RELATIONSHIP BETWEEN THE GOLGI APPARATUS,
GERL, AND SECRETORY GRANULES IN ACINAR
CELLS OF THE RAT EXORBITAL LACRIMAL GLAND

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ABSTRACT

The method of secretory granule formation in the acinar cells of the rat exorbital lacrimal gland was studied by electron microscope morphological and cytochemical techniques. Immature secretory granules at the inner face of the Golgi apparatus were frequently attached to a narrow cisternal structure similar to GERL as described in neurons by Novikoff et al. (Novikoff, P. M., A. B. Novikoff, N. Quintana, and J.-J. Hauw. 1971. *J. Cell Biol.* **50**:859–886). In the lacrimal gland, GERL was located adjacent to the inner Golgi saccule, or separated from it by a variable distance. Portions of GERL were often closely paralleled by modified cisternae of rough endoplasmic reticulum (RER), which lacked ribosomes on the surface adjacent to GERL. Diaminobenzidine reaction product of the secretory enzyme peroxidase was localized in the cisternae of the nuclear envelope, RER, peripheral Golgi vesicles, Golgi saccules, and immature and mature secretory granules. GERL was usually free of peroxidase reaction product or contained only a small amount. Thiamine pyrophosphatase reaction product was present in two to four inner Golgi saccules; occasionally, the innermost saccule was dilated and fenestrated, and contained less reaction product than the next adjacent saccule. Acid phosphatase (AcPase) reaction product was present in GERL, immature granules, and, rarely, in the innermost saccule, but not in the rest of the Golgi saccules. Thick sections of AcPase preparations viewed at 100 kV revealed that GERL consisted of cisternal, and fenestrated or tubular portions. The immature granules were attached to GERL by multiple connections to the tubular portions. These results suggest that, in the rat exorbital lacrimal gland, the Golgi saccules participate in the transport of secretory proteins, and that GERL is involved in the formation of secretory granules.

Morphologic, radioautographic, and biochemical studies of pancreatic exocrine cells (10, 30, 31, 32, 73, 75, 76, 82), hepatocytes (1, 14, 21, 62), salivary acinar cells (11, 23, 26), odontoblasts (83), and several other cell types have established that the intracellular transport and processing of exportable proteins occurs within membrane-delimited cytoplasmic compartments. After synthesis on attached ribosomes and transfer to the cisternal space of the rough endoplasmic reticulum (RER), the secretory proteins move through the RER to smooth-surfaced transitional regions at the periph-

ery of the Golgi apparatus. The proteins are then transferred to the Golgi apparatus, presumably by small vesicles, where they may undergo various modifications (7, 44), before concentration and packaging in secretory granules. The various steps involved in the synthesis and transport of secretory proteins have recently been reviewed by Palade (69).

The methods proposed for the packaging of secretory material into granules differ, however, depending upon the cell type studied. In many cells, the granules appear to form from the Golgi saccules, either as a swelling of the innermost saccule, or as dilations from the ends of two to three saccules at the inner face. In some cells, such as polymorphonuclear leukocytes (3) and pituitary mammotrophs (77), the granules may form as an aggregation of several smaller immature granules derived from the Golgi saccules. Jamieson and Palade (31) and Palade (69) have suggested that in the exocrine pancreas of the guinea pig the immature granules (condensing vacuoles) arise from the fusion of transfer vesicles from the transitional ER. However, Novikoff has recently suggested (52, 53, 54, 59, 60, 63) that in some secretory cells, including those of the exocrine pancreas, secretory material is packaged into granules by Golgi-endoplasmic reticulum-lysosomes (GERL), a Golgi-associated smooth membrane system characterized by the presence of acid phosphatase (AcPase) activity (65). Several other investigators have also described GERL, or GERL-like structures, in both exocrine (9, 23, 24, 25) and endocrine (16, 28, 38, 71, 72) cells, and AcPase reaction product is frequently present in forming or immature granules in a variety of secretory cells (4, 36, 47, 55, 56, 67, 70, 77, 78).

In the present study, we have examined the Golgi apparatus of the acinar secretory cells of the rat exorbital lacrimal gland, by electron microscope cytochemical methods, in order to determine the method of formation of secretory granules. Peroxidase, a secretory enzyme produced by these cells (27, 42), was utilized as a marker of the cellular compartments involved in the transport of secretory proteins. Thiamine pyrophosphatase (TPPase) activity served to mark the inner Golgi saccules, and AcPase activity identified GERL and structures arising from it. The results suggest that in these cells the Golgi saccules are involved in the transport of secretory proteins, and that the secretory granules are formed from GERL.

MATERIALS AND METHODS

Adult Sprague-Dawley rats, 240–550 g, of both sexes were used. The animals were anesthetized with chloral hydrate, 400 mg/kg, given intraperitoneally.

Fixation

For morphological and enzyme cytochemical studies of the exorbital lacrimal glands, fixation was usually achieved by vascular perfusion of room-temperature fixative solution (23). In a few cases, the lacrimal glands were fixed by immersion and mincing in the fixative. The fixative used was modified from Karnovsky (33), and contained 0.67–3.0% glutaraldehyde (Ladd Research Industries, Burlington, Vt.) and 1.0–2.0% formaldehyde (Ladd Research; or from paraformaldehyde powder, Fisher Scientific Co., Fair Lawn, N. J.) in 0.05–0.1 M sodium cacodylate buffer, pH 7.4. Total fixation time was 1–3 h. After fixation, the tissues were trimmed into small pieces and rinsed overnight in 0.1 M cacodylate buffer, pH 7.4, with 7% sucrose at 4°C.

Cytochemical Procedures

For enzyme cytochemical studies, the tissues were sectioned at 60–75 μm with a Sorvall TC-2 tissue sectioner, and rinsed several times in cacodylate-sucrose buffer. For the demonstration of endogenous peroxidase activity, the sections were incubated for 2–4 h at 37°C and pH 7.0 in the following medium: 20 mg of 3,3'-diaminobenzidine-tetra HCl (DAB, Sigma Chemical Co., St. Louis, Mo.); 10 ml of 0.05 M sodium cacodylate buffer, with 5% sucrose; and 0.2 ml of 1% H_2O_2. Controls consisted of sections preincubated with 10 mM KCN or 10 mM 3-amino-1,2,4-triazole followed by incubation in medium with the same concentrations of these inhibitors, or incubation in medium without H_2O_2.

TPPase and nucleoside diphosphatase (NDPase) activity were demonstrated by incubation at pH 7.2 in the Novikoff and Goldfischer medium (58) for 2 h at 37°C, with thiamine pyrophosphate, uridine-5'-diphosphate or inosine-5'-diphosphate (Sigma) as substrate. For demonstration of AcPase activity at pH 5.0, the sections were incubated for 60 min at 37°C in the medium described by Novikoff (48), with CMP (Sigma) as substrate. Incubation in the absence of substrate served as a control.

After incubation, the sections were rinsed several times in cacodylate-sucrose buffer. In some cases, the lead phosphate reaction product was converted to lead sulfide by treating the sections with dilute ammonium sulfide before postfixation.

Postfixation and Processing

Tissue blocks and incubated sections were postfixed for 1–2 h in 1% OsO_4 in Veronal-acetate buffer, pH 7.4, rinsed in 0.05 M maleate buffer, pH 5.2, and treated with 0.5% uranyl acetate in the same buffer for 1–2 h. After a brief rinse in distilled water, the tissues were

dehydrated in ethanol and embedded in Spurr's resin (79). Thin sections were cut on a Porter-Blum MT-2 microtome (DuPont Instruments, Sorvall Operations, Newtown, Conn.), stained with lead citrate and examined in an AEI EM 6-B, JEM 100-B, or JEM 100-C electron microscope. Thick sections, 0.2–0.3 μm, of tissues incubated for AcPase activity were examined without staining at 100 kV.

RESULTS

The acinar secretory cells of the rat exorbital lacrimal gland were relatively large compared to those of other exocrine glands, averaging about 23–28 μm from base to apex. The nucleus, usually oval in shape, but occasionally with an irregular, deeply indented border, was also quite large, up to 12–18 μm in its greatest dimension. Binucleated cells were frequently observed. Abundant quantities of RER filled the basal cytoplasm, and a variable number of membrane-bounded secretory granules, 0.4–1.5 μm in diameter, were located in the apical cytoplasm. In some cells, the secretory granules were serous-like and of moderate electron density, while in other cells the granules were mucous-like and of much lower density. However, no differences were observed in the cytochemical properties of the cells containing either type of granule. These cytological characteristics are in general agreement with previous morphological (74) and cytochemical (17, 27) investigations of the rat exorbital lacrimal gland.

Golgi Region

MORPHOLOGY

The large Golgi apparatus (Fig. 1) was located in the perinuclear cytoplasm either apical or slightly lateral to the nucleus, and appeared as several discrete stacks of saccules, usually oriented with their inner (mature or trans) face toward the apex or the central region of the cell. At the outer (forming of cis) face, ribosome-free portions of transitional ER closely approached the Golgi apparatus. Numerous small smooth-surfaced vesicles, presumably derived as buds from the transitional ER, were present between the transitional ER and the Golgi apparatus. Each individual Golgi stack consisted of five to seven saccules of irregular width and variable length. The saccules at the outer face (Figs. 1, 2, 7, 8, and 9) were usually of greater width than those at the inner face, and often appeared to be broken into several short segments. The inner two or three Golgi

saccules were generally narrower and more regular in outline, with only an occasional fenestration. In some cases, however, the innermost saccule was dilated, and heavily fenestrated or broken into short segments (Figs. 1, 7, 8).

Several immature secretory granules (condensing vacuoles; prosecretory granules) of variable size were usually found in the inner Golgi region (Figs. 1 and 2). They were frequently separated from the inner Golgi saccule by groups of small smooth vesicles and an occasional coated vesicle. The immature granules were frequently observed to be connected to a narrow cisterna of relatively uniform width (~25 nm) (Figs. 1 and 2), which was either adjacent to the inner Golgi saccule or separated from it by a variable distance, with a few intervening vesicles. Because of its morphology, relationship to the Golgi apparatus, and cytochemical properties (described below), we consider this cisterna to be equivalent to GERL. The membrane of GERL was usually more electron dense than that of the Golgi saccules, and portions of GERL appeared highly fenestrated, particularly near its juncture with the immature granules (Fig. 2). GERL often followed a curving path in the inner Golgi region, and occasionally wrapped around an immature granule (Fig. 9). GERL was frequently paralleled by cisternae of RER (Figs. 3 and 5). These cisternae often appeared modified, lacking ribosomes on the portion of their membrane adjacent to GERL (25).

Occasionally, a dense body, which often had a heterogeneous content of granular, membranous, and lipid-like material, was found in the inner Golgi region. These dense bodies resembled secondary lysosomes and residual bodies described in other cells. However, these dense bodies were most frequently found peripheral to the Golgi apparatus or in the basal cytoplasm.

CYTOCHEMISTRY

PEROXIDASE: Incubation of sections of lacrimal glands in DAB medium resulted in dense deposits of reaction product due to the activity of endogenous peroxidase, a secretory enzyme of the acinar cells. In the acinar cells (Fig. 3), reaction product was localized to cisternae of the nuclear envelope and RER, the transitional ER and vesicles between it and the Golgi apparatus, the Golgi saccules, immature, and mature secretory granules. The reaction product was most dense in the cisternae of the nuclear envelope and RER, and
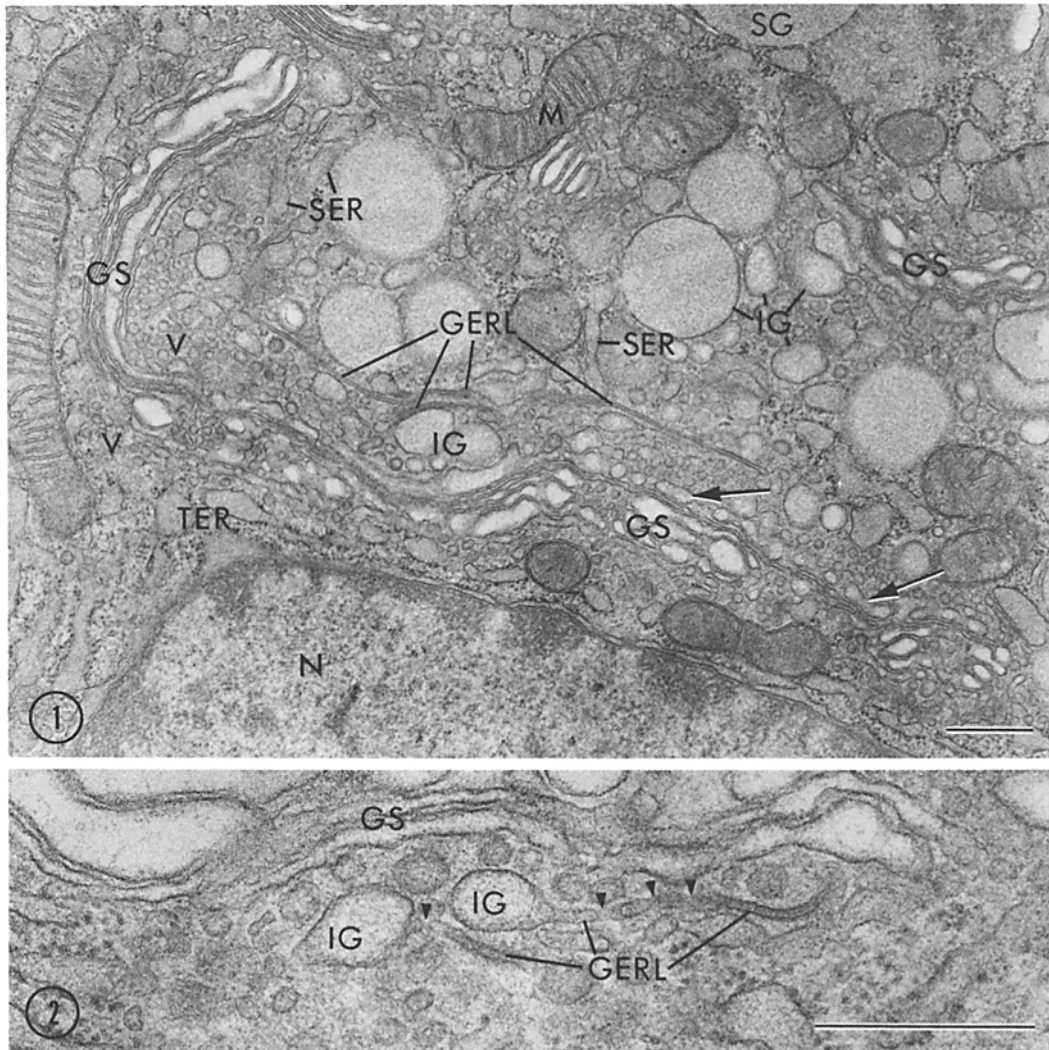
FIGURE 1 Golgi region of an acinar secretory cell of the rat exorbital lacrimal gland. The Golgi apparatus consists of stacks of 5-7 saccules (GS), numerous vesicles (V) between the transitional endoplasmic reticulum (TER) and the outer face, and immature secretory granules (IG) of varying size. The inner Golgi saccule (arrows) appears slightly dilated and fenestrated. Several short cisternae of RER and occasional smooth-surfaced cisternae (SER) are found in the inner Golgi region. Several segments of a membranous structure with a narrow cisternal space (GERL) are located in the inner Golgi region, but separated from the Golgi saccules by vesicles and immature granules. Nucleus (N); mitochondrion (M); secretory granule (SG). × 21,600.

FIGURE 2 Higher magnification of the inner Golgi region, showing two small immature granules (IG) which are continuous with segments of GERL. Fenestrations in GERL are indicated by the arrowheads. Several small vesicles separate the immature granules and GERL from the Golgi saccules (GS) × 58,300.

was least dense in the outer Golgi saccules. The inner Golgi saccules usually contained denser deposits of reaction product than the outer saccule. When the innermost saccule was dilated, as previously described, it was usually strongly reactive for peroxidase (Fig. 6).

GERL was usually free of reaction product (Figs. 3, 4, and 5), even though it was continuous

with immature granules that contained dense deposits. Occasionally, a small amount of reaction product was present in GERL (Fig. 4), particularly near its junction with a forming granule. The modified RER cisternae that paralleled GERL were intensely reactive for peroxidase (Figs. 3 and 5).

The deposition of peroxidase reaction product was only slightly diminished in control sections incubated in media containing amino-triazole or KCN. Omission of H_2O_2 from the medium completely prevented the reaction.

THIAMINE PYROPHOSPHATASE: After incubation with TPPase as substrate, two to four saccules at the inner face of the Golgi apparatus contained dense deposits of reaction product (Figs. 7-9). The most intense reaction usually occurred in the innermost saccule, with decreasing amounts of reaction product in saccules closer to the outer face. When the innermost saccule was dilated and/or fenestrated (Figs. 7 and 8), the amount of reaction product present in it varied from being the same or slightly less than that in the next adjacent saccule (Fig. 7) to being only a very light deposit (Fig. 8). GERL was consistently free of reaction product (Figs. 7-9). Immature granules were usually unreactive but occasionally contained a small amount of reaction product. No other intracellular organelle contained reaction product.

Incubation with uridine diphosphate or inosine diphosphate resulted in a distribution of reaction product similar to that observed with TPPase. The overall reaction of the Golgi saccules was slightly les than with TPPase, but a more consistent reaction was present in the immature granules. In some preparations, with either of the three substrates, reaction product was associated with the basal and lateral plasma membranes, and occasionally with the apical plasma membrane at the acinar lumen. In the absence of substrate, the acinar cells were free of deposits.

ACID PHOSPHATASE: Incubation for AcPase activity resulted in deposits of reaction product in GERL, immature secretory granules, and lysosomes (Figs. 10-15). The structure of GERL and its relationships to the immature granules were best visualized in thick (0.2-0.3 μm) sections viewed at 100 kV (Figs. 10-13). In low magnification micrographs (Fig. 10), the position and large extent of GERL at the inner face of the Golgi apparatus were clearly evident. When

GERL was cut in cross section (Figs. 10, 11, and 13) it appeared as thin dense lines with numerous dilations of variable size, which were small immature secretory granules filled with reaction product. When GERL was more or less parallel to the section plane (Figs. 10 and 12), it appeared as a broader gray area, again with closely adjacent, dense, and immature granules. At higher magnification, GERL was observed to consist of two discrete portions: a platelike cisternal portion (Figs. 11-13), which often followed a curving path through the inner Golgi region; and a portion which was fenestrated or consisted of anastomosing tubules (Figs. 11 and 12) to which the immature granules were attached through multiple connections (Fig. 12). The tubular or fenestrated portions and attached granules were continuous with the peripheral region of the cisternal portion (Figs. 11 and 12). It has not yet been determined whether separate cisternal portions are in continuity through an interposed tubular portion or whether each cisternal-tubular complex exists as a separate unit.

Occasionally, GERL appeared to enclose a round or oval profile of unstained, homogeneous content (x in Fig. 10). This was interpreted as an unreactive secretory granule, or a portion of the modified RER which parallels GERL, surrounded by a GERL cisterna.

Examination of thin sections of tissues incubated for AcPase activity confirmed the presence of reaction product in small immature granules which were in continuity with GERL (Fig. 14). Large immature granules were usually unreactive. The Golgi saccules were also unreactive, except occasionally for the innermost saccule (Fig. 15).

Reaction product was also present in variable amounts along the basal and lateral cell membranes, and in the acinar lumina. The cytoplasm and nuclei frequently had a light sprinking of reaction product, but this was assumed to be nonspecific. No deposits were seen in sections incubated without substrate.

DISCUSSION

The results of our structural and cytochemical studies of the acinar secretory cells of the rat exorbital lacrimal gland, summarized in Fig. 16, indicate that GERL, a smooth-surfaced membranous structure located in the inner Golgi region, is intimately involved with the formation of secretory granules. GERL can be identified by its unique

FIGURES 3-6   Figs. 3-6 illustrate the distribution of reaction product of the secretory enzyme, peroxidase, in the Golgi region of lacrimal acinar cells incubated in DAB-H₂O₂ medium at pH 7.0.

FIGURE 3   Peroxidase reaction product is present in the nuclear envelope (*NE*), the rough endoplasmic reticulum (*ER*), vesicles (*V*) between the transitional *ER* and the Golgi apparatus, the Golgi saccules (*GS*), immature (*IG*), and mature secretory granules (*SG*). A slight increase occurs in the density of the reaction product in the Golgi saccules from the outer to the inner face. At the right center, a portion of GERL (arrowheads) is seen which is free of reaction product. At the upper left, another portion of GERL (arrowheads) contains only a small amount of reaction product at one end. Segments of modified RER containing dense reaction product (arrows) closely parallel GERL. The cytoplasmic staining around the mature granules is probably due to diffusion of enzyme. Mitochondria (*M*) are unreactive. × 35,700.

structural and cytochemical properties. It consists of cisternal and fenestrated or tubular portions, is frequently spatially separated from the Golgi sac-

cules, and is closely related to modified cisternae of the RER. Immature secretory granules are in continuity with GERL through multiple tubular
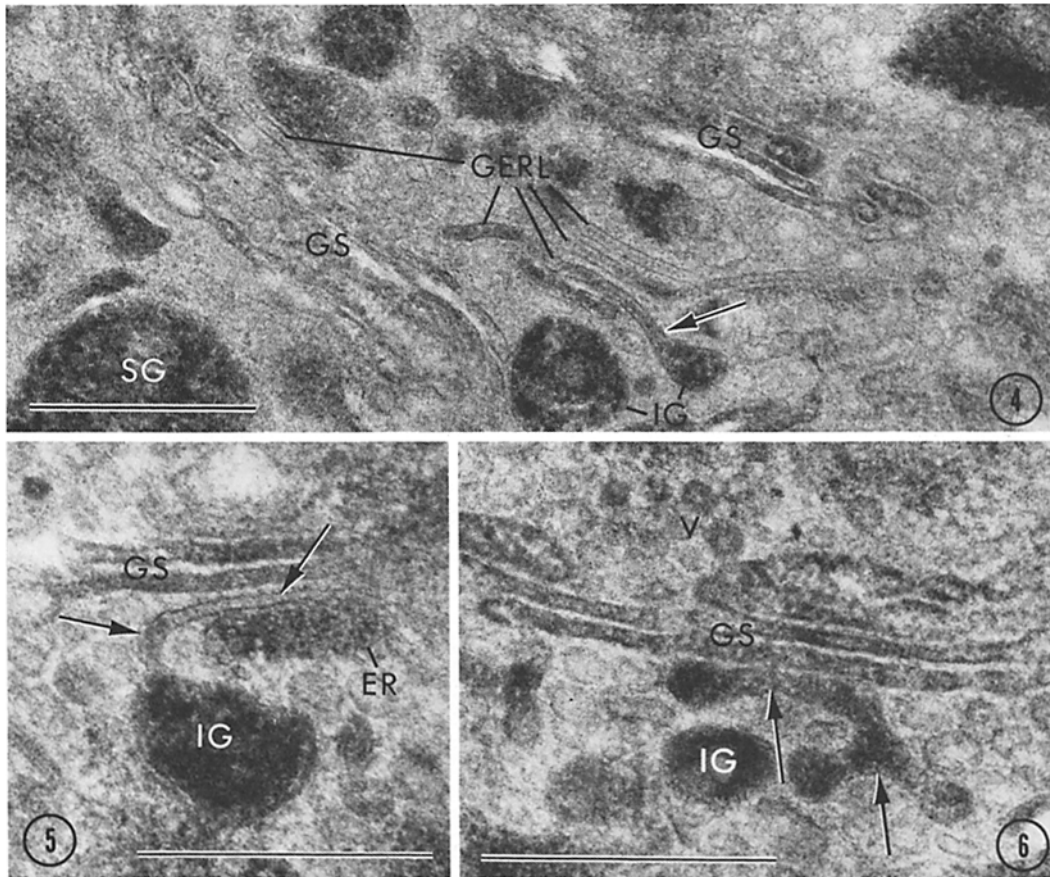
FIGURE 4   Peroxidase reaction product is present in Golgi saccules (*GS*), immature granules (*IG*), and mature secretory granules (*SG*). Several segments of GERL are seen, some of which are free of reaction product while others contain a variable amount. One portion of GERL (arrow) is continuous with a forming granule. × 59,800.
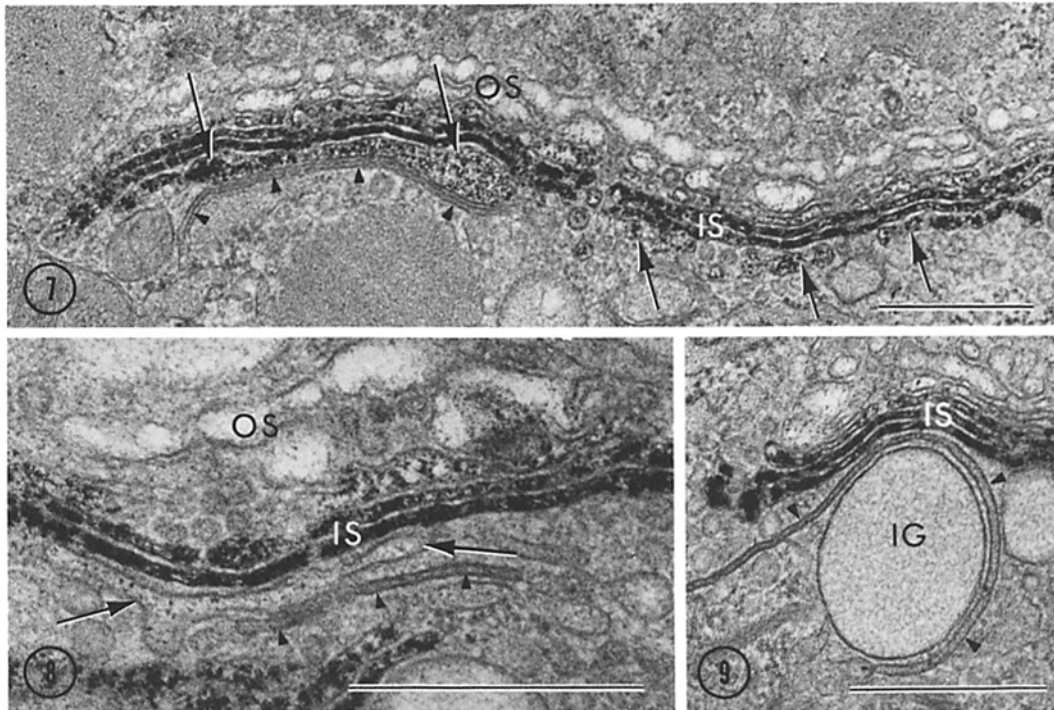
FIGURE 5   A small portion of the Golgi apparatus shows peroxidase reaction product in two of the saccules (*GS*) and the immature granule (*IG*). GERL (arrows), in this case adjacent to the inner saccule, is unreactive except for a small amount of reaction product near its juncture with the immature granule. A cisterna of modified RER closely parallels GERL. × 78,000.

FIGURE 6   The Golgi saccules (*GS*), a few vesicles (*V*) and an immature granule (*IG*) contain peroxidase reaction product. The innermost saccule (arrows), which is dilated and irregular, is strongly reactive. × 77,400.

connections. Reaction product of AcPase activity is localized in GERL and the immature granules forming from it, while TPPase reaction product is restricted to the inner Golgi saccules. Secretory proteins, as judged by the localization of peroxidase reaction product, are only infrequently present in GERL, despite its continuity with forming granules.

GERL was originally described in neurons by Holtzman et al. (29), Novikoff (49, 50, 51), and Novikoff et al. (65), where it is considered to be a specialized portion of smooth ER, continuous with the RER through smooth-surfaced tubules. Dense body-type lysosomes appear to be formed by the anastomosing tubular regions of GERL in these cells. Similar structural and/or functional properties have been described for GERL in hepatocytes (18, 43, 61, 64), epithelial cells of the vas deferens (20), megakaryocyts (6), alveolar macrophages (45), and lutein cells (68). Although its

FIGURES 7-9 Figs. 7-9 illustrate the distribution of reaction product of TPPase activity in the Golgi region of lacrimal acinar cells incubated for 2 h at pH 7.2.

FIGURE 7 TPPase reaction product fills two of the inner saccules (IS), is spotty in the next adjacent saccule, and is absent from the outer saccules (OS). The innermost saccule (arrows) contains a variable amount of reaction product. To the left of center the innermost saccule is dilated, while to the right it appears fenestrated. GERL (arrowheads), which lies adjacent to the innermost saccule, is unreactive. × 41,100.

FIGURE 8 A similar distribution of reaction product is seen in the Golgi saccules. The irregular innermost saccule (arrows) contains only light deposits, and the narrow cisterna of GERL (arrowheads) is unreactive. Inner saccules (IS); outer saccules (OS). × 78,800.

FIGURE 9 Heavy deposits of TPPase reaction product are present in three inner saccules (IS). A portion of GERL (arrowheads), which wraps around the immature granule (IG), is free of deposits. × 51,500.

basic structural and cytochemical characteristics are similar to those of GERL in neurons and other cell types, GERL in lacrimal acinar cells differs from the former in two important respects. First, GERL in lacrimal cells appears to form secretory granules, and has no obvious relationship to the lysosomal system. Morphologically recognizable secondary lysosomes are most frequently located peripheral to the Golgi apparatus or in the basal cytoplasm (cf. references 17 and 27). The counterpart of the primary lysosome in these cells has not yet been identified. In other cells, primary lysosomes are believed to be AcPase-positive, coated or smooth-surfaced vesicles located in the Golgi region (15, 20, 46, 57, 77). While similar vesicular profiles are observed in lacrimal acinar cells, it is possible that some of these may represent sections through the tubular portion of GERL. Second, it is not clear whether GERL in lacrimal acinar cells is in continuity with the ER; while occasional micrographs have suggested such connections, we have as yet been unable to verify their existence. However, GERL does exhibit a close topographical relationship with the ER, being closely paralleled by modified RER cisternae. The significance of this association is unknown.

GERL, or structures similar to GERL as we

have described it in lacrimal acinar cells, has now been observed in several different secretory cells, including those of the adrenal medulla (28), anterior pituitary (16, 71), supraoptic neurons (72), thyroid follicular cells (60), a transplantable insulinoma (63), pancreatic β-cells (52, 53) and exocrine cells (25, 52, 53, 59) and both serous and mucous salivary gland cells (9, 23, 24, 25). In most of these cells, GERL was closely related to forming secretory granules. Novikoff (52, 53) and Novikoff et al. (54, 59, 60, 63) have recently suggested that GERL of secretory cells functions as a processing and packaging system for secretory material. Proteins packaged by GERL could thus conceivably by delivered directly from the ER to secretory granules, by-passing the Golgi saccules. However, the lack of a suitable marker for secretory proteins in most of the cells previously studied precludes determination of their localization in the Golgi region.

We have taken advantage of the presence of the secretory protein peroxidase (27, 42) in the lacrimal acinar cells, utilizing it as a marker of the intracellular compartments containing secretory protein(s) and thus those compartments which are presumably involved in protein transport and processing. Although several studies have dealt with peroxidase localization in exocrine cells (2, 8, 13, 26, 34, 53, 80), including developing (27) and adult (17) rat exorbital lacrimal gland, the structure of the Golgi apparatus frequently was poorly preserved, and/or there was little or no peroxidase reaction product in the Golgi saccules. This has led some investigators to suggest that the Golgi saccules do not significantly contribute to the transport or condensation of peroxidase (26, 27). However, our results on peroxidase localization in lacrimal acinar cells suggest that the Golgi saccules in these cells do in fact participate in the transport and, possibly, concentration of secretory proteins. These cytochemical findings are similar to those reported for the localization of peroxidase and other enzymes in differentiating polymorphonuclear leukocytes, monocytes, and eosinophils (4, 5, 46, 66), the B apoprotein of plasma lipoproteins in hepatocytes (1), and exocrine secretory proteins in the bovine pancreas (35), and provide additional support for the general function of the Golgi saccules in secretory protein transport.

The apparent involvement of GERL of lacrimal acinar cells in secretory granule formation raises the question of how the secretory proteins reach the granules. Although kinetic evidence for se-
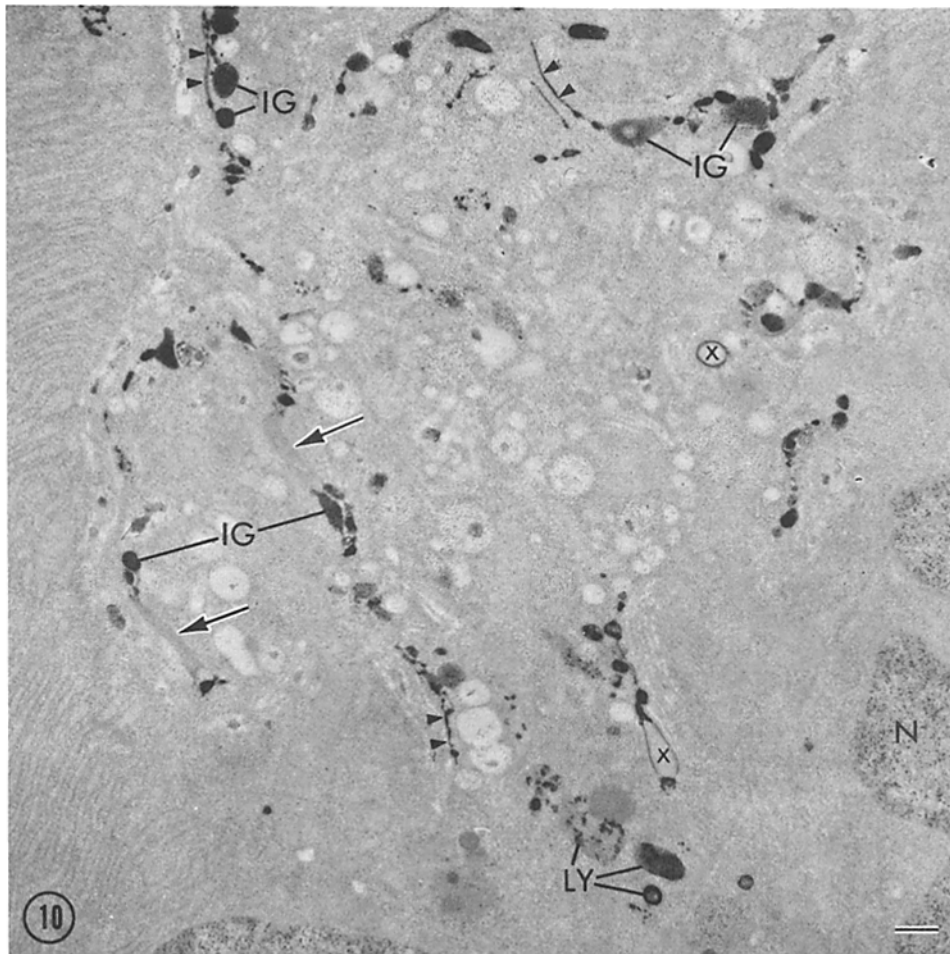
quential transport of protein in these cells is currently unavailable, on the basis of our morphological and cytochemical observations several possible routes can be considered: (a) direct transfer from the RER, through GERL. However, the presence of peroxidase reaction product in the Golgi saccules, and the lack of continuities between the ER and GERL suggest that this route is of little significance, at least for peroxidase; (b) transfer from the Golgi saccules to the forming granules through undetected connections or via vesicular transport, and reaction product in GERL is due to "backflow" of peroxidase from the granules; or (c) passage from the Golgi saccules through GERL and into the granules.

While (b) above cannot be ruled out at present, our observations tend to support the (c) mechanism. The lack of reaction product in GERL may be due to an effective concentration of peroxidase which is below the limit of detection in our cytochemical preparations. This could result from a rapid transport of peroxidase through GERL, or from a cytochemical artifact, such as complexing with other molecules, differential inhibition by the fixative, or inability of the constituents of the incubation medium to reach the enzyme. As an alternative hypothesis, protein transfer may be effected by conversion of the inner Golgi saccule to GERL. Granule formation may be initiated by the inner saccule, which then collapses, forcing its contents into the granules while the remainder of the saccule is converted to GERL. The dilatation and fenestration of the innermost saccule may represent a stage in conversion; furthermore, its cytochemical properties, i.e., weak to moderate deposits of AcPase and TPPase reaction product, are intermediate between those of the rest of the inner saccules and GERL. Additionally, since TPPase and NDPase are presumably membrane-bound, the occasional observation of reaction product of these enzymes in immature granules suggests that the granule membranes may initially have been part of the inner Golgi saccules. Conversion of the innermost saccule to GERL implies a turnover, or movement of saccules through the Golgi stack. This process has been suggested for a number of cells (14, 39, 44, 75, 81, 83), and is an essential part of membrane flow hypotheses regarding the conversion of intracellular membranes from ER-like to plasma membrane-like (22, 40, 41). However, arguments against the conversion of Golgi membranes to GERL have been presented by Novikoff et al. (65), and experimental

data on the composition, synthesis, and turnover of intracellular membranes of pancreatic exocrine cells obtained by Meldolesi (37) have been interpreted as contrary to the concept of membrane flow. The resolution of this problem obviously requires considerable additional effort.

An essential consideration in assessing the significance of the present results is whether or not GERL, as it exists in the lacrimal acinar cells, is in fact a separate and distinct entity. Obviously, it is closely related to the Golgi apparatus, and might simply be considered as the innermost saccule. However, its structural and cytochemical characteristics are substantially different from those of the Golgi saccule per se. For example, it is frequently separated from the stack of saccules, while

FIGURES 10-15   Figs. 10-15 demonstrate the distribution of AcPase reaction product in the Golgi region of acinar cells incubated for 1 h at pH 5.0 with CMP as substrate. Fig. 10-13 are unstained 0.2 μm sections observed at 100 kV.

FIGURE 10   At low magnification, the large size of the Golgi apparatus and extensive nature of the AcPase-positive elements of GERL can be seen. The thin dense profiles (arrowheads) are GERL cisternae sectioned perpendicularly, while the broader less dense areas (arrows) are GERL cisternae which lie more or less parallel to the section plane. The round to oval structures closely related to GERL are immature secretory granules (IG). A few lysosomes (LY) containing reaction product are present at the bottom of the micrograph. The two structures labeled x are interpreted as sections through cup-shaped portions of GERL which enclose an unreactive granule or modified cisterna of RER. Nucleus (N). × 11,300.
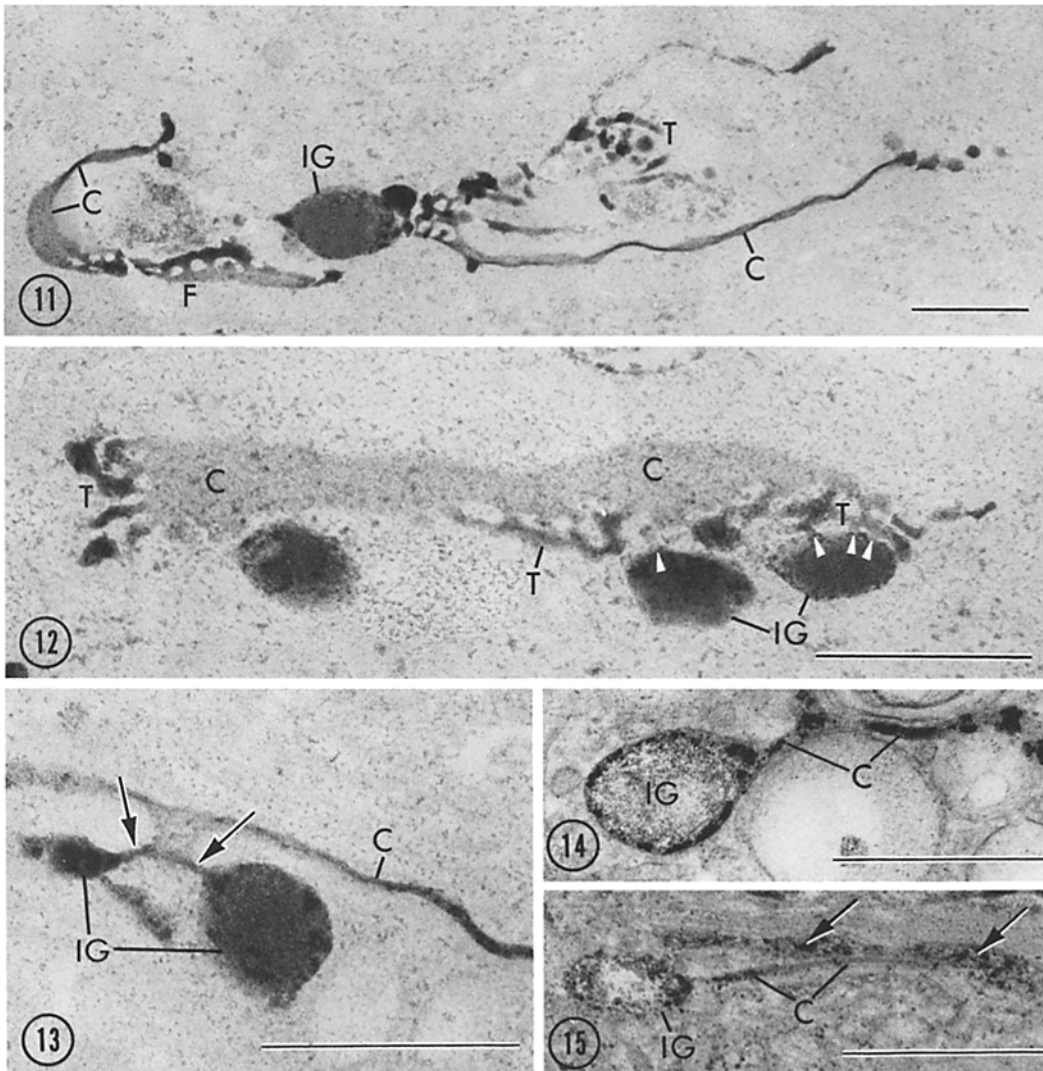
FIGURE 11   Fenestrated (F) and tubular (T) portions of GERL containing dense reaction product are continuous with cisternal portions (C). At the left, the cisternal portion curves to lie partially in the section plane. The immature granule (IG) is closely related to a region of branching or anastomosing tubules. × 30,700.

FIGURE 12   Tubular portions (T) of GERL are located at the periphery of a cisternal portion (C), which lies in the plane of the section. Immature granules (IG) appear continuous (arrowheads) with the peripheral tubular portions. × 56,800.

FIGURE 13   Immature granules (IG) are clearly continuous (arrows) with GERL. The cisternal portion (C) lies primarily perpendicular to the section plane. × 68,300.

FIGURE 14   The cisternal (C) portion of GERL is continuous with an immature granule (G) containing moderate deposits of reaction product. × 55,800.

FIGURE 15   Reaction product is present in an immature granule (IG), the cisternal (C) portion of GERL, and the innermost Golgi saccule (arrows). × 54,800.
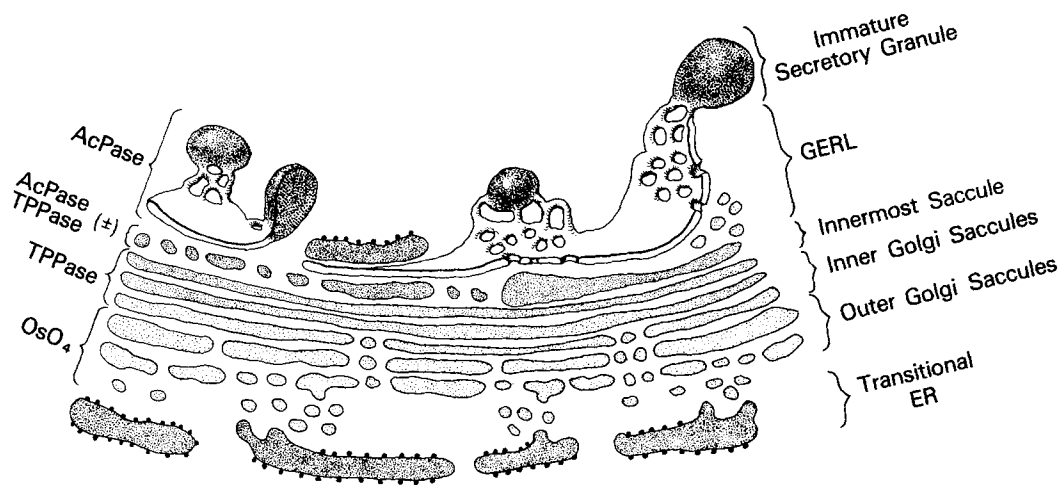
FIGURE 16 Diagrammatic representation of the morphological and cytochemical features of the Golgi apparatus and GERL of acinar cells of rat exorbital lacrimal gland. GERL is located in the inner Golgi region and consists of cisternal and fenestrated or tubular portions. Forming secretory granules are attached to the tubular portions of GERL. Secretory proteins, i.e., peroxidase (indicated by the stippling), are present in the RER, Golgi saccules, and immature granules, but usually not in GERL. GERL and the immature granules are reactive for AcPase, while the inner Golgi saccules are reactive for TPPase, and NDPase activities. The outer saccules and vesicles between the RER and Golgi apparatus contain dense deposits after osmium impregnation (our unpublished observations). Occasionally, the innermost Golgi saccule is dilated and fenestrated, with cytochemical properties intermediate between those of the remaining inner saccules and GERL.

separations between adjacent saccules have not been observed. It lacks TPPase activity, which in many cells is considered to be a cytochemical marker of the inner Golgi saccules (12, 19, 56, 58). Further, if GERL was actually the innermost saccule, one might reasonably expect it to contain a greater concentration of peroxidase than the rest of the Golgi saccules as it gives rise to the secretory granules. Admittedly, GERL in the lacrimal acinar cells does not fully meet the definition of GERL as formulated from the studies of neurons by Holtzman et al. (29), Novikoff (49, 50, 51), and Novikoff et al. (65). However, because of the diverse properties and functions of the various cells in which GERL has been observed, differences in its function and relationships can reasonably be expected. Therefore, we feel that GERL should be recognized as a distinct entity in lacrimal acinar cells.

In conclusion, cytochemical studies of the acinar secretory cells of the rat exorbital lacrimal gland indicate that GERL is intimately related to forming secretory granules. The secretory enzyme, peroxidase, is apparently transported and may be initially concentrated by the Golgi saccules; transfer of peroxidase to the immature secretory gran-

ules may involve conversion of the innermost Golgi saccule to GERL. The immature granules are in continuity with GERL through multiple tubular connections, and are strongly reactive for AcPase activity, a marker enzyme of GERL. These results, therefore, suggest that at least one function of GERL, and possibly the major function of it in exorbital lacrimal gland cells, is the formation of secretory granules.

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