STUDIES OF THE SECRETORY PROCESS IN THE MAMMALIAN EXOCRINE PANCREAS

I. The Condensing Vacuoles

ALEX B. NOVIKOFF, MICHIO MORI, NELSON QUINTANA, and ANA YAM

From the Pathology Department, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461. Dr. Mori's present address is the Department of Pathology, Sapporo Medical College, Sapporo City, Japan.

ABSTRACT

Phosphatase cytochemistry was used to distinguish between the Golgi apparatus and GERL (considered as a specialized region of endoplasmic reticulum [ER] at the inner [trans] aspect of the Golgi stack) in pancreatic exocrine cells of guinea pig, rat, rabbit, and hamster. The trans element of the Golgi stack exhibits thiamine pyrophosphatase (TPPase) but no acid phosphatase (AcPase) activity. In contrast, GERL shows AcPase but no TPPase activity. The nascent secretory granules, or condensing vacuoles, are expanded cisternal portions of GERL. Continuities of condensing vacuoles with rough ER are suggested, and it is proposed that some secretory components may have direct access to the condensing vacuoles from ER. Connections of Golgi apparatus with GERL were not seen.

KEY WORDS condensing vacuoles · enzyme cytochemistry · GERL · Golgi apparatus · phosphatases · secretion

Palade and his collaborators (9, 19–23, 46) showed that, in pancreatic exocrine cells of the guinea pig, zymogen granules arise as irregular "condensing vacuoles" in the Golgi zone. Within the condensing vacuoles the dilute secretory proteins become progressively concentrated. As concentration occurs, the condensing vacuoles become spherical and they develop into mature zymogen granules. Subsequently, the zymogen granules empty their contents into the acinar lumen by exocytosis. When [^{3}H]leucine was injected into either untreated animals or animals fasted overnight and refed, or when pancreatic slices were exposed to media containing [^{3}H]leucine, autoradiographic (ARG) grains were not observed over the Golgi "complex" (9, 19–22). It was therefore concluded that the transport of newly synthesized proteins to the condensing vacuoles did not involve the Golgi "stacks." Instead, small vesicles were thought to convey these proteins to the condensing vacuoles, effectively bypassing the Golgi stacks (23). Jamieson and Palade (23) subsequently reported that when tissue slices of fasted guinea pig pancreas were stimulated by the secretogogue, carbamylcholine, for 3 h, ARG grains were found over the flattened sacs of the Golgi stack. Thus, they proposed that in the stimulated gland the direct pathway to the condensing vacuoles was replaced by one that involved the Golgi apparatus. Palade (46) considers this to be "variations on a theme," the theme established by the work on unstimulated cells. It was suggested that in the rat, as described by van Heyningen (54), and in a few other species, the secretory pathway resembled that in the stimulated tissue slices (23, 46).

Because ultrastructural cytochemistry can give

accurate localizations, phosphatase cytochemistry has provided new insights into details of the secretory pathway involving the condensing vacuoles. We investigated exocrine pancreatic cells not only in the guinea pig and rat, but also in the rabbit, hamster, and mouse. One phosphatase activity was used to identify the innermost or trans (11) element of the Golgi stack, another phosphatase activity to delineate GERL. The Golgi stacks seen in electron micrographs are views resulting from sectioning through the Golgi apparatus randomly. In the pancreatic acinar cell this organelle is found in a limited area of the cytoplasm, generally known as the cytocentrum or Golgi zone; in contrast, in many vertebrate neurons (32, 45) the Golgi apparatus is more widely distributed in the cytoplasm.[1]

In many mammalian cell types the unequivocal distinction in electron micrographs between elements of the Golgi stacks and GERL elements can best be made in material incubated for thiamine pyrophosphatase (TPPase) and acid phosphatase (AcPase) activities, respectively (45, 34). GERL is considered to be a specialized area of endoplasmic reticulum (*ER*), adjacent to the trans Golgi element (*G*), rich in hydrolase activity and from which a variety of lysosomes (*L*) appear to form (34). Some of the results have been reported in abstract form (39, 35, 40). Observations on the C57 black mouse and its mutant, beige, are described elsewhere.[2]

MATERIALS AND METHODS

Albino male guinea pigs, ~400–420 g, were obtained from Marland Farms, Hewett, N. J.; female rats, ~150 g, from Holtzman Co., Madison, Wis.; female hamsters, ~100 g, from Charles River, Lakeview, Newfield, N. J.; and male rabbits, ~2.5 kg, from Whaley Summit View Farms, Belvidere, N. J. Two guinea pigs were fasted for about 48 h and then given access to food for about 90 min before sacrifice. In some experiments a small piece of pancreas was removed from animals lightly anesthetized (with ether) and cut into small cubes in cold 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4, and fixed for 1–2 h. A large part of the pancreas was held in a spread condition in the animal for several minutes while cold aldehyde fixative was dropped on the organ. The fixative was the formaldehyde-glutaraldehyde fixative of Karnovsky (24) as modified by Miller and Herzog (27). The partially fixed material was then immersed in ice-cold fixative for a total fixation time of 90 min. In most experiments the pancreas was fixed initially by perfusion. Under light ether anesthesia, isotonic saline was perfused through the aorta for 1 min (with the posterior vena cava cut below the diaphragm to serve as an outlet for the perfusate) followed by the formaldehyde-glutaraldehyde fixative for 3–5 min, both at room temperature, at a flow rate of 2–3.5 ml/min. Portions of the pancreas were then fixed by immersion in the same fixative at ice temperature for a total fixation time of 90 min.

The further processing of both osmium tetroxide-fixed and aldehyde-fixed tissue, including *en bloc* staining with uranyl acetate, was done as described elsewhere (26), as was the preparation of sections for incubation for phosphatase activities. TPPase activity was demonstrated by the procedure of Novikoff and Goldfischer (38), and AcPase activity by the CMP procedure of Novikoff, as described elsewhere (26). Thin sections were stained for 5–10 min in lead citrate (50); thin sections of tissues incubated for TPPase or AcPase activities were also examined without lead staining (Fig. 7). Sections were examined in the Elmiskop I microscope at 80 kv or the Philips 300 microscope at 80 kv. The results illustrated did not vary depending upon the position of the cells in relation to the exposed surface of the nonfrozen ("chopper") sections. In a few cases, "semi-thin" sections (0.5–2.0 μm) of such incubated tissues were examined, without lead staining, in the electron microscope, as in earlier studies on neurons (44). The cytochemical controls consisted of incubation of sections in complete TPPase and AcPase media lacking substrate; in all instances electron-opaque deposits were not present in sections of such controls.

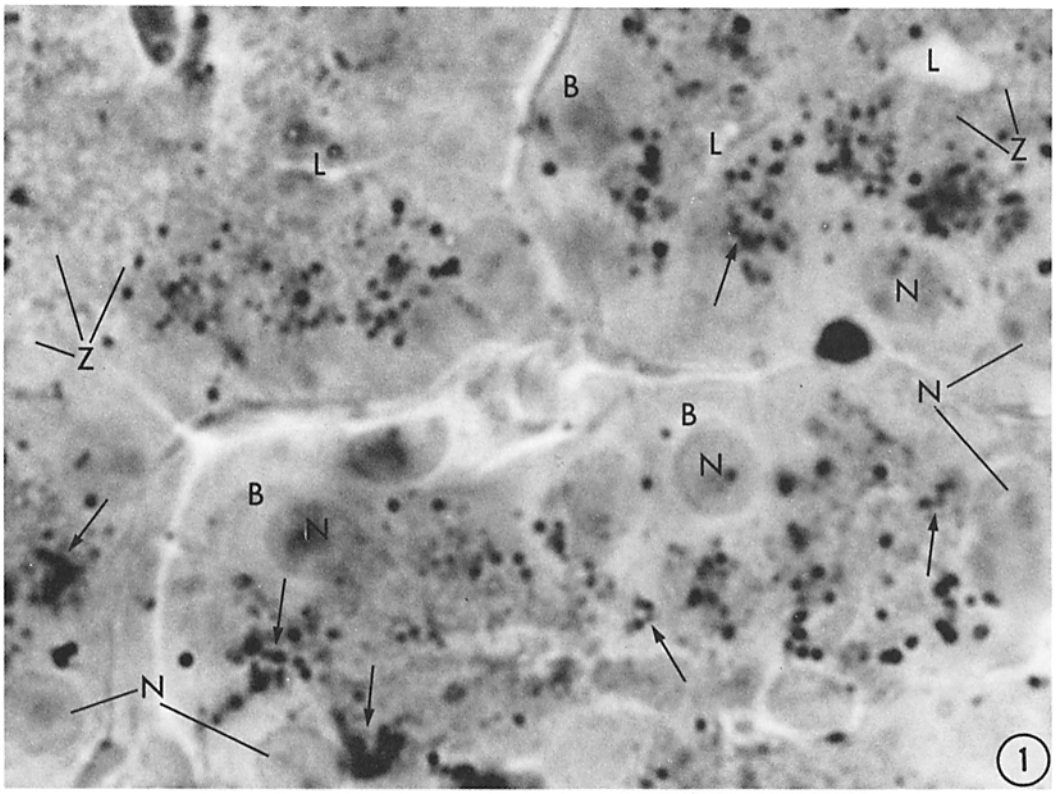
Light microscope examination of 2–7 μm sections of Epon-embedded tissue incubated for TPPase or AcPase activities was performed after visualization of the reaction product by treatment with ammonium sulfide, heated on a hot plate. Frozen sections were also incubated for the two phosphatase activities but the results are not illustrated here. They were essentially like those seen in the Epon-embedded sections, and like those published in early papers from this laboratory on frozen sections of pancreatic and parotid exocrine cells (38, 31, 36).

RESULTS

Essentially similar results were obtained with guinea pigs, rats, rabbits, and hamsters, with light microscopy of AcPase activity (Fig. 1) and TPPase activity (Fig. 2), and with electron microscopy of unincubated material (Figs. 3–6), and of material incubated for AcPase activity (Figs. 7, 9, 11–13,

[1] For an interesting account of the historical development of our knowledge of the Golgi apparatus, see the review by Beams and Kessel (5) and the recent monograph by Whaley (57). To appreciate the contributions of phosphatase cytochemistry to this development, see reference 4 for TPPase and reference 44 for AcPase.

[2] Novikoff, A. B., N. Quintana, and M. Mori. Manuscript submitted for publication.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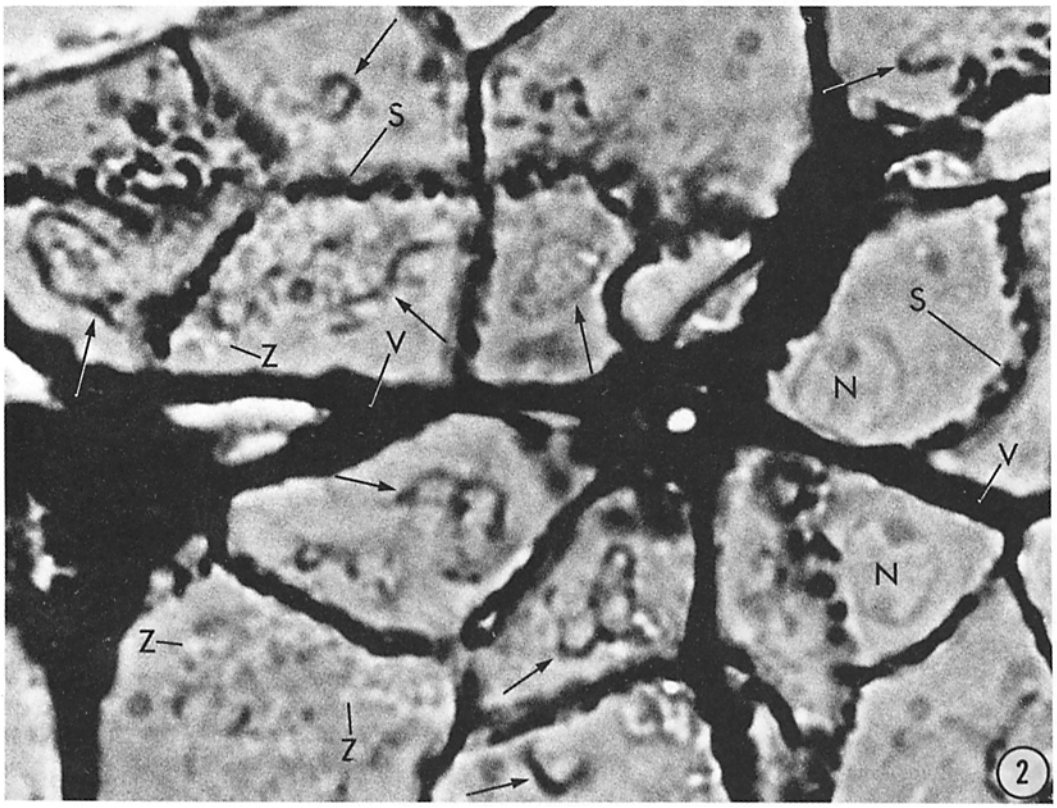
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and 15) and for TPPase activity (Figs. 8, 10, 14, and 16).

As summarized by Palade (46), the pancreatic exocrine cell is highly polarized, with rough ER occupying an extensive basal region and the zymogen granules located in the apical portion of the cell (Fig. 1, upper right). The number of zymogen granules varies with the physiological state of the animal. When many are present, the Golgi zone is both above and lateral to the nucleus.

Figs. 1 and 2 are light micrographs which, because they cover such large areas of the tissue and because the sections are much thicker than those generally employed for electron microscopy, reveal features that are difficult to appreciate from electron micrographs. In pancreatic exocrine the lysosomes resolvable by light microscopy consist of autophagic vacuoles (Fig. 9), and in normal pancreas these are very scarce. Thus, the AcPase preparations reveal condensing vacuoles almost exclusively. Fig. 1 shows how numerous these vacuoles are. Portions of the cisternal elements of GERL are barely evident (arrows, Fig. 1).

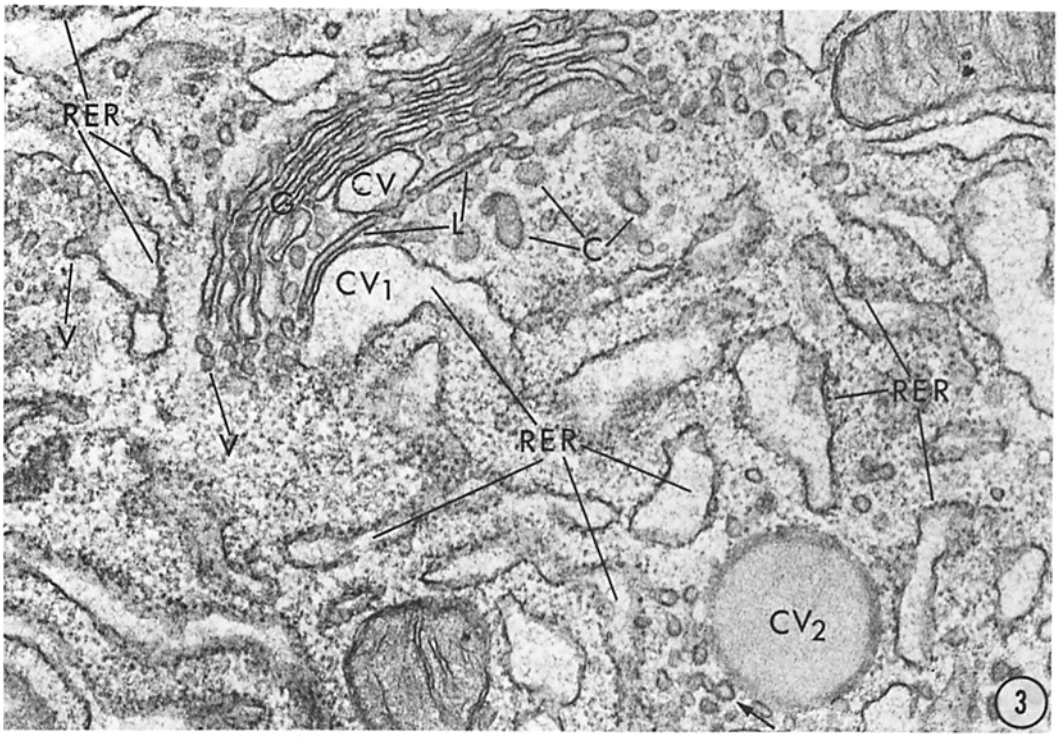
The trans element of the Golgi apparatus, because it is broader than GERL, is more readily evident when TPPase preparations are viewed by light microscopy. The three-dimensional aspect of the Golgi apparatus, suggested in Fig. 2 (arrows), can be better appreciated when focusing at the microscope than can be illustrated with a photograph taken at one level of focus. However, the photograph makes evident why so many Golgi stacks can be seen in thin sections viewed by electron microscopy.

It should be noted that the zymogen granules show neither AcPase (Fig. 1) nor TPPase activity (Fig. 2).

Preliminary studies of 0.5- to 2.0-μm sections indicate that the trans element of the Golgi apparatus in pancreatic acinar cells does not possess the geometrically regular "polygonal compartments" described in neurons of rat dorsal root ganglia (45) and also that the TPPase-positive trans Golgi element has numerous branches and fenestrations. Because the Golgi apparatus and accompanying GERL twist as they course through the cytoplasm, GERL may appear above the Golgi stack in one region and below it in an adjacent region (Fig. 9). But always GERL remains trans to the Golgi stack (see Fig. 40 in reference 45).

FIGURE 1 Epon section, ca. 7 μm thick, of rat pancreas. Fixation: perfusion and immersion, formaldehyde-glutaraldehyde, as indicated in text. Incubation: AcPase medium, 25 min at 37°C. A striking feature of such preparations is the absence of reaction product from the zymogen granules. This micrograph was taken with the iris diaphragm reduced to the point where some of the zymogen granules were barely evident (*Z*). Further reduction of the iris opening would make many more of them visible but would cause undesirable overlap of structures possessing reaction product. The ER shows no reaction product so that the base of the cell (*B*), where the ER is concentrated (46), appears clear. The lumina of the acini are indicated at *L*. The nuclei (*N*) are evident because of their optical diffraction and not because they are AcPase-positive. The great majority of the structures with reaction product (black) are condensing vacuoles. This identification is established by electron microscopy (Figs. 7, 9, 11–13, and 15). It is possible to know the condensing vacuoles by light microscopy only because in the pancreatic acinar cells the number of lysosomes is extremely small (see Fig. 9 and text). Other than its condensing vacuoles, most of the AcPase-positive GERL (Figs. 7, 9, 11–13, and 15) and coated vesicles are below the resolving power of the light microscope. However, a few regions of GERL are visible (arrows), probably because the cisternae are so sectioned that their wide aspects are exposed to view. Because the trans element of the Golgi apparatus is considerably broader, the distribution of the Golgi apparatus is readily seen in sections incubated for TPPase activity (Fig. 2). × 2,200.

FIGURE 2 Epon section, ca. 2 μm thick, of hamster pancreas. Fixation: as in Fig. 1. Incubation: TPPase medium, 90 min at 37°C. Arrows indicate portions of the Golgi apparatus that are visible at this focal plane; other portions became evident when the focal plane was changed. Two nuclei (*N*) are barely visible. The few zymogen granules (*Z*) seen in this plane of section and with this iris opening are without reaction product. Whereas the acinar lumina are barely recognizable in routine sections, incubation of aldehyde-fixed sections in lead-containing media with TPP (or with various nucleoside phosphates, as summarized in reference 37) at neutral pH and visualization with ammonium sulfide, reveals the branching lumina with great clarity. This luminar system is sometimes referred to as "secretory capillaries" (55). Portions are seen at *S*. The rest of the plasma membrane also shows reaction product (cf. Fig. 14). Also intensely stained by such media are blood capillaries (37) with which the pancreas is richly endowed (*V*). × 2,600.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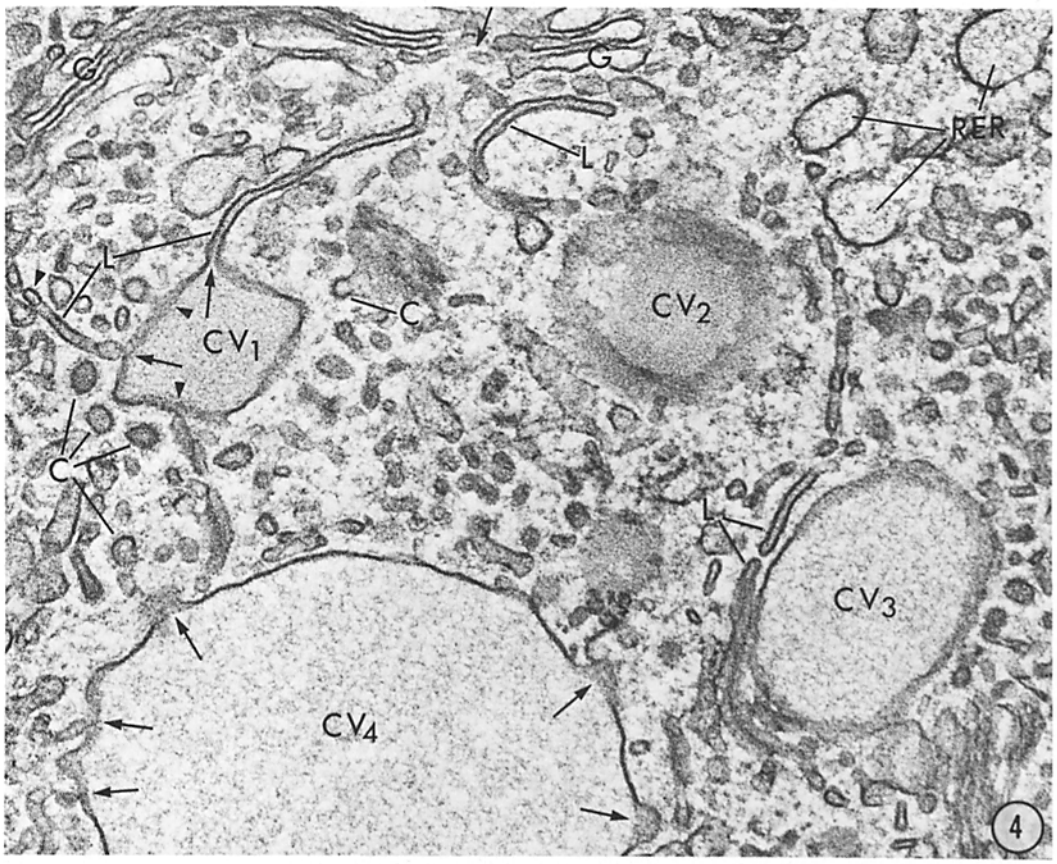
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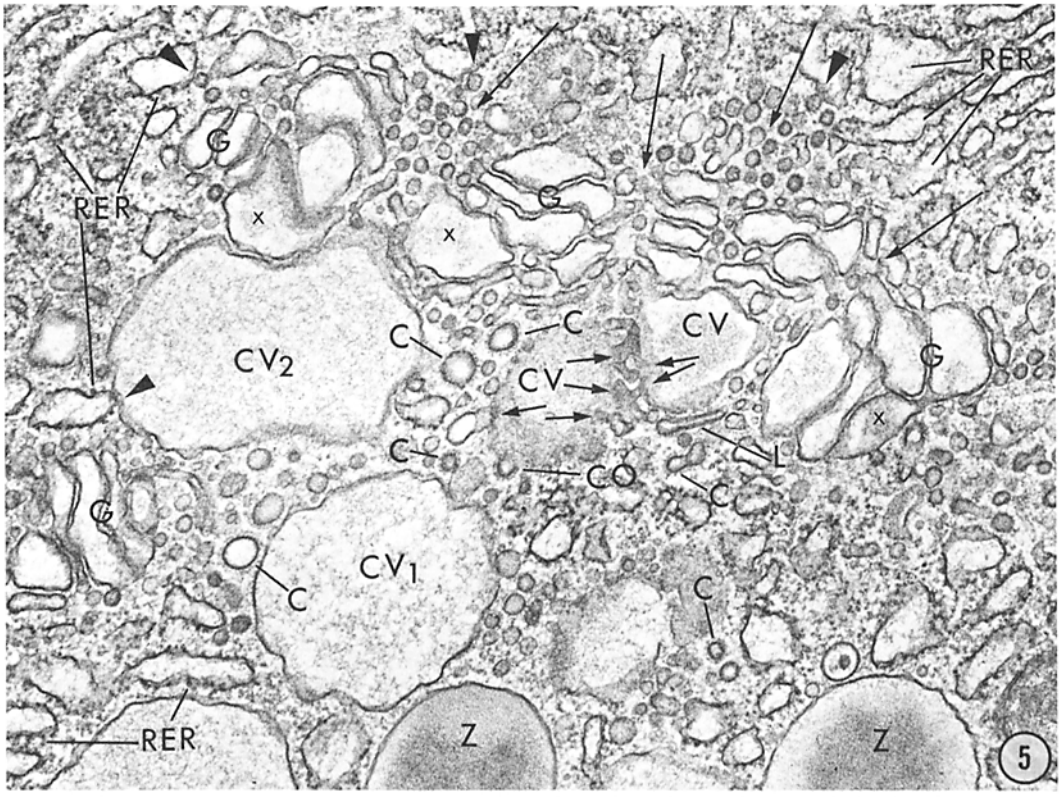
Naturally, the electron microscope images are basically similar to those of Caro and Palade (9), Jamieson and Palade (19–23), and Palade (46), but certain features are emphasized by us. One relationship we wish to stress is that rough ER is directed towards the trans aspects of the Golgi stacks (Figs. 3–10, 15, and 16) as well as towards the cis aspects (Figs. 3, 5, 6, 8–10, 13, 15, and 16). The ER courses through regions where the Golgi stacks are interrupted in the particular plane of section. Such interruptions may be considered as passageways through which rough and smooth ER connect with GERL. The passageways may be very small (as at the arrows in Figs. 4 and 5), of moderate size (as in Figs. 5, 9, 10, 12, 15, and 16), or large (as in Figs. 3, 4, 6, and 8). The large interruptions may well correspond to the spaces seen in TPPase preparations by light microscopy (Fig. 2) within the anastomosing trans element, but a three-dimensional model reconstructed from serial sections will be required to establish such matters. Without morphometric measurements on serial sections, the relative extent to which rough ER goes to cis or trans aspects of the Golgi stack cannot be stated.

The two distinctive structures of GERL are the dilated portions, the condensing vacuoles of Caro and Palade (9), and the flattened lamellae of smooth ER which bear a striking resemblance to the rigid lamellae described by Claude (10) in rat hepatocytes. By serial sectioning, Claude showed these regions to be lamellar rather than tubular. Because they were straight and their bounding membranes appeared parallel, with relatively uniform distances between them, Claude described them as rigid. In the pancreatic acinar cells the distance (cisternal space) separating the two surfaces of the rigid lamella membrane is 200–300 Å. It is highly likely that the rigid lamellae are cisternal rather than tubular since they are generally seen as long structures in thin sections (Figs. 3–5 and 7–16).

Figs. 3–6 were chosen to present, as best as possible with four illustrations, a sense of the variations encountered in the structure of Golgi apparatus and GERL in electron micrographs. Tangential sections are more frequent than perpendicular sections through the organelles. Condensing vacuoles vary greatly in shape, tending to be more angular when smaller but always possessing rounded areas in which the delimiting membranes appear thicker than in rough ER (Figs. 3–6). Ribosomes are not found on the membranes delimiting the condensing vacuoles. Rigid lamellae frequently lie nearby, e.g., near *CV* and CV_1 in Fig. 3, and near CV_1 and CV_3 in Fig. 4. Continuities between condensing vacuoles and rigid lamellae are common. The clearest one in the illustrations

FIGURE 3 A portion of a pancreatic exocrine cell of the rabbit. Fixation: 1% OsO_4-phosphate buffer, pH 7.4, 2 h. Rough endoplasmic reticulum (*RER*) is abundant at both cis and trans aspects of the Golgi stack (*G*). Small vesicles (*V*) are seen at the lateral edges of the stack and at both its cis and trans aspects; at the bottom right the arrow indicates a tubule that could easily appear as a vesicle in an adjacent section. Labeled components of GERL are coated vesicles (*C*), rigid lamellae (*L*) and condensing vacuoles (*CV*, CV_1, and CV_2). Note that at its upper left portion, CV_1 is rounded and is delimited by a thick membrane on which there are no ribosomes. Note also that CV_1 is adjacent to a rigid lamella. In these three respects it is like *CV*. Because CV_2 is cut tangentially, it does not reveal these features. × 38,000.

FIGURE 4 A portion of a pancreatic exocrine cell of the guinea pig, fasted 43 h and refed 95 min. Fixation: 1% OsO_4-phosphate buffer pH 7.4, 80 min. Only small portions of Golgi stacks are seen (at *G* and also at the lower right corner of the figure). Note that vesicles and larger membranous structures separate GERL from the Golgi stack. Labeled portions of GERL include coated vesicles (*C*), rigid lamellae (*L*), and condensing vacuoles (*CV*). In CV_1 the upper arrow indicates the continuity of a rigid lamella and vacuole. At the lower arrow the continuity is not so evident, and at the two arrowhead areas in CV_1 and the five arrows in vacuole CV_4 such suggestions are even less firm in the absence of serial sections. An arrow at the top center of the figure indicates a small interruption in the Golgi stack in this plane of section. The coated vesicle (*C*) near the center of the figure is attached to a tangentially sectioned membranous structure, probably a condensing vacuole; see *CO* in Fig. 5 and arrowhead in CV_2 of Fig. 6. Note that at the upper right the rough ER (*RER*) is directed to the trans aspect of the Golgi stack. The arrowhead at the left border of the figure, above center, indicates that a transverse section of a rigid lamella would appear as a vesicle, somewhat flattened. The rigid lamella to the left of CV_3 appears fenestrated, but this was not studied by serial sections. × 50,000.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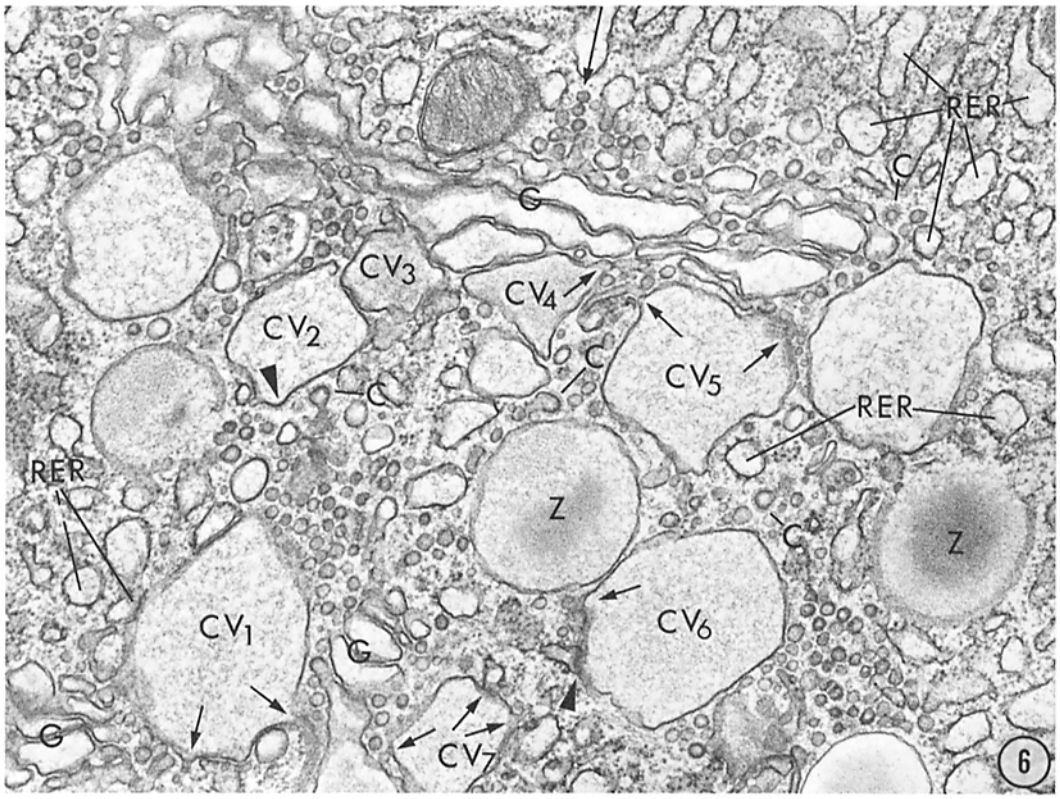
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is seen at the upper arrow in CV_1, in Fig. 4. Others, less clear, are indicated in the legends. Coated areas of condensing vacuole membranes are seen occasionally, as in CV_2, Fig. 6 (arrowhead). In Fig. 5, *CO* is probably such a coated area in a tangential section of the condensing vacuole, *CV*. GERL (condensing vacuoles and rigid lamellae) is seen somewhat more frequently lying adjacent to the Golgi stack (Figs. 3, 5, and 6) than at some distance from the stack (Figs. 3–6). When GERL is seen separated from the Golgi stack, vesicles and other membranous structures lie in the areas separating the two organelles. As emphasized in reports on a variety of animal cells from this laboratory (especially see reference 45), such vesicles and other structures are not seen between the adjacent elements that compose the Golgi stack.

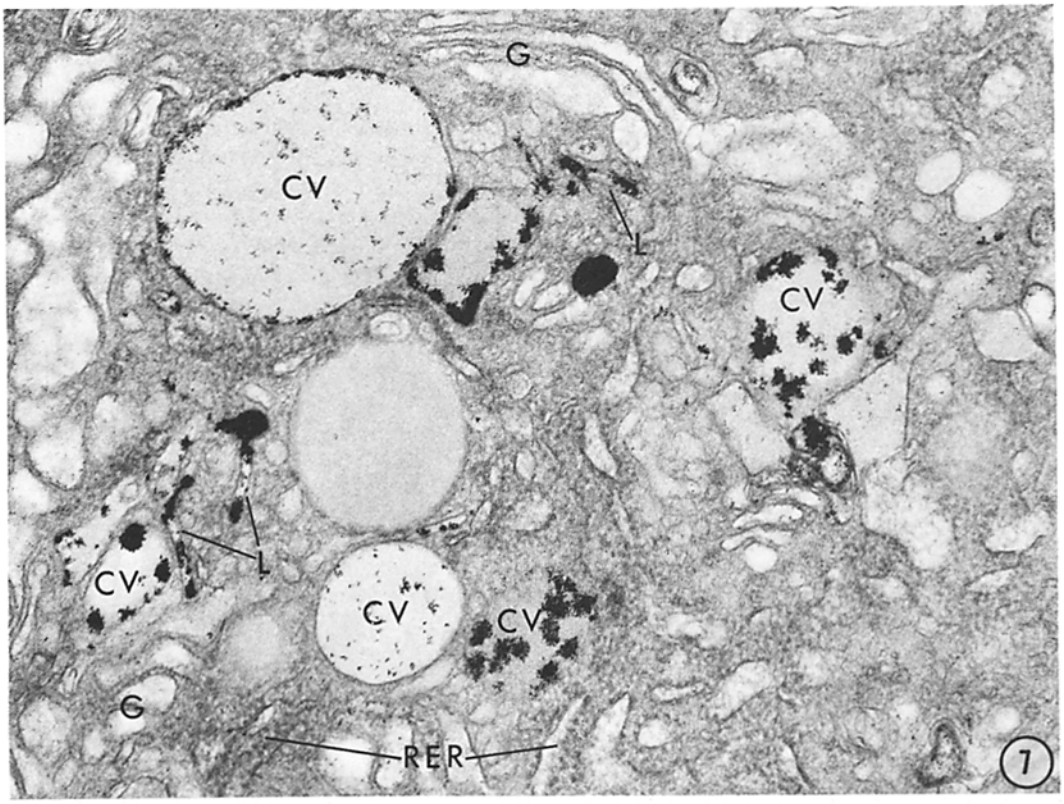
In addition to the frequent continuities of condensing vacuoles with smooth ER (rigid lamellae), the vacuoles often show close proximities to rough ER (arrowhead in CV_2 in Fig. 5, and arrowhead outside CV_6 in Fig. 6). Since serial sections plus specimen tilting, were not performed, it is not possible to assert that continuities between the two structures probably existed in these instances. However, in our interpretation, such continuity is clearly seen in Fig. 3 where an unusually patent connection between rough ER and a condensing vacuole is seen, at CV_1. That the structure, CV_1, is indeed a condensing vacuole and not a dilated area of rough ER is strongly indicated by three features: (*a*) the rounded region (upper left) has a delimiting membrane which is thicker than that of rough ER; (*b*) ribosomes are absent from this membrane; and (*c*) the proximity of the structure to a rigid lamella (*L*). Indeed, it is possible that CV_1 was connected to *CV* by a rigid lamella, as is seen for the two vacuoles labeled *CV* in Fig. 5 and in Fig. 14.

The distinction between these smooth membrane structures constituting GERL (see reference 34)—condensing vacuoles and rigid lamellae—and the Golgi stacks can readily be made when they are separated from the Golgi stacks by some distance because of the vesicles and other membranous structures between GERL and Golgi stacks in the areas of separation.

There are always regions where the smooth membrane structures (condensing vacuoles and rigid lamellae) lie against the Golgi stack. Even here, the distinction between trans Golgi element

FIGURE 5 A portion of a pancreatic exocrine cell of the hamster. Fixation: 1% OsO_4-phosphate buffer, pH 7.4, 1 h. Cisternae of rough ER (*RER*) are seen extending towards the cis face of the Golgi stack (*G*). Arrows indicate small passageways in the Golgi stack in which tubules or vesicles separating from rough ER are seen. The rough ER (*RER*) at the upper left is directed towards GERL, in a passageway across the Golgi stack (see Results). Within CV_2 the arrowhead indicates the contiguity of rough ER and the vacuole. At *L* a rigid lamella is seen, not connected to a condensing vacuole in the plane of section. Numerous coated vesicles (*C*) are seen. One (*CO*) appears continuous with the tangentially sectioned condensing vacuole; cf. *C* near center of Fig. 4 and coated area at arrowhead in CV_2 of Fig. 6. Also labeled are two zymogen granules (*Z*); note that in tissue post-fixed in OsO_4-phosphate after initial aldehyde fixation, as in this figure and in Fig. 6, the content of the zymogen granules is heterogeneous in contrast to the granules of material fixed only in aldehyde (Figs. 9, 10, and 16). The three structures marked by small *x*'s are probably enlargements of the trans Golgi element. Note that they resemble the condensing vacuoles in shape and in inner content. × 34,000.

FIGURE 6 A portion of a pancreatic exocrine cell of the hamster. Fixation: 1% OsO_4-phosphate buffer, pH 7.4, 1 h. At upper right, the rough ER (*RER*) is directed towards one of the passageways in the Golgi stack (*G*); only a portion of the RER is seen in the passageway at the left edge of the figure. Note that the contents within the ER cisternae and within the condensing vacuoles (*CV*) are similar, whereas the contents of the Golgi elements are less electron opaque (cf. Fig. 13). Arrows within CV_1, CV_4, CV_5, CV_6, and CV_7 indicate regions where smooth ER might have been continuous with the condensing vacuoles in other planes of section, but in the absence of serial sections this cannot be asserted. Within CV_2 the arrowhead indicates a coated area; cf. appearance of coated vesicles, *C* near the center of Fig. 4 and *CO* in Fig. 5. An arrowhead outside CV_6 indicates the contiguity of rough ER membrane and of condensing vacuole membrane. The long arrow near the top center of the figure indicates a region of smooth ER and/or vesicles in apparent transition from ER to the cis element of the Golgi stack. Also labeled are coated vesicles (*C*) and zymogen granules (*Z*). × 28,000.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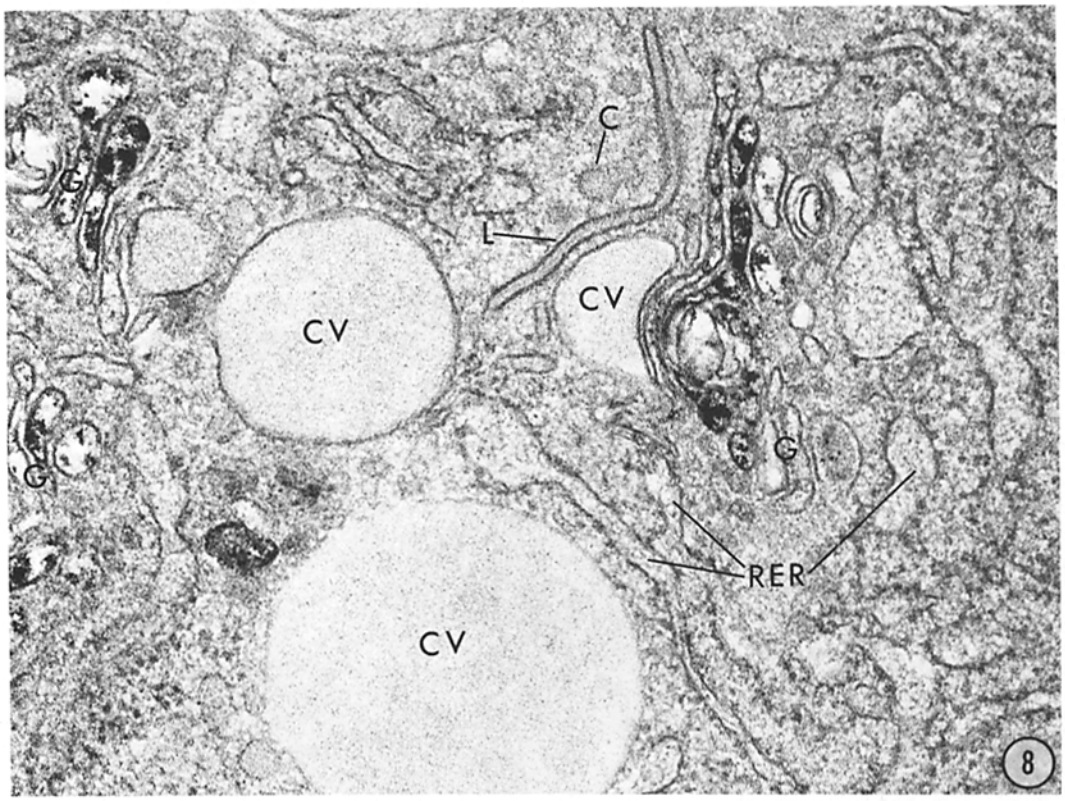
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and GERL is unequivocal when ultrastructural cytochemistry is used. The smooth membrane structures display AcPase activity (Figs. 7, 9, 11–13, and 15) and not TPPase activity (Figs. 8, 10, 14, and 16). In contrast, the trans element of the Golgi stack adjacent to GERL structures reveals TPPase activity (Figs. 8, 10, 14, and 16) and not AcPase activity (Figs. 7, 9, 11–13, and 15). The mature zymogen granules, as with light microscopy (Figs. 1 and 2), show neither AcPase nor TPPase activity (Figs. 9, 10, and 16); the same is true of rough ER (Figs. 7–10 and 13–16).

The condensing vacuoles are frequently seen to be directly continuous with rigid lamellae (Figs. 4, 6, 13, 14, and 16). In some instances in both unincubated (Fig. 6) and incubated (Fig. 13) material, the inner contents of the ER and of the condensing vacuoles of GERL resemble each other, and these contents are more electron-opaque than those of the Golgi elements.[3]

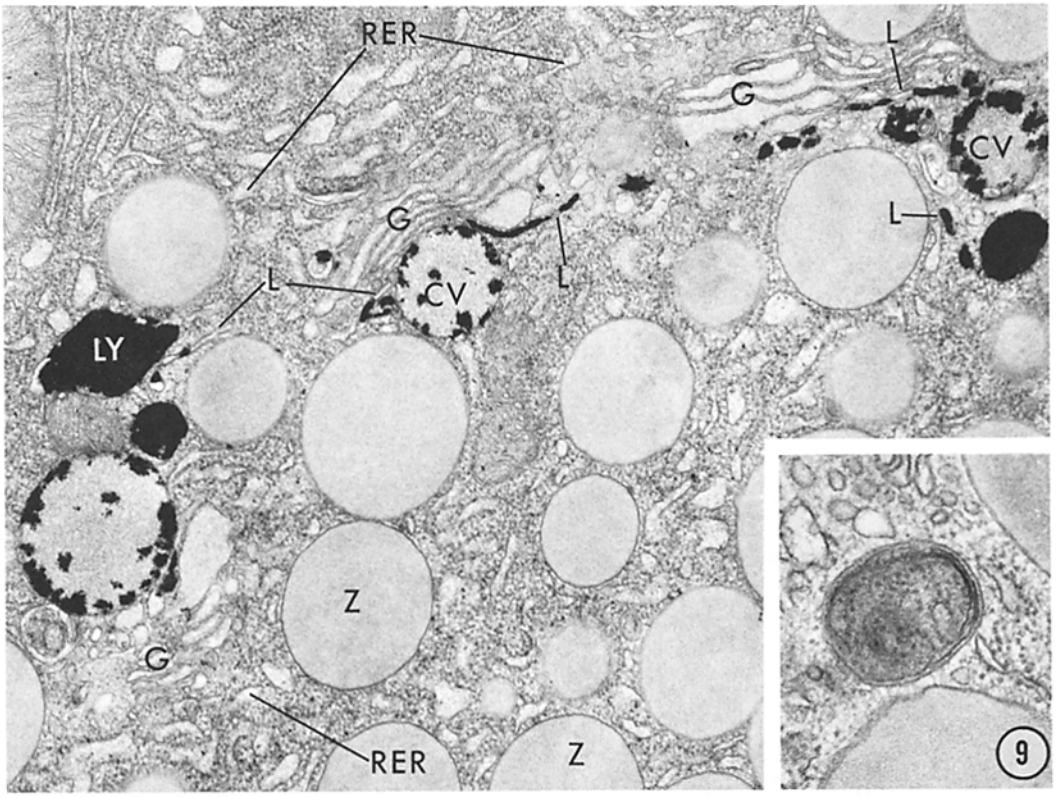
Intensive search for structural connections between the Golgi apparatus, especially its trans element, and condensing vacuole, rigid lamellae, or other smooth ER elements of GERL failed to reveal any connections. There are some dilatations in the trans element of the Golgi stack, sometimes with an electron-opaque material resembling that in the condensing vacuoles (Fig. 10, arrow; also Fig. 5) but these are rare. On the other hand, close proximities of portions of the trans element to the condensing vacuoles of GERL are always found (Figs. 5–12). However, as emphasized above, rough ER approaches close to the trans aspects of the Golgi stacks. Most importantly, there is always a sharp distinction between Golgi stacks and GERL, without any intermediate or overlapping, in their phosphatase activity. All condensing vacuoles and other GERL structures in the Golgi zone show AcPase activity (Figs. 7, 9, 11–13, and 15); none shows TPPase activity (Figs. 8, 10, 14, and 16). The trans element of the Golgi apparatus always shows TPPase but no AcPase activity.

In the pancreatic exocrine cells, as in other cell types studied in our laboratory, lysosomes apparently form from GERL. AcPase-positive coated vesicles are generally considered to be lysosomes (33). Such coated vesicles are numerous in the Golgi zone of these exocrine cells (Figs. 3–6, 8, 10–12, and 16). They display AcPase activity (Figs. 11 and 12) and the images suggest that they arise from GERL (Figs. 12) (see reference 49). The origin of the autophagic vacuoles, structures encountered rarely in the pancreatic exocrine cells of the four species studied (Fig. 9), was not investigated. Their rapid conversion to residual bodies is suggested by the inset in Fig. 9 where membrane arrays characteristic of many residual bodies (33) are already present, while many ribosomes and probably ER membrane fragments are still evident in the autophagic vacuole.

Small vesicles without coats are often seen attached to ER (Figs. 3 and 5) as if leading to the cis Golgi element of the Golgi stack (Fig. 6). Other uncoated vesicles are probably transverse sections of smooth ER (small arrowhead at the left edge of Fig. 4). There are many apparent vesicles at regions of the Golgi stacks which appear, in thin sections, as lateral edges of the elements (Figs. 3, 5, 6, 10, 12, and 16), but it is difficult to know whether any are transverse sections of smooth tubules. Even the artifactual breakdown of tubules into vesicles during fixation should be con-

[3] Two guinea pigs were injected with carbamylcholine, but the dose proved insufficient to induce abundant zymogen discharge from the pancreatic acinar cells. In these cells, the condensing vacuoles appeared much larger than in the other guinea pigs studied. These cells also showed a higher electron opacity in the ER and condensing vacuoles than in the contents of the Golgi elements. This contrast was much greater than that illustrated in Figs. 6 and 13.

FIGURES 7 and 8 Portions of pancreatic exocrine cells of the guinea pig. Fixation: as in Fig. 1. Incubations: Fig. 7, AcPase medium, 40 min at 37°C, thin section unstained; Fig. 8, TPPase medium, 90 min at 37°C, thin section stained with lead. No portion of the Golgi stack (*G*) shows AcPase activity (Fig. 7). AcPase activity is shown by components of GERL: condensing vacuoles (*CV*), rigid lamellae (*L*) and coated vesicles (not seen in this section; but see *C* in Fig. 8 and also Figs. 11 and 12). The innermost (trans) Golgi element shows TPPase activity (Fig. 8). Such activity is not shown by any components of GERL. The rough ER (*RER*) shows neither AcPase nor TPPase activity. Note the extent of ER directed towards the trans aspect of the Golgi stack, probably in a large interruption in the stack (cf. Fig. 2). Fig. 7, × 48,000; Fig. 8, × 50,000.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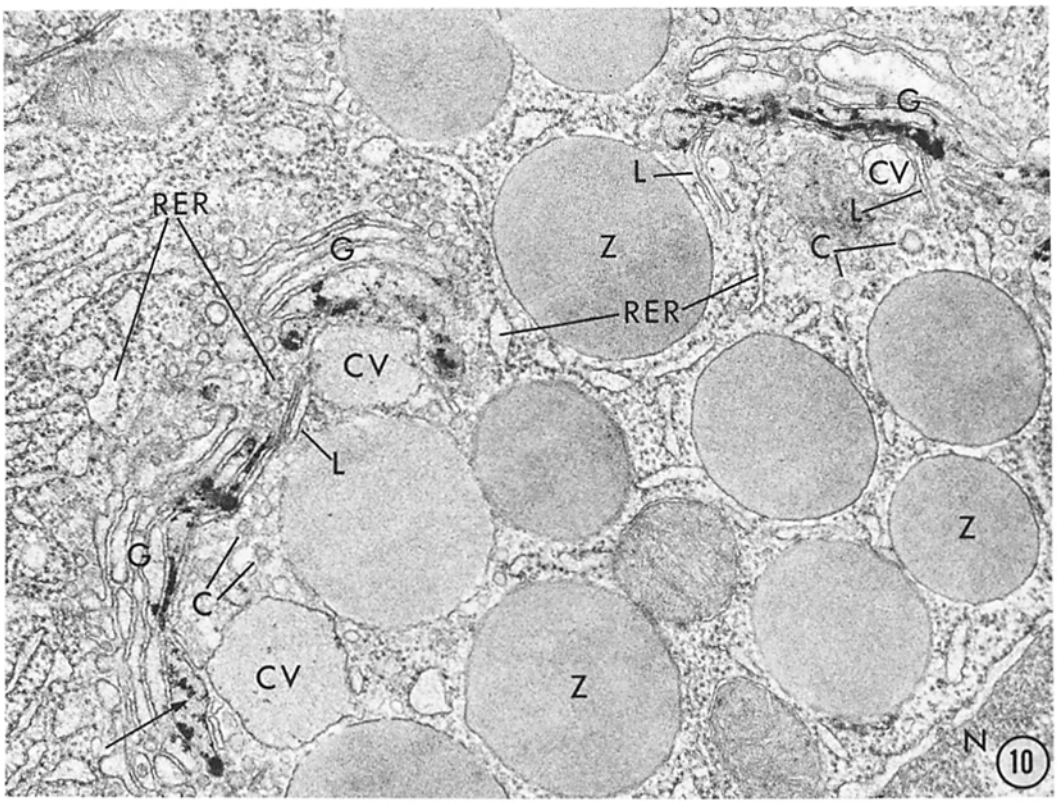
G
L
CV
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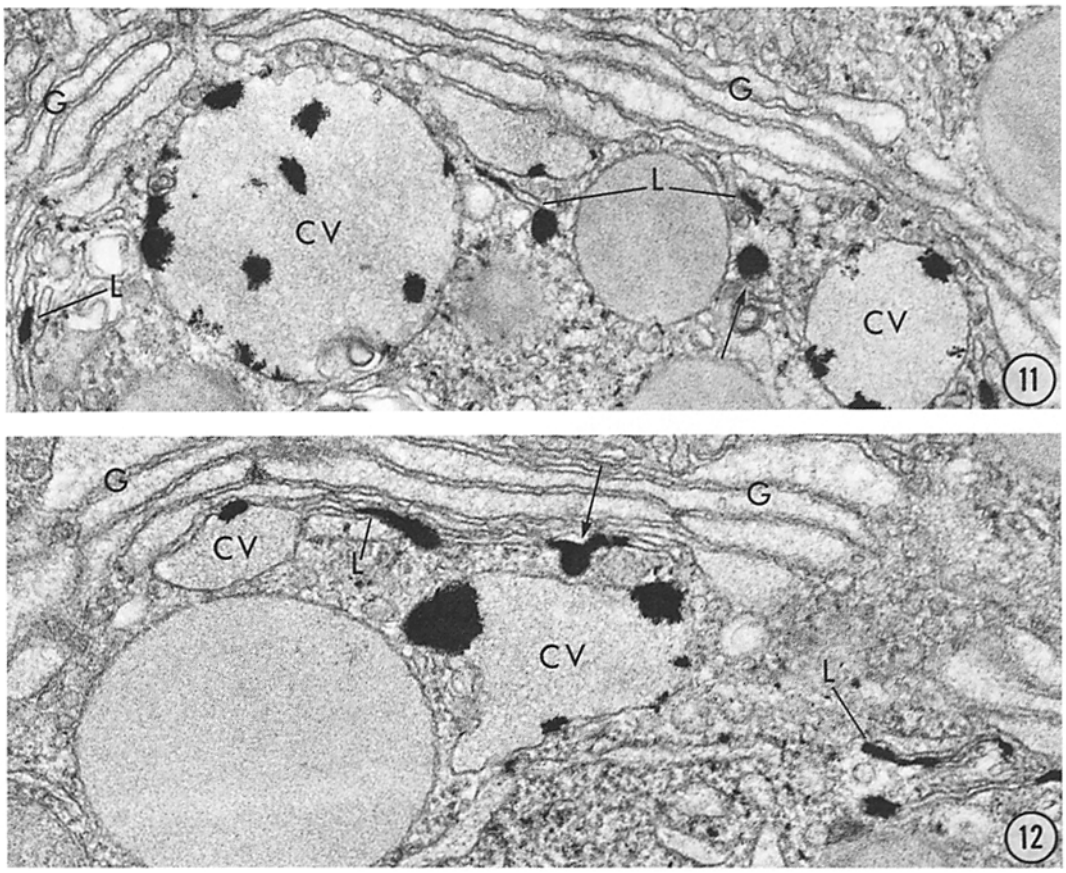
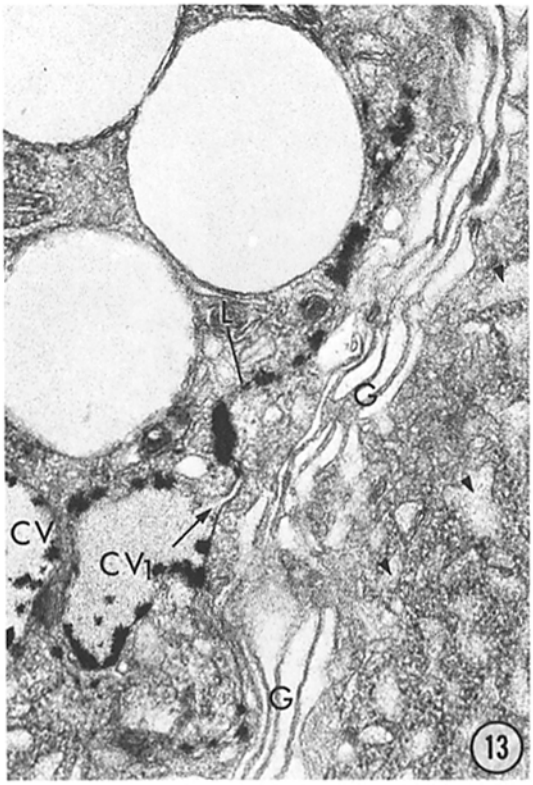
10



FIGURES 11 and 12 Portions of pancreatic exocrine cells of the rat. Fixation: as in Fig. 1. Incubations: AcPase medium, 25 min at 37°C; thin sections stained with lead. Reaction product is seen in GERL, including condensing vacuoles (*CV*), rigid lamellae (*L*), and coated vesicles (arrows). In Fig. 11 the coated vesicle appears to be separate from the cisternal portion of GERL, whereas in Fig. 12 it is still attached to GERL. Reaction product is not present in the Golgi stack (*G*), nor in small vesicles at the lateral edges of the Golgi stack or in rough ER (both unlabeled). × 46,000.

FIGURES 9 and 10 Portions of pancreatic exocrine cells of the rat and, in the inset of Fig. 9, of the guinea pig. Fixation: as in Fig. 1, except for the inset where the fixation was as in Fig. 5. Incubations: Fig. 9, AcPase medium, 25 min at 37°C, thin section stained with lead; Fig. 10, TPPase medium, 90 min at 37°C, thin section stained with lead. The Golgi stack (*G*) shows no AcPase activity, while its trans element shows TPPase activity. In Fig. 9, AcPase activity is shown by the two components of GERL seen in the field: condensing vacuoles (*CV*) and rigid lamellae (*L*). In Fig. 10, GERL structures, including coated vesicles (*C*), condensing vacuoles (*CV*), and rigid lamellae (*L*), do not show TPPase activity. The rough ER (*RER*) and mature zymogen granules (*Z*) show neither AcPase nor TPPase activity. Note, in Fig. 10, that rough ER (*RER*), portions of GERL, and a zymogen granule (*Z*) are seen in a passageway across the Golgi stack; this is not as evident in Fig. 9. The arrow in Fig. 10 indicates a dilated region in the trans element of the Golgi stack. In Fig. 9, *LY* indicates an autophagic vacuole (see inset); when a very light print was made from this negative, membranes—some resembling mitochondrial membranes—were evident within the vacuole; cf. Fig. 12 in reference 34. The inset of Fig. 9 is of unincubated material. The center of the inset is occupied by an autophagic vacuole containing ribosomes and probably some ER membranes. At the upper right of the autophagic vacuole, a small membrane array characteristic of residual bodies is seen (cf. reference 33). Fig. 9, × 26,000; Inset, × 63,000; Fig. 10, × 26,000.


L
G
CV
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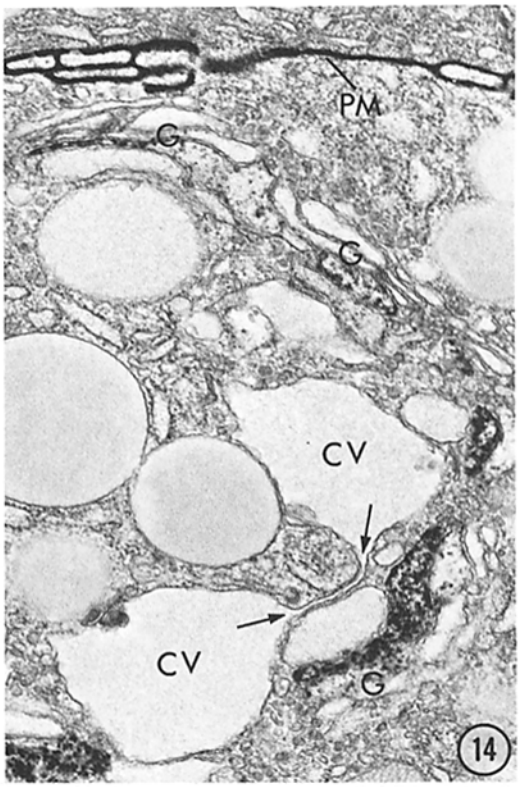
G
13


PM
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CV
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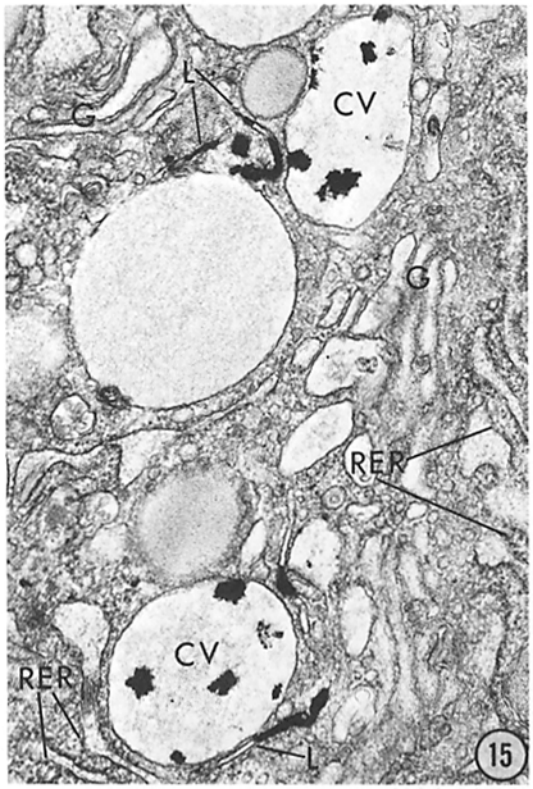
14


L
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RER
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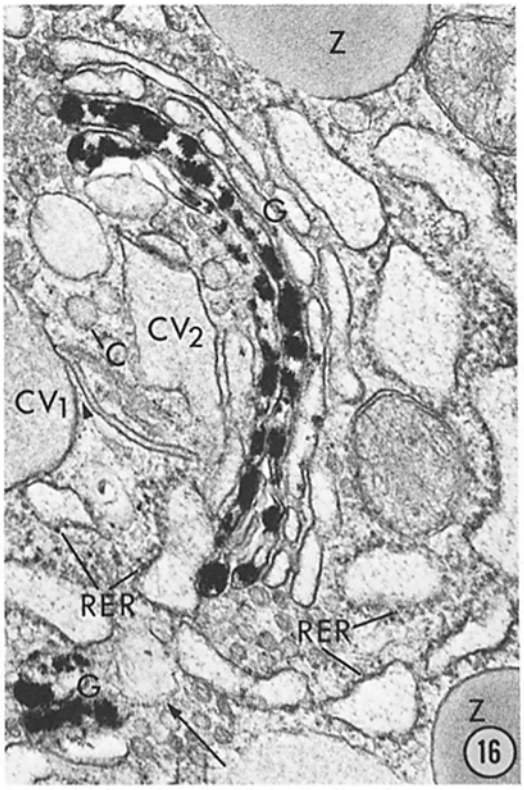
15


Z
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CV2
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CV1
RER
RER
G
Z
16

sidered a possibility. This may account, in part, for images such as seen at the long arrow in Fig. 6 (see reference 51).

An observation worth noting is that, in the two fasted and refed guinea pigs, some rough ER cisternae contained intracisternal granules, up to 300 nm in diam, as described by Palade (47). No change occurred in either TPPase or AcPase localizations in these guinea pigs.

DISCUSSION

The concept of condensing vacuoles as the nascent form of zymogen granules (9, 19, 20) remains vital to understanding the secretory process in the pancreatic exocrine cell (46). The results presented here, however, require that we view afresh the origins and structural relations of these vacuoles. In pancreatic exocrine cells of guinea pig, rat, rabbit, and hamster, the findings were the same. By conventional electron microscopy and by ultrastructural cytochemistry, the condensing vacuoles were demonstrated to be dilated portions of GERL.

Strong support for our findings in the pancreas exocrine cells comes from the work of Hand and Oliver (17, 18) on the exocrine cells of five different salivary glands in three species. As we have done in the work reported here as well as in other cell types in earlier studies (43) and in mouse pancreatic exocrine cells described elsewhere,[2] these investigators used AcPase and TPPase activities to distinguish between GERL and Golgi apparatus. They considered "immature secretory granules," i.e., the condensing vacuoles, to be part of GERL. In their studies, condensing vacuoles and tubular elements displayed AcPase but not TPPase activity; the trans Golgi element showed TPPase but not AcPase activity, and mature zymogen granules showed neither phosphatase activity. Also like us, Hand and Oliver found no structural connections between Golgi elements and GERL.

It should be noted that static electron micrographs cannot exclude the possibility that, by some kind of "membrane flow" (12, 29, 57), the trans element of the Golgi apparatus can be converted into GERL with its condensing vacuoles and rigid lamellae. Indeed, some support for this possibility may be seen in the proximity of the condensing vacuoles and distended portions of the GERL element, the similarity in their shapes, and, infrequently, in their inner contents. On the other hand, the condensing vacuoles are often far removed from the Golgi apparatus while still attached to the rigid lamellae, as in Fig. 4. The inner contents of the condensing vacuoles resemble those of the ER more frequently than those of the Golgi elements. Most importantly, as in the case of the neurons of dorsal root ganglia (44), any such membrane flow would require that the trans element of the Golgi apparatus drastically change its structure, and lose its demonstrable TPPase activity and gain demonstrable AcPase activity.

The exocrine cells of the rat exorbital lacrimal and parotid glands studied by Hand and Oliver (17, 18) offer an important advantage over pancreatic exocrine cells because the enzyme, peroxidase, is one of their secretory products, and because peroxidase activity is readily demonstrable by the 3,3′-diaminobenzidine procedure of Gra-

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FIGURES 13–16 Portions of pancreatic exocrine cells of the hamster (Figs. 13 and 14) and the rabbit (Figs. 15 and 16). Fixation: as in Fig. 1. Incubations: Figs. 13 and 15, AcPase medium, 45 min (Fig. 13) and 30 min (Fig. 15), 37°C (thin sections stained with lead); Figs. 14 and 16, TPPase medium, 90 min, 37°C (thin sections stained with lead). The Golgi stack (*G*) shows no AcPase anywhere, while its trans element shows TPPase activity. In the upper part of Fig. 15 and lower part of Fig. 16 (arrow), portions of rough ER and GERL are present in passageways across the Golgi stack. In Fig. 13 the contents of rough ER (arrowheads) and condensing vacuole (*CV*) are more electron-opaque than the contents of the Golgi elements; cf. Figs. 5 and 6. AcPase reaction product is seen in components of GERL, evident in Figs. 13 and 15; condensing vacuoles (*CV*) and rigid lamellae (*L*). The arrow in Fig. 13 indicates a continuity between condensing vacuole and a rigid lamella. Contiguity of these two structures is seen at the arrowhead in Fig. 16; cf. Fig. 4. Other rigid lamellae are indicated by *L* in Fig. 15. GERL structures, including coated vesicles (*C*, Fig. 16), do not show TPPase activity. The rough ER (*RER*) shows neither AcPase nor TPPase activity. Small vesicles, even adjacent to a TPPase-positive Golgi element, show no phosphatase activity (Fig. 16). Note, in Fig. 14, a rigid lamella connecting two condensing vacuoles (arrows), and at *PM*, reaction product at the plasma membrane. Fig. 13, × 42,000; Fig. 14, × 23,500; Fig. 15, × 31,000; Fig. 16, × 48,000.

ham and Karnovsky (16). Peroxidase is present in the ER, in the Golgi apparatus, and in both immature and mature secretory granules. The immature granules are connected to tubules of GERL, and both these structures show AcPase activity. However, interpretation of the peroxidase localizations is complicated. Although GERL apparently packages the secretory product, "GERL was usually free of reaction product . . . or contained only a small amount . . ., even though it was in continuity with immature granules which were reactive" (17). Hand and Oliver write, "In view of its apparent role in secretory granules formation, the general absence of secretory proteins in GERL, as judged by the localization of peroxidase reaction product, is somewhat surprising. The distribution of reaction product in the Golgi saccules suggests that they participate in the transport and initial concentration of the secretory proteins. The method of transfer of the secretory proteins from the inner saccule to the forming granule remains obscure" (17).

Thus, the situation in these two exocrine secretory cells is more complicated than in eosinophilic leukocytes (3) and other leukocytes (e.g., references 1 and 30), where the peroxidase-positive granules are packaged by Golgi elements which possess peroxidase activity, as expected.

Kraehenbuhl et al. (25) present impressive evidence that all zymogen granules of bovine pancreas contain trypsinogen, chymotrypsinogen A, carboxypeptidase A, RNase, and DNase. However, the evidence for the involvement of the Golgi apparatus in packaging the granules is compromised by the difficulty of identifying Golgi components (see Fig. 8 of reference 25).

That GERL is part of the ER, continuous with rough ER, remains a hypothesis rather than an established fact. However, not only Fig. 3 in this publication, which we consider as an unequivocal connection between a condensing vacuole and rough ER, but other recent micrographs serve to strengthen the hypothesis. Fig. 9 in reference 41 is a micrograph of the Golgi zone of a hepatocyte in the beige mouse, in which Essner and Oliver (13) have described a much enlarged GERL. We interpret the micrograph, as we have Fig. 1 of an insulinoma cell in reference 44, Figs. 19 and 20 of a thyroid epithelial cell in reference 42, and Fig. 1 of a rat hepatocyte (see footnote 4) to show areas of rough ER where degranulation of ribosomes is occurring, in areas of transition from rough ER to GERL, free of ribosomes. In our interpretation, such degranulation is probably occurring between the upper line from *RER* and CV_1 in Fig. 3. Fig. 11 of a rat megakaryocyte published by Bentfeld and Bainton shows AcPase reaction product "in a smooth-membrane portion of a cisterna (ser) (presumably part of GERL), which is continuous with the RER (rer)" (6).

From the results presented in this publication, it is possible to propose the hypothesis that some secretory material moves from the rough ER to the condensing vacuoles, without passing through either the Golgi apparatus or some "lock-gate" mechanism (46) in the Golgi zone. It remains to be learned whether mechanisms exist, currently undetectable by electron microscopy, by which contributions may be made by the Golgi apparatus to this secretory material. In this connection, it might be highly informative if a cytochemical procedure for the demonstration of ultrastructural localizations of glycosyl transferases were to become available. Some of these transferases are known to be highly concentrated in subcellular fractions, isolated from a variety of cell types, that are rich in portions of the Golgi apparatus (14, 29, 52).

This uncertainty regarding contributions to secretory product by the Golgi apparatus in cell types where GERL appears to concentrate and package secretory and other material should not be extended to the many cell types where Golgi elements do appear to function in these capacities. Among others, these include odontoblasts (56), neutrophils (2), as well as the leukocytes mentioned earlier, and a variety of plant cells (57; also reference 8).

A test of the hypothesis, by ARG, might be possible by selecting rigid lamellae that are separated from both Golgi stacks and condensing vacuoles by distances greater than the size of silver grains. This would require a sufficiently high concentration of the particular secretory material in the rigid lamellae, a relatively slow transfer rate through the lamellae, and an appropriate radioactive precursor.

Biochemical data are required to assess some of the observations recorded here and to appreciate their functional implications. Many important questions might be answered with sufficiently purified isolated subcellular fractions. Are other hydrolytic enzymes associated with AcPase activity

[4] Novikoff, P. M., and A. Yam. Manuscript in preparation.

in condensing vacuoles and coated vesicles? Do these include proteases capable of catalyzing "limited proteolysis" reactions (see discussions in references 43, 53, and 49) such as are involved in proinsulin to insulin conversion and in a rapidly expanding number of similar intracellular conversions?

Coated vesicles have recently been isolated from brain and other tissues (48). Such vesicles are abundant in pancreatic acinar cells and may prove to be readily isolated. Although our findings establish the relation of coated vesicles to GERL and although observations on other cell types suggest that these vesicles probably function as primary lysosomes (33), their roles in pancreatic acinar cells remain to be established. The possibility that they carry AcPase and other hydrolytic enzymes to condensing vacuoles seems most unlikely from the electron microscope observations. The images are more consistent with vesicle separation from, rather than merger with, condensing vacuoles and other portions of GERL (Figs. 3–5 and especially Fig. 12); see Discussion in reference 47.

Comparison of isolated condensing vacuoles and mature zymogen granules might reveal that the zymogen granules, despite the absence of cytochemically demonstrable activity, in fact do possess AcPase detectable by biochemical assay. Maturation of the granule might, for currently unknown reasons, mask the activity of this enzyme during the cytochemical procedure; for a listing of cell types in which this type of "latency" has been observed, see reference 7.

Were it possible to isolate rigid lamellae of sufficient quantity and purity, their special properties could be studied. This might elucidate the mechanisms responsible for keeping the membrane flattened, separated by 200–300 Å.

Other questions arise, most of which have been alluded to in the Results. Aside from coated vesicles, what other types of vesicles are present in the Golgi zone? Do any apparent vesicles arise by the breakdown of tubules during fixation as seen in the toad spinal ganglia studied by Rosenbluth (51)? How can we account for the absence of reaction product, as illustrated in Fig. 16 and observed invariably, in the small vesicles adjacent to the TPPase-positive trans Golgi element? Is this because the vesicles do not, as is often assumed, arise from all Golgi elements?; or do the vesicles from the trans element, as from others, differentiate as they separate from the Golgi element? Will the smooth-membraned structures described by Jamieson and Palade (23) after stimulation of pancreas slices by carbamylcholine, at a dose sufficient to degranulate the exocrine cells, show AcPase activity? These structures appear to us to be hypertrophied elements of GERL.

Our observations suggest that in secretory cells such as exocrine pancreas cells, GERL may play an important role in transforming macromolecules that are subsequently secreted. Recently, one of us (34) has listed a variety of macromolecular transformations in which GERL seems to be involved.

In the Results above, uncertainties regarding the three-dimensional geometry of the Golgi apparatus and of GERL were alluded to. In the neuron study from our laboratory (44), as in other studies, e.g., in plant cells (28) and in epididymis (15), diagrammatic representations were drawn, but models were not constructed from serial sections. To our knowledge, such reconstructions have been published only for the Golgi apparatus in cells of maize roots. These were prepared by J. E. Kephart and appear as Fig. 31 in the monograph of Whaley (57). In the pancreatic acinar cell, the Golgi apparatus and associated GERL are far more complex. Two uncertainties in the pancreas cells which require construction of a model are: (*a*) the nature of the different "passageways," the localized areas where the Golgi stack is interrupted and through which the ER gains access to GERL; and (*b*) the relations of what are generally referred to as lateral Golgi vesicles to these passageways. Such a model will serve to define more precisely what is meant by terms such as lateral edges and fenestrations. Generally, fenestration is used for interruptions in individual Golgi elements or in ER at the cis face of the Golgi apparatus and in GERL at the trans face (e.g., see references 28, 12, 15, and 44).

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