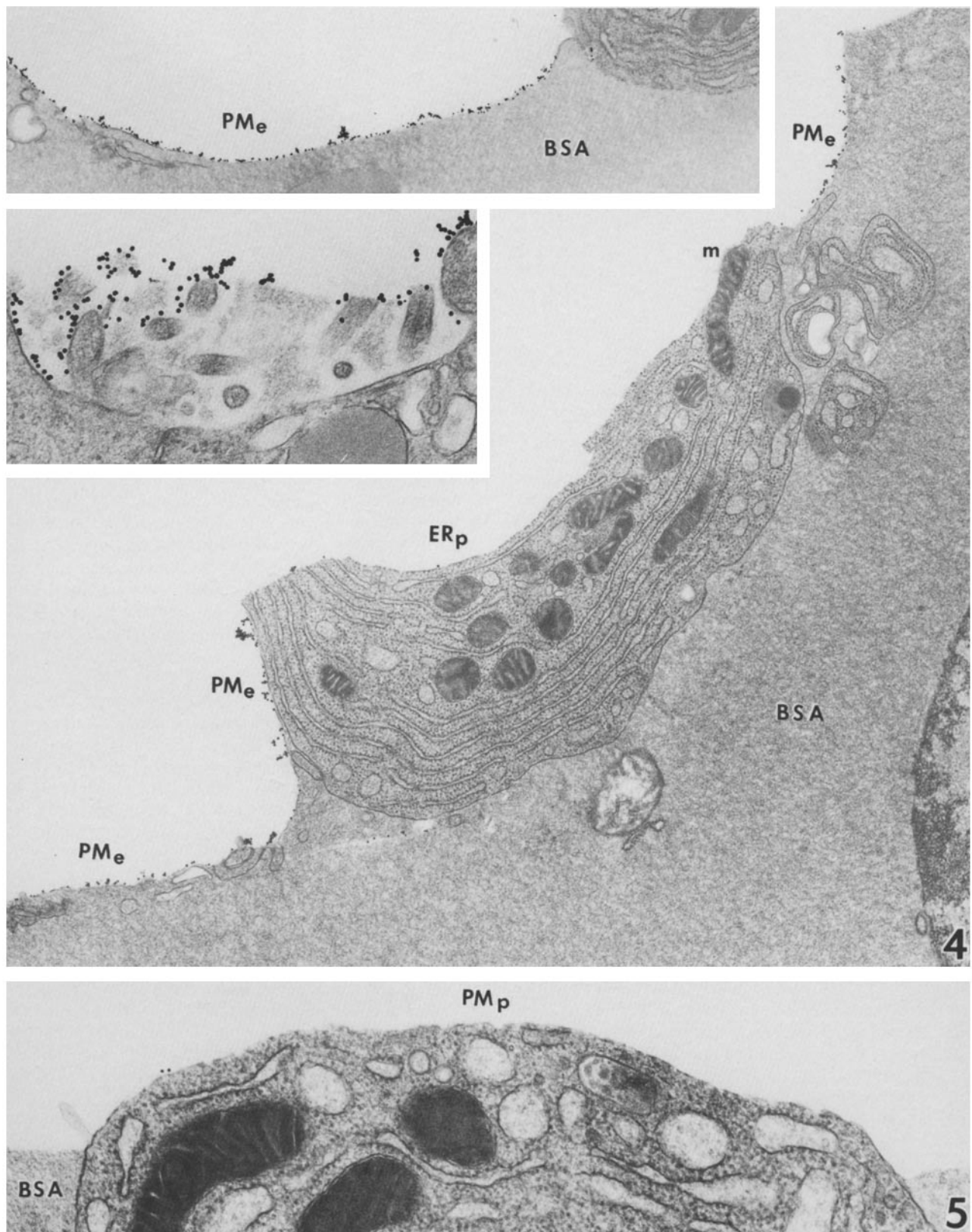
FIGURES 4 AND 5   Thin-section views of freeze-fractured exocrine pancreatic cells treated with WGA and labeled with a colloidal gold/ovomucoid complex. Most of the label is associated with the exoplasmic face of the plasma membrane (PM_e). The endoplasmic reticulum (ER_p, Fig. 4) and mitochondria (m) are virtually unlabeled. *Lower inset* in Fig. 4 shows partial penetration of gold particles into the luminal matrix with surface labeling of apical membrane and microvilli. Fig. 4, × 22,000 (*upper inset*, × 17,000; *lower inset*, × 52,000); Fig. 5, × 40,000.
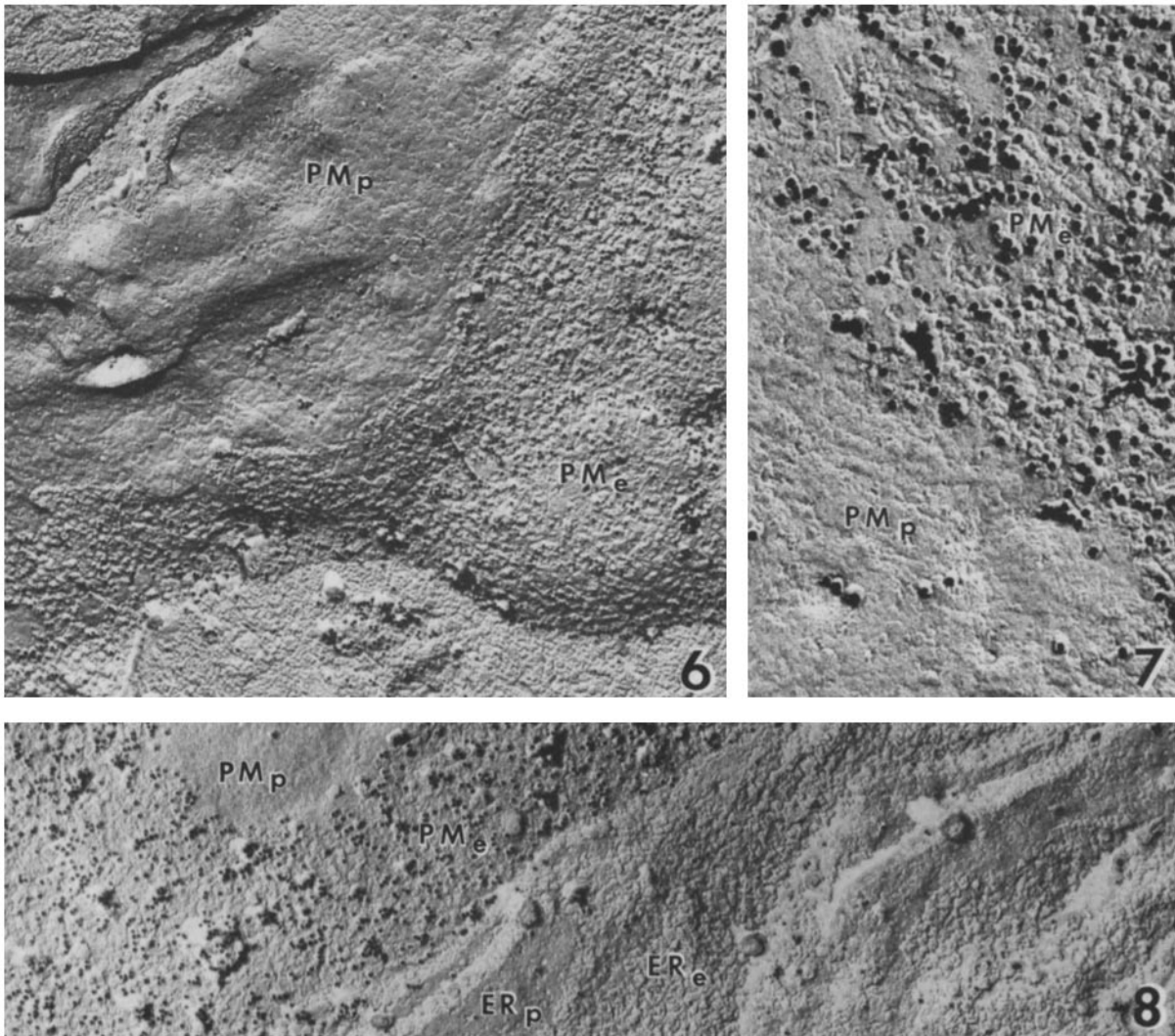
FIGURES 6–8   Colloidal gold labeling of WGA binding sites on fractured plasma membranes of exocrine (Figs. 7 and 8) and endocrine (Fig. 6) pancreatic cells. In plasma membranes the protoplasmic faces (PM_p) show very little label, whereas the exoplasmic faces (PM_e) are densely labeled. Occasional label can be seen on both P and E faces of fractured endoplasmic reticulum (Fig. 8, ER_p and ER_e) Fig. 6, × 33,000; Fig. 7, × 80,000; Fig. 8, × 32,000.

fractured endoplasmic reticulum (Fig. 16) or nuclear envelope membranes (for detailed analysis of Con A labeling of nuclear envelope membranes, see reference 16). Labeling could also be observed on the surface of the luminal membrane and microvilli (Fig. 17).
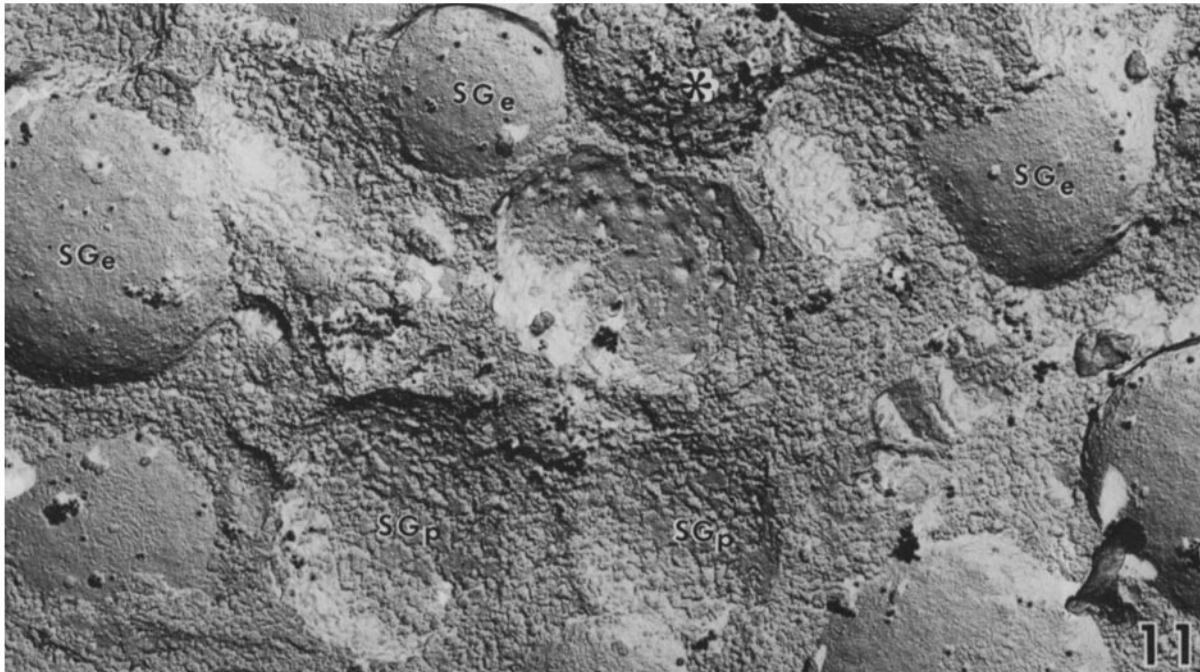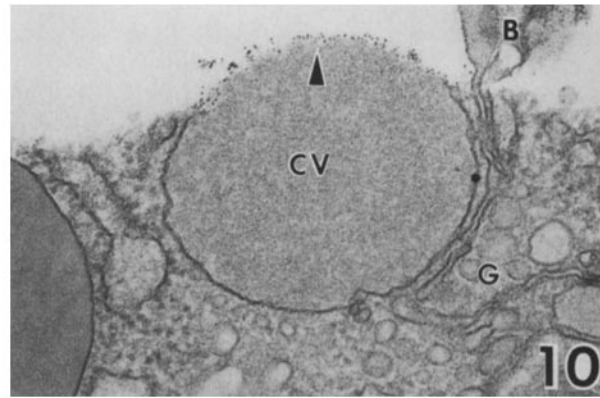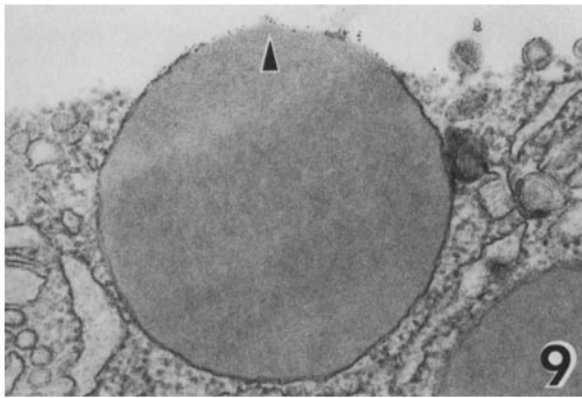
ENDOCRINE PANCREAS

Freeze-fractured endocrine pancreatic cells are labeled by WGA in a manner similar to that of exocrine cells. The exoplasmic faces of plasma membranes are densely labeled as seen both in thin sections (Fig. 19) and in replicas of critical-point-dried cells (Fig. 22). Labeling of the protoplasmic face is sparse but appears heavier than that of the fractured nuclear envelope membranes where only occasional gold particles are observed (Fig. 22). Labeling of the secretory granules is clear but at first difficult to interpret (see Fig. 23). In thin sections, colloidal gold particles label intensely the exoplasmic face of the secretory granule membranes (Fig. 20). Occasionally, this freeze-fractured membrane face appears disrupted and the label is seen associated with the inner surface of the vesicle (Fig. 21, arrow). In these thin sections some of the gold particles appear also to label the granule core itself (Fig. 21, arrowhead).

Close observation of the distribution of the label in replicas of critical-point-dried preparations (Figs. 22, 23) shows that the granules are not labeled and indicates that the apparent label probably results from remnants of the exoplasmic half of secretory vesicle collapsed over the granules themselves. In these preparations, exoplasmic fracture faces of the secretory vesicles are conspicuously absent and replaced by a system of spherical or ovoid craters intensely labeled by colloidal gold (Figs. 22 and 23). Many of these craters contain the unlabled granule core inside but in many others it is absent (presumably removed during specimen preparation). In some cases, the granule cores are displaced from their original positions and located over unrelated membranes (Fig. 22, arrowhead). Protoplasmic faces of the vesicles could only be tentatively identified as concave calottes with a texture slightly less rough than that of the surrounding cytoplasm (Figs. 22 and 23). They are virtually unlabeled.

DISCUSSION

We introduce here the combination of thin-section and critical-point-drying fracture-label to study the relative distribution

FIGURES 9 AND 10   Ferritin label of WGA binding sites on freeze-fractured membranes of secretory granules, exocrine pancreas. Moderate label is observed over the exoplasmic face of mature secretory granules (Fig. 9, arrowhead). Fig. 10 shows an immature secretory granule or condensing vacuole (CV) with an exoplasmic face densely labeled by ferritin-WGA conjugate. Membranous blebs or blisters (B) emerge occasionally from cross-fractured membranes. G, Golgi apparatus. Fig. 9, × 50,000; Fig. 10, × 55,000.
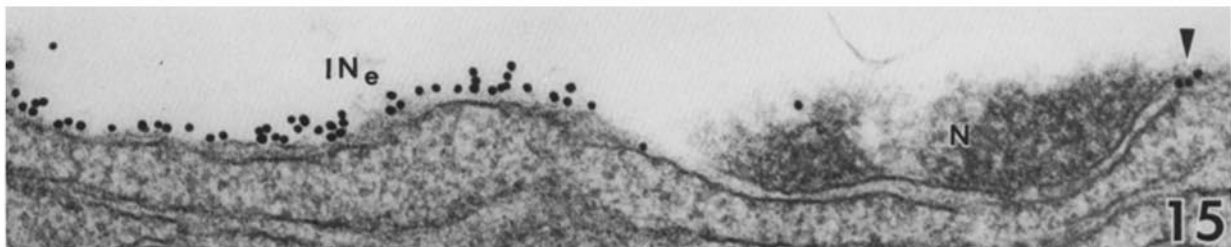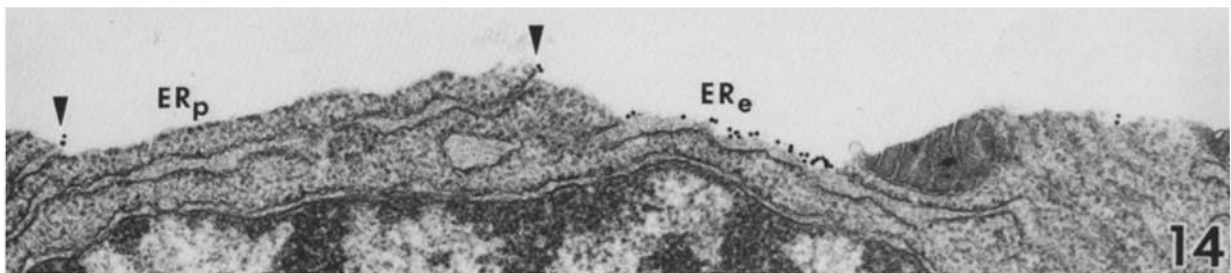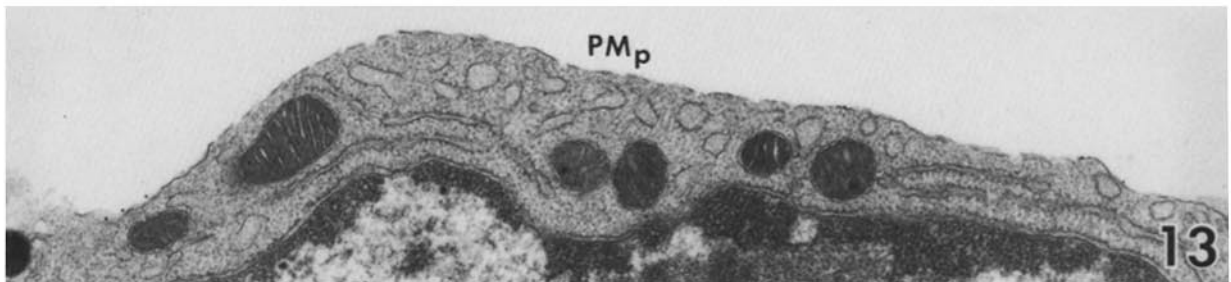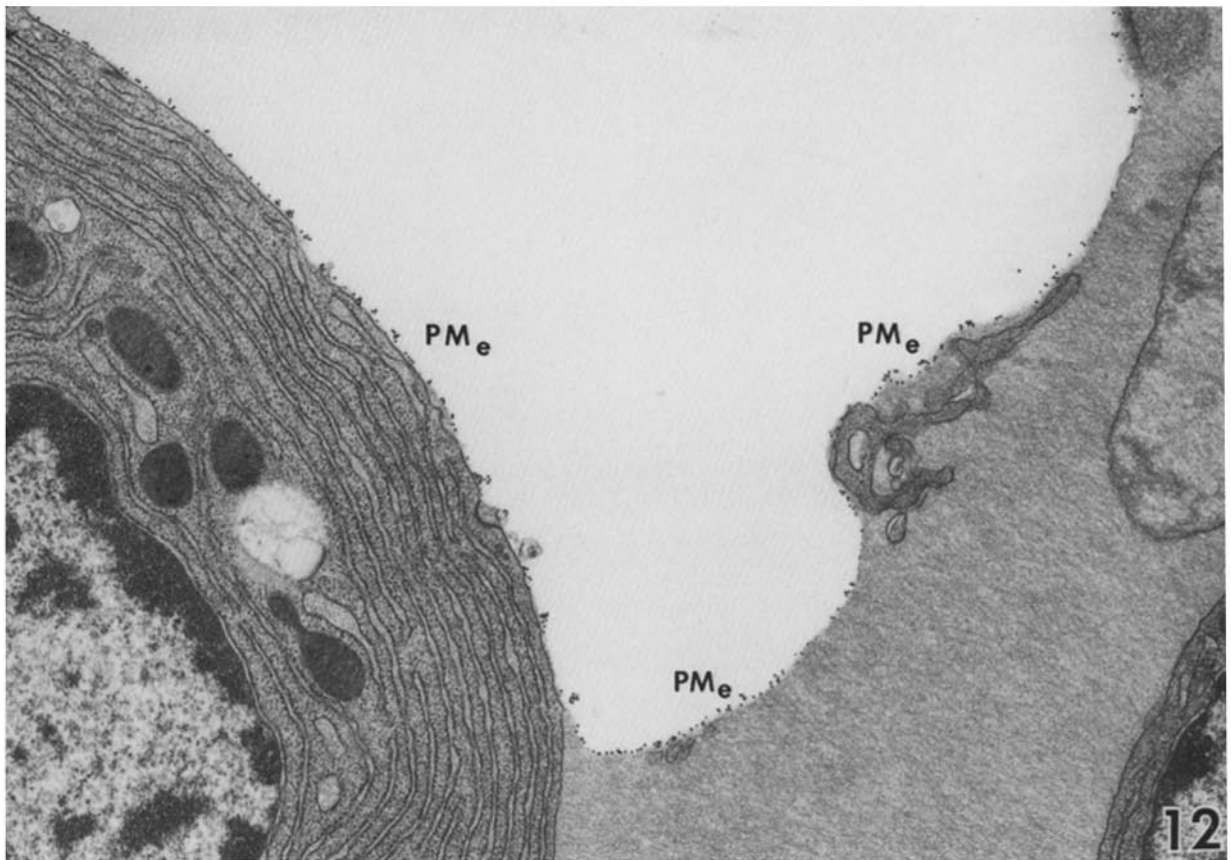
FIGURE 11   Colloidal gold label of WGA binding sites on freeze-fractured membranes of secretory granules, exocrine pancreas. Replica of critical-point-dried preparation shows moderate colloidal gold label of most exoplasmic fracture faces (SG_e), very dense label in some (asterisk), and occasionally over the protoplasmic faces (SG_p). × 42,000.

Ultrastructural Aspects

and partition of WGA and Con A binding sites on the fracture faces of plasma and intracellular membranes of exocrine and endocrine pancreatic cells. These fracture-label techniques allow the nonaveraged localization of chemical groups exposed through the structural dissection of biological membranes that occurs upon freeze-fracture.

Ultrastructural Aspects

The analysis of the thin-section morphology of membrane fracture faces was the object of previous publications (15, 16). The present results show that, as with other membranes, in pancreatic cells thin sections of fracture faces appear as interrupted unit membrane profiles. As discussed, this is the probable result of the reorganization of membrane components after freeze-fracture-induced splitting of the membrane bilayer that could occur upon thawing.

Observation of platinum-carbon replicas of fractured, critical-point-dried material shows that, although the overall appearance resembles that of conventional freeze-fracture preparations (including finer details such as nuclear pores or tight-junction strands), the fine structure of the membrane fracture faces suffers marked alterations. In particular, the membrane particles—conspicuous and important structures clearly seen in conventional freeze-fracture—are not observed. The protoplasmic faces appear relatively smooth but are interrupted by a variable complement of erratic fissures. It is likely that the structure of the faces as seen in critical-point-dried specimens also reflects the reorganization postfracture of membrane components as inferred from thin-sectioned material and, in addition, alterations that may occur during or after critical point drying. The general morphology of the fractured, critical-point-dried membranes of pancreatic cells is qualitatively similar to that reported in other cells, including erythrocytes (14; Pinto

FIGURES 12-15   Colloidal gold–peroxidase label of Con A binding sites on freeze-fractured exocrine pancreas. Dense labeling is observed on the exoplasmic faces of plasma membranes (Fig. 12, PM_e), endoplasmic reticulum (Fig. 14, ER_e), and inner nuclear envelope (Fig. 15, IN_e). Only occasional label can be observed on the protoplasmic faces of the plasma membrane (Fig. 13, $PM$and of endoplasmic reticulum (Fig. 14, ER_p). Label can also be observed on cross-fractured endoplasmic reticulum (Fig. 14, arrowheads) or nuclear envelope (Fig. 15, arrowhead). N, nuclear matrix. Fig. 12, × 16,000; Fig. 13, × 27,000; Fig. 14, × 43,000; Fig. 15, × 80,000.
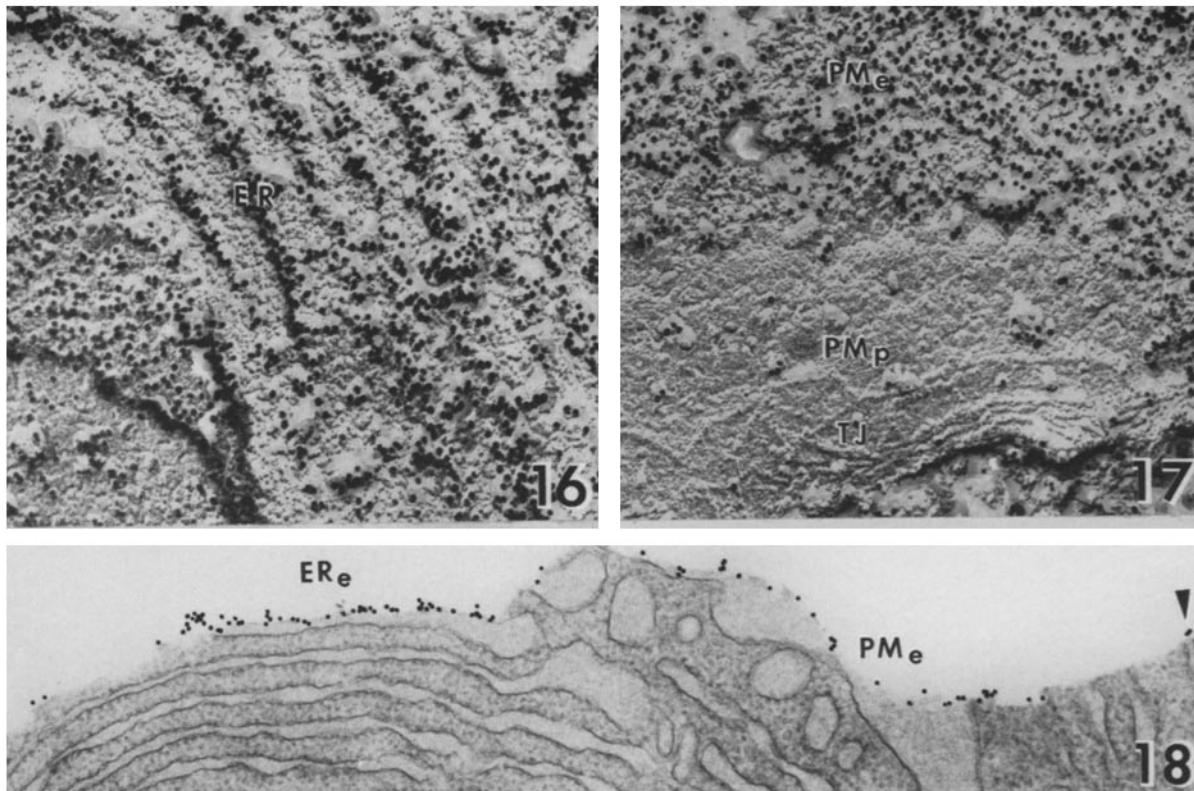
FIGURES 16 AND 17   Replicas of critical-point-dried exocrine pancreas after freeze-fracture and colloidal gold labeling of Con A binding sites. The exoplasmic face of the plasma membrane (PM_e) and an oblique fracture of endoplasmic reticulum (ER) are heavily labeled. Light label on the protoplasmic face of the plasma membrane (PM_p) is indifferent to the tight-junction band. Fig. 16, $\times$ 60,000; Fig. 17, $\times$ 60,000.

FIGURE 18   Thin section of Con A fracture-labeled exocrine pancreatic cells shows higher density of label on the exoplasmic face of freeze-fractured endoplasmic reticulum (ER_e) than on that of the plasma membrane (PM_e). Cross-fractured endoplasmic reticulum is labeled (arrowhead), $\times$ 42,000.

da Silva and Torrisi, manuscript in preparation). In consequence, we believe that the processes that appear operative during postfracture manipulation lead to similar alterations that are expressed in a characteristic morphology. Although better preservation of the conventional freeze-fracture detail is typical of membranes from fractured specimens freeze-substituted in osmium/acetone solutions, their cytochemical labeling in the absence of aqueous solutions appears unfeasible.

In replicas of critical-point-dried pancreatic cells, the exoplasmic faces of freeze-fractured membranes are clearly distinct from the protoplasmic faces: generally, they display a coarse texture, and erratic fissures are not apparent. Again, these fracture faces resemble exoplasmic faces of critical-point-dried, freeze-fractured membranes in other cells (14). The preservation of these exoplasmic faces appears worse than that of protoplasmic faces, possibly because they lack the compact support that is provided by cytoplasmic components underneath the protoplasmic face. Physical support by a compact material might also explain why the exoplasmic faces of secretory granules in the exocrine pancreas are generally well preserved.

The probable existence of reorganization processes postfracture makes it clear that the fracture faces as observed in thin-sectioned or critical-point-dried, freeze-fractured specimens cannot be equated with those of conventional freeze-fracture preparations. In consequence, and although the conventional terminology of fracture faces is extended here to our thin-sectioned and critical-point-dried preparations, it is clear that

the "exoplasmic" and "protoplasmic" faces described and discussed here must be taken in light of the discussion above, and the results of cytochemical labeling must be interpreted with caution. Even with this proviso, the interpretation of fracture-label results is, operationally, feasible and useful.

Freeze-fracture provides a dissection of membrane components into two different and readily identifiable sets (P and E faces) as it splits plasma and intracellular membranes into asymmetric halves (4, 13). Upon fracture, each face is free from the peripheral membrane components associated with the other membrane half. In addition, chemical groups at the outer surface that are part of integral, membrane-traversing proteins can be labeled on the protoplasmic face as they can be dragged during the fracture process across the exoplasmic half of the membrane and labeled on the protoplasmic face (15). Partition of exoplasmic surface sites associated with integral membrane-traversing proteins appears, however, to depend on local conditions such as the effectiveness of anchoring to components at the cytoplasmic surface: for instance, in human erythrocytes Con A binding sites (associated with Band III component) are preferentially labeled on the protoplasmic face (15) and WGA (associated with glycophorin) on the exoplasmic face (Pinto da Silva and Torrisi, manuscript in preparation).

Cytochemical Labeling

Although labeling of plasma membranes of exocrine and endocrine pancreatic cells by both WGA and Con A is similar,
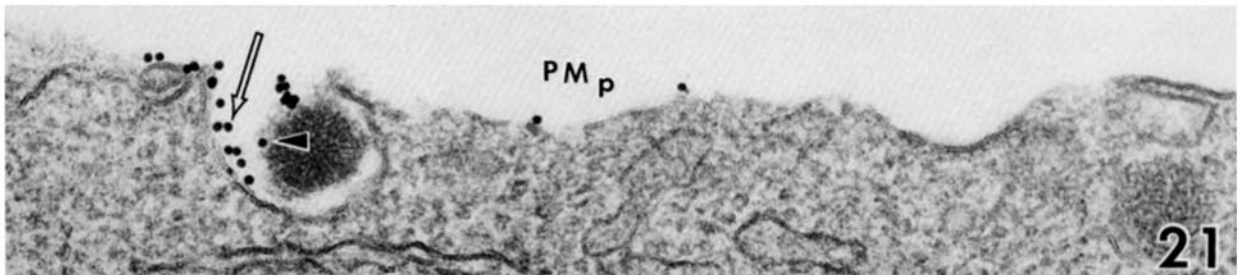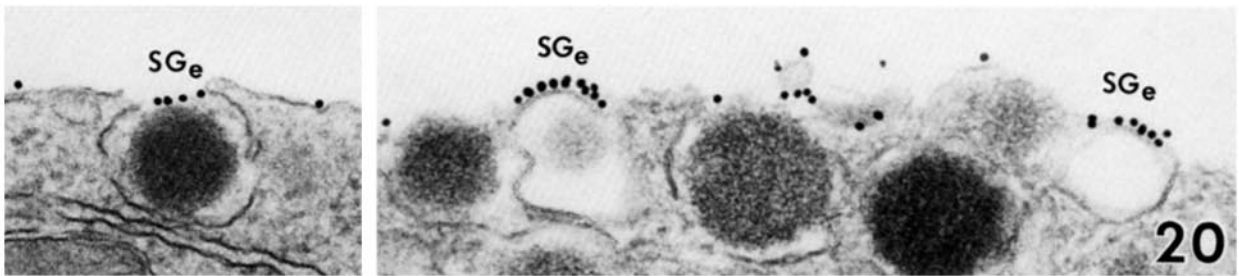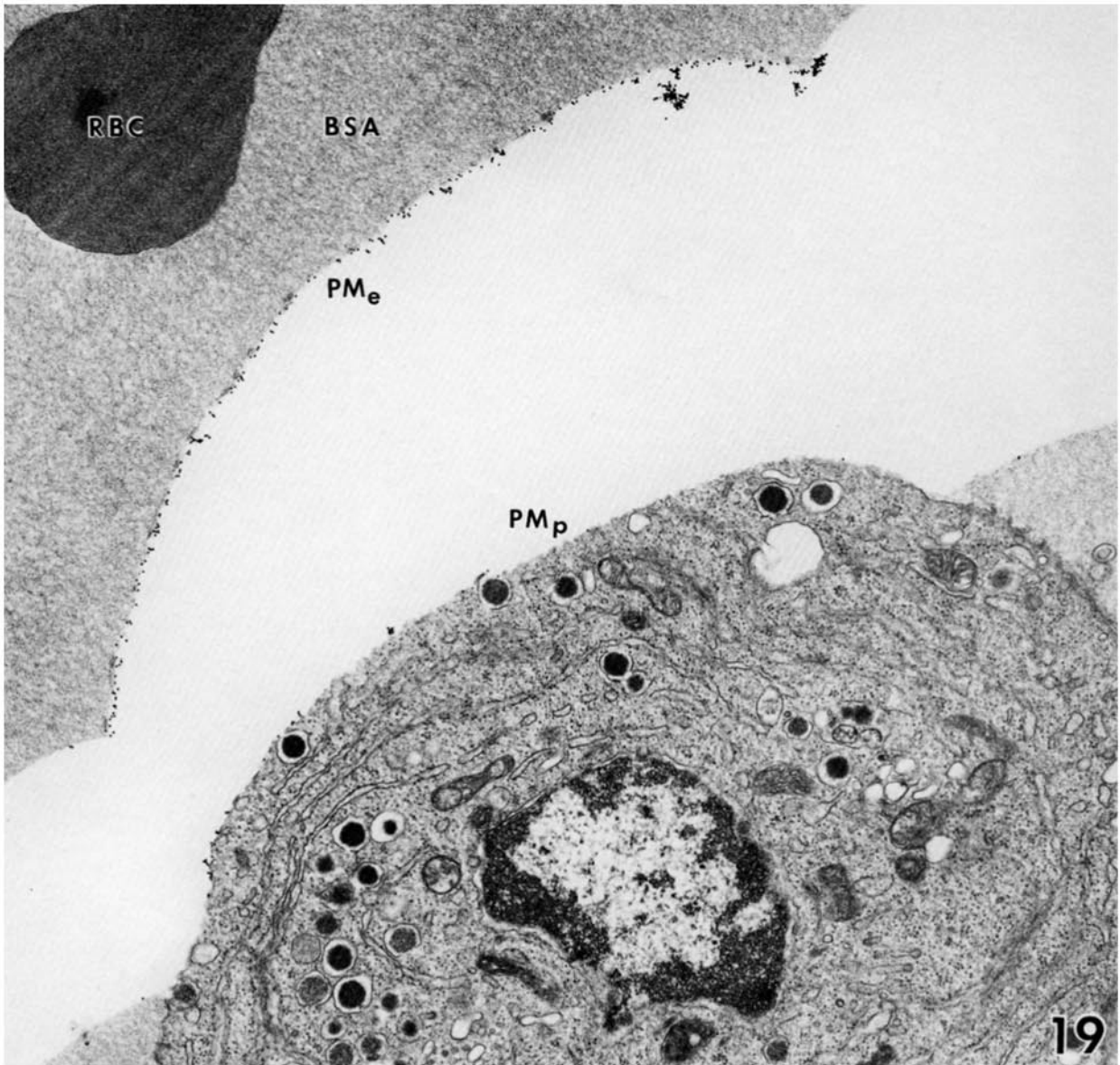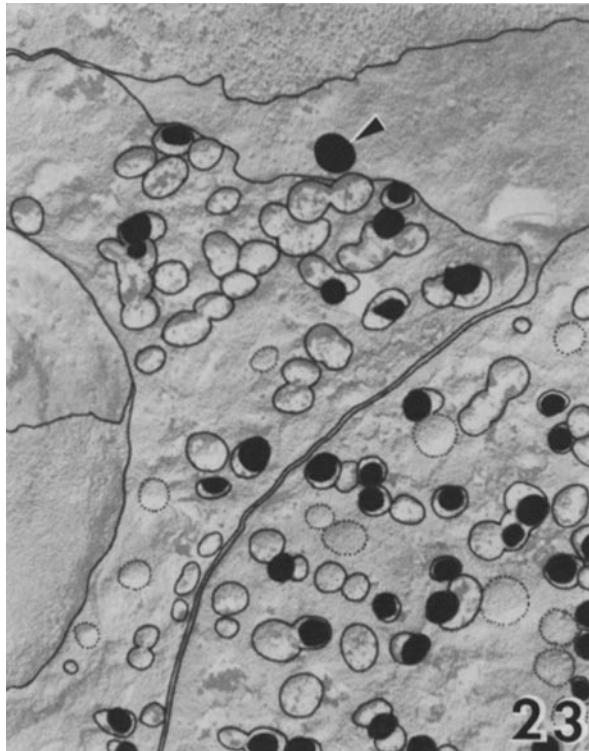
FIGURES 19–21   Colloidal gold labeling of WGA binding sites on freeze-fractured endocrine pancreatic cells. In fractured plasma membranes most of the label is observed over the exoplasmic face (Fig. 19, PM_e), very little on the protoplasmic face (Fig. 19, PM_p). Label is heavy over the exoplasmic face of fractured secretory granules (Fig. 20 and *inset*). In some cases the label penetrates into the vesicle interior and labels its surface (Fig. 21, arrow). Labeling of granule core (Fig. 21, arrowhead) is atypical (see also Figs. 22 and 23). *RBC*, erythrocyte embedded in BSA gel. Fig. 19, × 18,000; Fig. 20, × 77,000 (*inset* at left, × 74,000); Fig. 21, × 80,000.
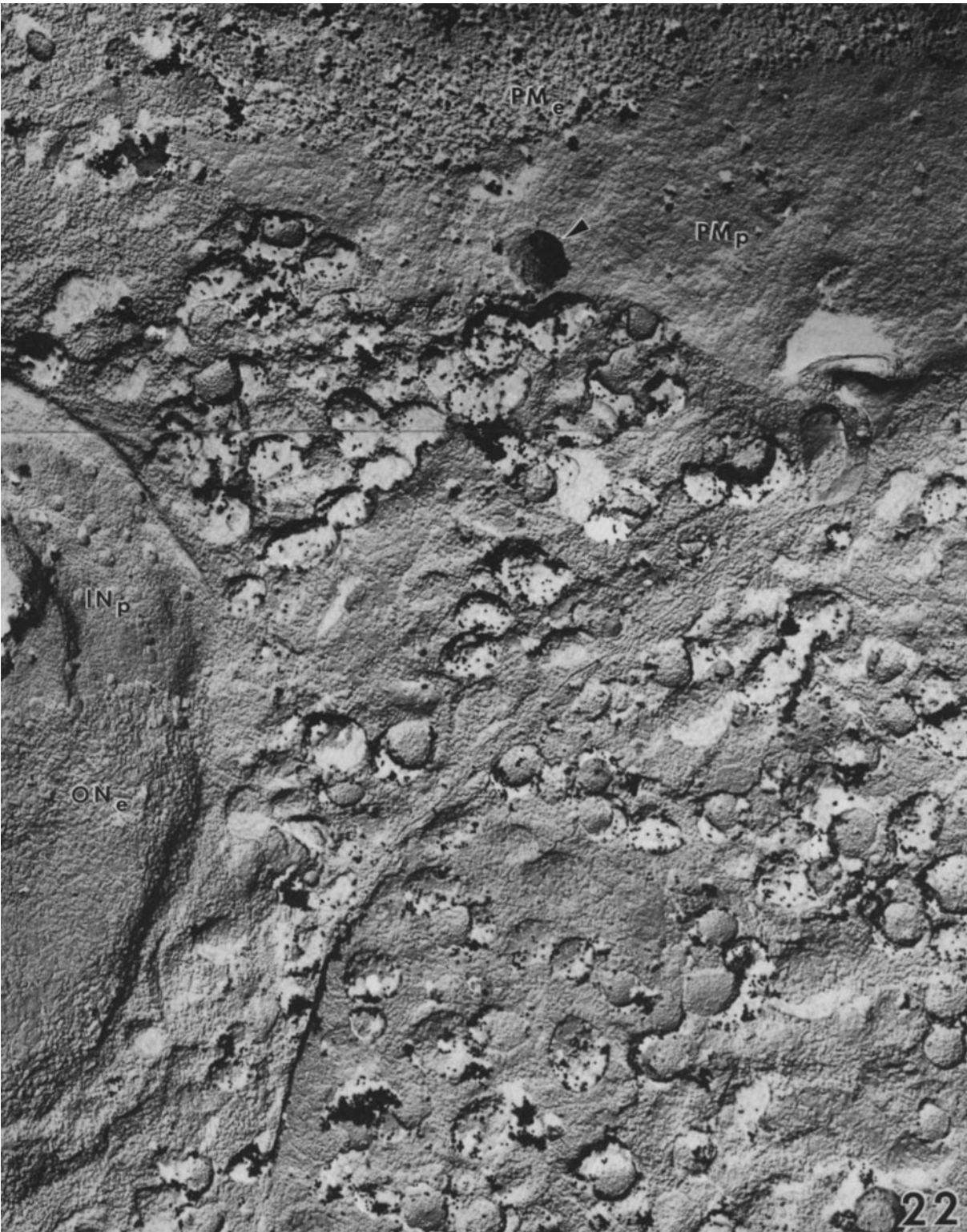
important differences in the labeling characteristics of the intracellular membranes of these cells were detected.

On plasma membranes almost all of the label was confined to the exoplasmic faces, showing that Con A and WGA binding sites are mostly restricted to the exoplasmic half of the split membranes. These results indicate the presence of the label over the outer surface of the cell where it may be associated with peripheral membrane components or with integral components that, upon fracture, partition with the exoplasmic half of the split membranes. Lectin binding sites may be rendered accessible thorugh structural reorganization postfracture. On the protoplasmic faces, scant labeling observed might correspond to sites associated with integral membrane proteins that upon fracture are dragged across the exoplasmic half of the

FIGURES 22 AND 23 Colloidal gold labeling of WGA binding sites on freeze-fractured endocrine pancreatic cells. In the plasma membrane, most of the label is associated with the exoplasmic face (PM_e) although some is seen over the protoplasmic face (PM_p). Virtually no label is seen over the face of freeze-fractured nuclear envelope membranes (IN_p, protoplasmic face of inner nuclear envelope membrane; ON_e, exoplasmic face of outer nuclear envelope membrane). In secretory vesicles almost all of the label is associated with the inner surface of secretory granule membranes (Fig. 23, continuous outlines). Little or no label is seen over the granule core solid circles (Fig. 23). Exoplasmic faces of fractured secretory granule membranes are not apparent and may have collapsed or have been lost postfracture. This explains why the label penetrated into the vesicle interior (see also Fig. 21) and why the granule core is visualized. Many granule cores are released from the vesicle interior, leaving empty vesicles behind. A displaced granule core can be seen over the protoplasmic face of the plasma membrane (Fig. 22 and 23, arrowhead). Protoplasmic faces of fractured secretory vesicles are difficult to distinguish from cross-fractured cytoplasm but are tentatively recognized as concave, spherical depressions with a size similar to that of fractured secretory vesicles (Fig. 23, dotted outlines). × 35,000.

membrane, in a manner similar to that observed on the protoplasmic faces of human erythrocyte membranes (15) or of subpopulations of human leukocytes (Torrisi and Pinto da Silva, manuscript in preparation).

Over fractured cytoplasmic membranes, clear differences were observed between the binding by WGA and that by Con A. Labeling of the endoplasmic reticulum by Con A but not by WGA is of particular interest. It is likely that Con A binds to mannosyl residues associated with nascent peptide chains during the synthesis of membrane and secretory glycoproteins (12, 18, 19, 23, 24) and, possibly, also to lipid-oligosaccharide molecules that participate in the process of cotranslational transfer of sugar residues to the nascent polypeptides (22).

WGA fails to significantly label[1] endoplasmic reticulum membranes and nuclear envelope (6, 19, 26–28), presumably because the terminal sialic acid–containing trisaccharide is added after transfer to the Golgi apparatus where the glycosyl transferases that catalyze the terminal steps of glycosylation of glycoproteins and, also, glycolipids appear to be located (5, 8, 17, 20). Our results are not consistent with the proposed reflux of complete, sialic acid–containing glycoproteins from the Golgi apparatus to the endoplasmic reticulum (3, 7, 25, 29). In the nuclear envelope the pattern of labeling is identical to that of the endoplasmic reticulum, as expected from the affinity of these two membrane systems (11, 26–28). In addition, Con A binding sites are uniformly distributed over all of the endoplasmic reticulum membranes, suggesting the widespread distribution of mannosyl-bearing molecules. Although we have not been able to locate fractured Golgi membranes, WGA labeling of the secretory vesicles as well as the plasma membranes of exocrine and endocrine pancreas is also consistent with the addition of terminal sialic acid–containing trisaccharides in the Golgi region. Because the removal of mannosyl residues that precedes the implantation of the terminal saccharides is only partial, the labeling of plasma membranes by Con A is also expected.

Labeling by WGA of fractured secretory vesicles of endocrine and exocrine pancreas requires careful interpretation. In exocrine pancreas, a significant minority of the vesicles had an exoplasmic face with a coarse structure and dense labeling, whereas the majority had an unusually smooth exoplasmic face and much sparser label. These results parallel those derived from conventional freeze-fracture preparations which show that zymogen granule membranes have a remarkably low density of membrane particles compared to the endoplasmic reticulum, Golgi, or immature granule (condensing vacuole) membranes (21). It appears, in consequence, that the low density of both WGA label and membrane particles might reflect a relative scarcity of intramembrane components in mature zymogen granules. In endocrine pancreas, labeling of secretory granule membranes seems to be associated with the exoplasmic face or with internal membrane surfaces but fails to label the granule core. In critical-point-dried preparations the convex exoplasmic fracture faces in these granules are not observed, as they may have been removed or collapsed during manipulation of the specimen. This is reasonable to expect, as this exoplasmic face is not supported by a matrix underneath, the secretory granule being physically separated from the membrane by an aqueous space.

In conclusion, our results show that thin-section and critical-point-drying fracture-label provides complementary views of the distribution of lectin binding sites on the fracture faces of plasma and intracellular membranes. Our results are consistent with recent biochemical and cytochemical investigations on the mechanism of glycosylation of membrane glycoproteins and, possibly, glycolipids. The techniques appear sensitive, of high resolution, and potentially useful for pursuing further the topological analysis of membrane components as well as that of the cellular spaces they delimit. The expansion of the application of fracture label to the localization of membrane

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[1] Although thin sections of WGA-labeled preparations rarely show occasional labeling of endoplasmic reticulum membranes (Fig. 4), larger membrane areas in critical-point-dried preparations show a few colloidal gold particles. At present, the significance of this occasional label cannot be assured but equal partition of label between exoplasmic and protoplasmic faces may indicate nonspecific adherence.

receptors and antigens appears feasible and is being actively investigated.

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