# Endocytic Pathways at the Lateral and Basal Cell Surfaces of Exocrine Acinar Cells

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ABSTRACT    In parotid acinar cells, horseradish peroxidase (HRP) administered via the main excretory duct is endocytosed from the apical cell surface in smooth C- or ring-shaped vesicles (Oliver, C. and A. R. Hand. 1979. *J. Cell Biol.* 76:207). These vesicles ultimately fuse with lysosomes adjacent to the Golgi apparatus. The present investigation extends these findings and examines the uptake and fate of intravenously injected HRP from the lateral and basal cell surfaces of resting and stimulated parotid and pancreatic acinar cells from rats and mice. Isoproterenol and pilocarpine were used to stimulate the parotid gland and the pancreas, respectively. HRP was internalized in smooth and coated vesicles primarily in areas of membrane infoldings. Both the number of coated vesicles and the amount of tracer internalized increased markedly following secretagogue administration. In both resting and stimulated cells, the HRP was rapidly sequestered in a unique system of basally located lysosomes that possess trimetaphosphatase activity, but not acid phosphatase activity. At 1-3 h after HRP administration, reaction product was also found in multivesicular bodies, vesicles, and lysosomes adjacent to the Golgi apparatus. With time, more HRP was localized in Golgi-associated lysosomes. By 6-7 h, tubules in the apical cytoplasm of stimulated cells contained HRP reaction product. When native ferritin was administered retrogradely and HRP injected intravenously, both tracers could be localized in the same lysosome after 4-5 h, indicating that material taken in from all cell surfaces mixes in Golgi-associated lysosomes. The results of this study suggest that two separate and distinct endocytic pathways exist in exocrine acinar cells: one involves membrane retrieval from the apical cell surface; and the other is a stimulation-dependent process at the lateral and basal cell surfaces.

In secretory cells, membrane retrieval from the cell surface may aid the cells in maintaining a relatively constant size and shape (2, 19) despite the addition of considerable membrane to the surface during secretion, as well as providing a source of membrane for new secretory granule formation (13, 15, 23-25, 42). In cells as highly polarized as exocrine acinar cells, membrane retrieval could be restricted primarily to the apical cell surface, where secretion occurs and membrane insertion is most extensive. Previous studies employing electron-dense markers (13, 15, 29) have shown that membrane is internalized from the apical cell surface in small endocytic vesicles (see Fig. 4, reference 29). Intracellularly, this membrane may be degraded in lysosomes or recycled during secretory granule production. However, the uptake of membrane from the lateral and basal cell surfaces has not been thoroughly investigated. In the present investigation, the uptake and fate of intravenously

injected horseradish peroxidase (HRP) was followed in resting and stimulated pancreatic and parotid acinar cells from rats and mice. Endocytosis at the lateral and basal surfaces was distinct from that at the apical surface, with tracer being initially sequestered in a unique lysosomal system at the base of the cells. Ultimately, the HRP was localized in typical secondary lysosomes adjacent to the Golgi apparatus. Furthermore, endocytosis at the lateral and basal surfaces proved to be largely dependent on secretagogue administration.

## MATERIALS AND METHODS

Inc., Warrington, PA) into the main excretory duct of either the parotid gland or the pancreas as previously described (29). The maximum hydrostatic pressure was calculated to be 16 mm Hg. Intraperitoneal injections of isoproterenol (20 mg/kg b.wt) or pilocarpine (40 mg/kg b.wt) were used to stimulate secretion of parotid and pancreatic acinar cells, respectively. At varying time intervals, from 30 min to 24 h, after HRP administration, the tissue was fixed by vascular perfusion of fixative containing 2% glutaraldehyde (Ladd Research Industries, Burlington, VT), 2% formaldehyde (Ladd Research Industries), 0.025% calcium chloride in 0.1 M cacodylate buffer (pH 7.4). Following perfusion, the glands were removed, immersed in fresh fixative, and cut into 1 × 1 × 5 mm strips. After 1 h of fixation, the tissue was rinsed in 0.1 M cacodylate buffer (pH 7.4) containing 7% sucrose (sucrose buffer) and stored overnight in sucrose buffer at 4°C. The tissue was then chopped into 75-μm thick sections using a Smith-Farquhar TC-2 tissue sectioner (DuPont Instruments-Sorvall Biochemical Div., Dupont Co., Newtown, CT) and incubated either for 45 min at room temperature to demonstrate peroxidase activity (7) or for 40 minutes at 37°C for trimetaphosphatase activity (28). Controls for peroxidase activity included sections from glands that had not received HRP, but that were incubated in full medium, and sections from glands that had received HRP, but that were incubated in medium without hydrogen peroxide. For trimetaphosphatase activity, controls consisted of sections incubated without substrate or incubated in the presence of $10^{-4}$ M zinc acetate. Reaction product was not observed in any of the control sections for either peroxidase or TMPase. In animals that received both HRP and ferritin, during chopping sections were divided into two groups, one section being incubated in full medium and the adjacent section processed without incubation for peroxidase activity. Additionally, incubated and unincubated sections of glands from animals that had received HRP, but not ferritin, were examined for the presence of endogenous ferritinlike particles. Although lysosomes in these sections did contain small particles, they were easily distinguished from the native ferritin on the basis of their size and density. After incubation, the sections incubated for trimetaphosphatase activity were rinsed in 1% sodium sulfide in sucrose buffer in order to visualize the reaction product for light microscopy. All the sections were then postfixed for 1 h at room temperature in 1% osmium tetroxide in sucrose buffer, or in 1% osmium tetroxide, 1.5% potassium ferrocyanide (20) in distilled water or sucrose buffer. The tissue was dehydrated through a graded series of ethanols and propylene oxide and embedded in Spurr's resin (34). The distribution of reaction product in the tissue was assessed in 1-μm thick sections. Thin sections of selected areas were cut with a diamond knife, mounted on bare copper grids, and examined in a JEM 100C electron microscope either unstained or lightly stained with Reynold's lead citrate (32) and/or uranyl acetate.

## RESULTS

The fate of the intravenously injected HRP was examined in parotid and pancreatic acinar cells from rats and mice. With the exception of minor morphological differences, tracer uptake was the same for both cell types whether from rats or mice. The most striking differences were observed between resting and stimulated acinar cells. Compared to resting cells (Fig. 1), significantly more HRP was internalized by the stimulated cells (Fig. 2). Despite this difference in the amount of tracer internalized, the basic endocytic pathway appeared to be the same in both resting and stimulated cells. Therefore the following description applies to both resting and stimulated cells unless otherwise stated. HRP was endocytosed primarily from areas of plasma membrane infolding (Fig. 3). The HRP was frequently localized in coated pits and vesicles, which were more numerous in stimulated cells (Fig. 4a and b). Once internalized, the tracer rapidly gained access to a basally located system of anastomosing tubules, 200–220 nm in diameter (Fig. 5), which were interposed between the cisternae of the rough endoplasmic reticulum, often in close association with cytoplasmic microtubules and mitochondria (Fig. 5c). These tubules have been shown to be part of a unique lysosomal system
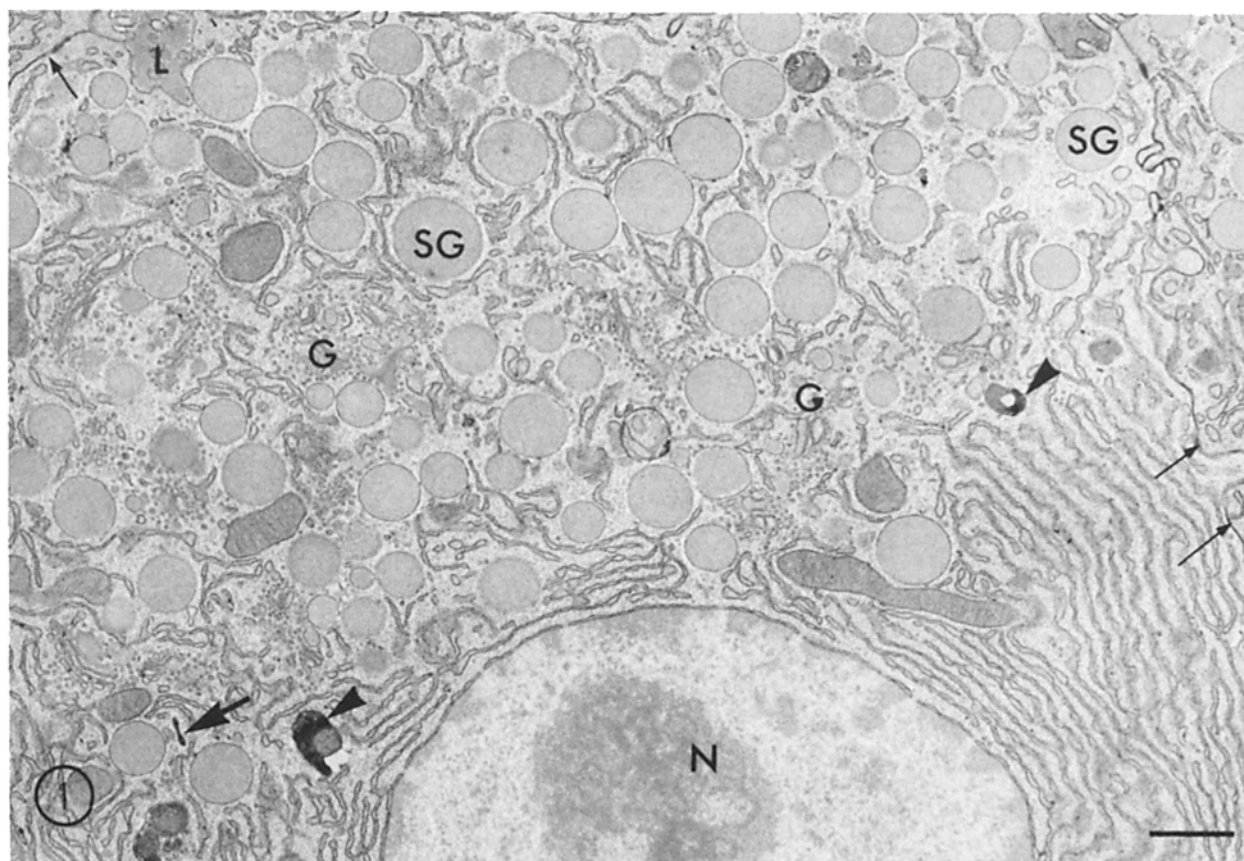


FIGURE 1   Resting-mouse pancreas. 3 h after HRP. Little HRP has been internalized. Reaction product is present in lysosomes (arrowheads) and a tubule (arrow) near the Golgi apparatus (G). (L, lumen; N, nucleus; SG, secretory granule; small arrows, extracellular space). Bar, 1 μm. × 11,000.
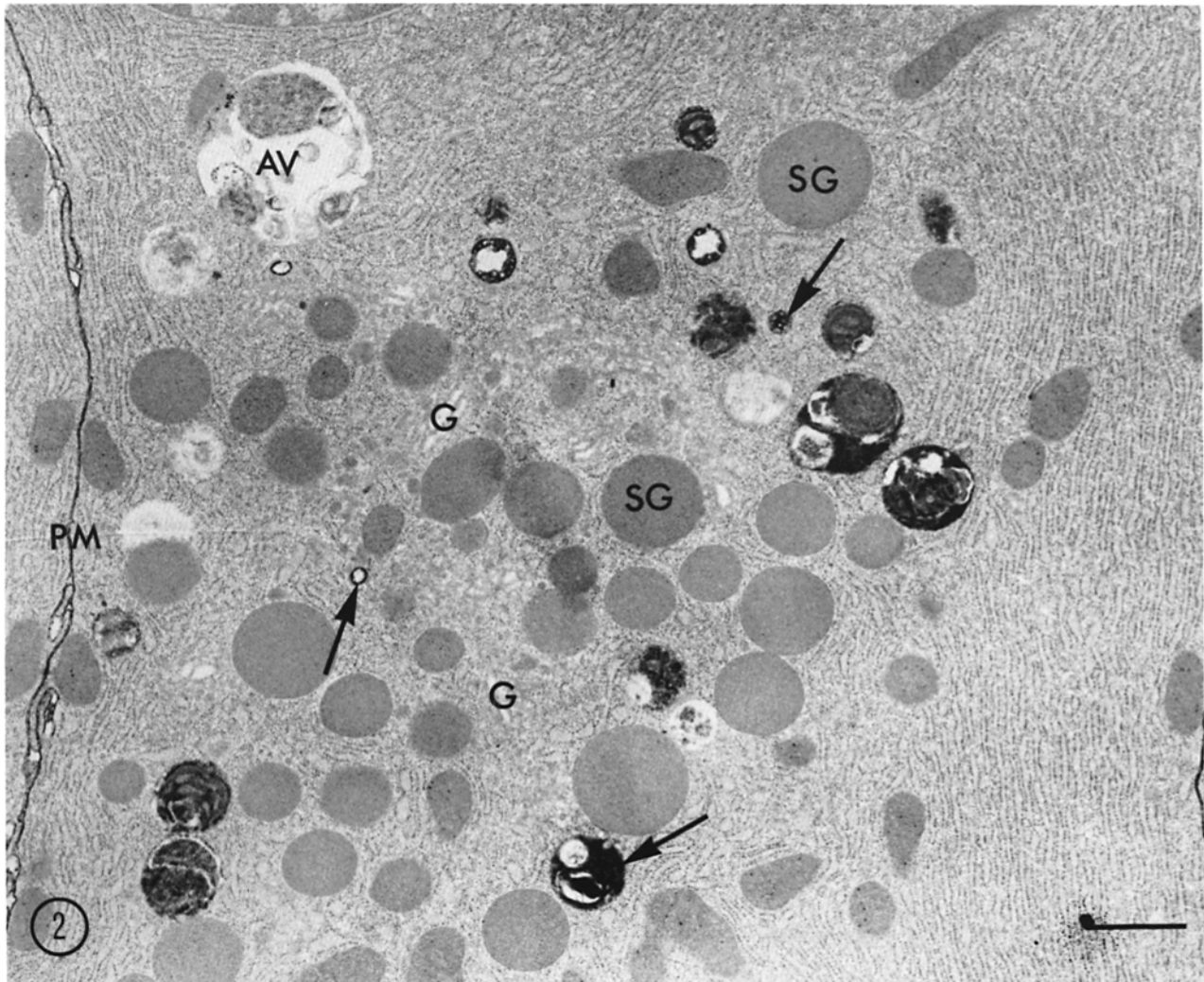
FIGURE 2  Stimulated mouse pancreas. 3 h after HRP and pilocarpine. Compared to the resting gland (Fig. 1), more peroxidase (arrows) is present in Golgi-associated vesicles, dense bodies, and autophagic vacuoles (*PM*, plasma membrane; *G*, Golgi apparatus; *SG*, secretory granule; note the unreactive autophagic vaucole (*AV*). Bar, 1 μm. × 14,000.
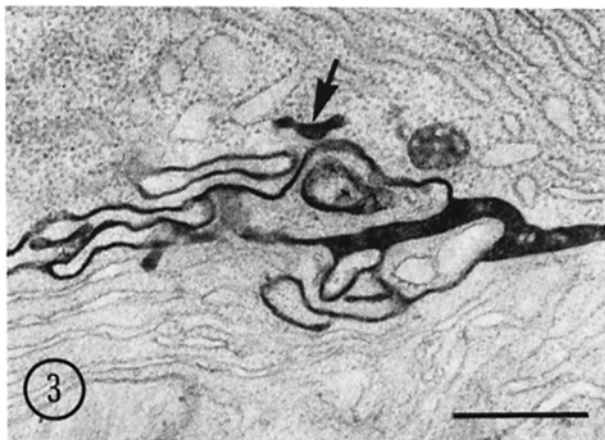


FIGURE 3  Resting mouse pancreas. 3 h after HRP. Reaction product is localized in infoldings of the plasma membrane, elongated tubules (arrow), and a multivesicular body. Bar, 0.5 μm. × 37,000.
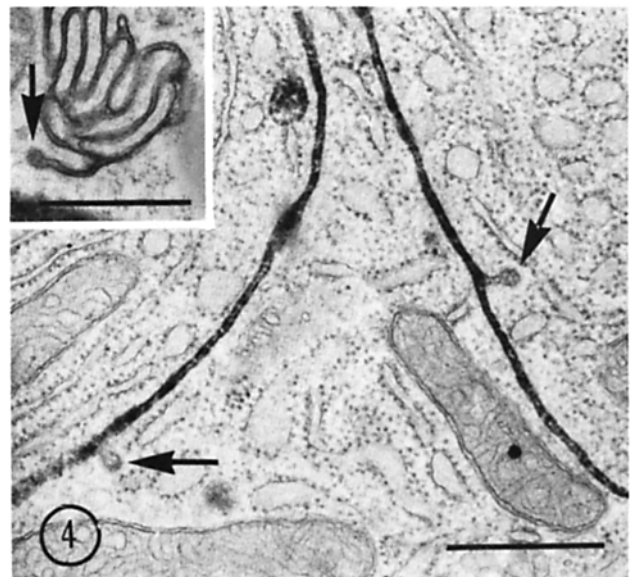


FIGURE 4  Stimulated rat pancreas. 1 h after HRP and pilocarpine. HRP is internalized in coated vesicles (arrows) which arise from the plasma membrane, infolding (inset). Bar, 0.5 μm. × 40,000.

that possesses trimetaphosphatase activity (Fig. 5*d* and *e*), but not acid phosphatase activity (27, 28). At 1–3 h after injection, tracer was also localized in multivesicular bodies adjacent to the plasma membrane (Fig. 6*a*) and in multivesicular bodies
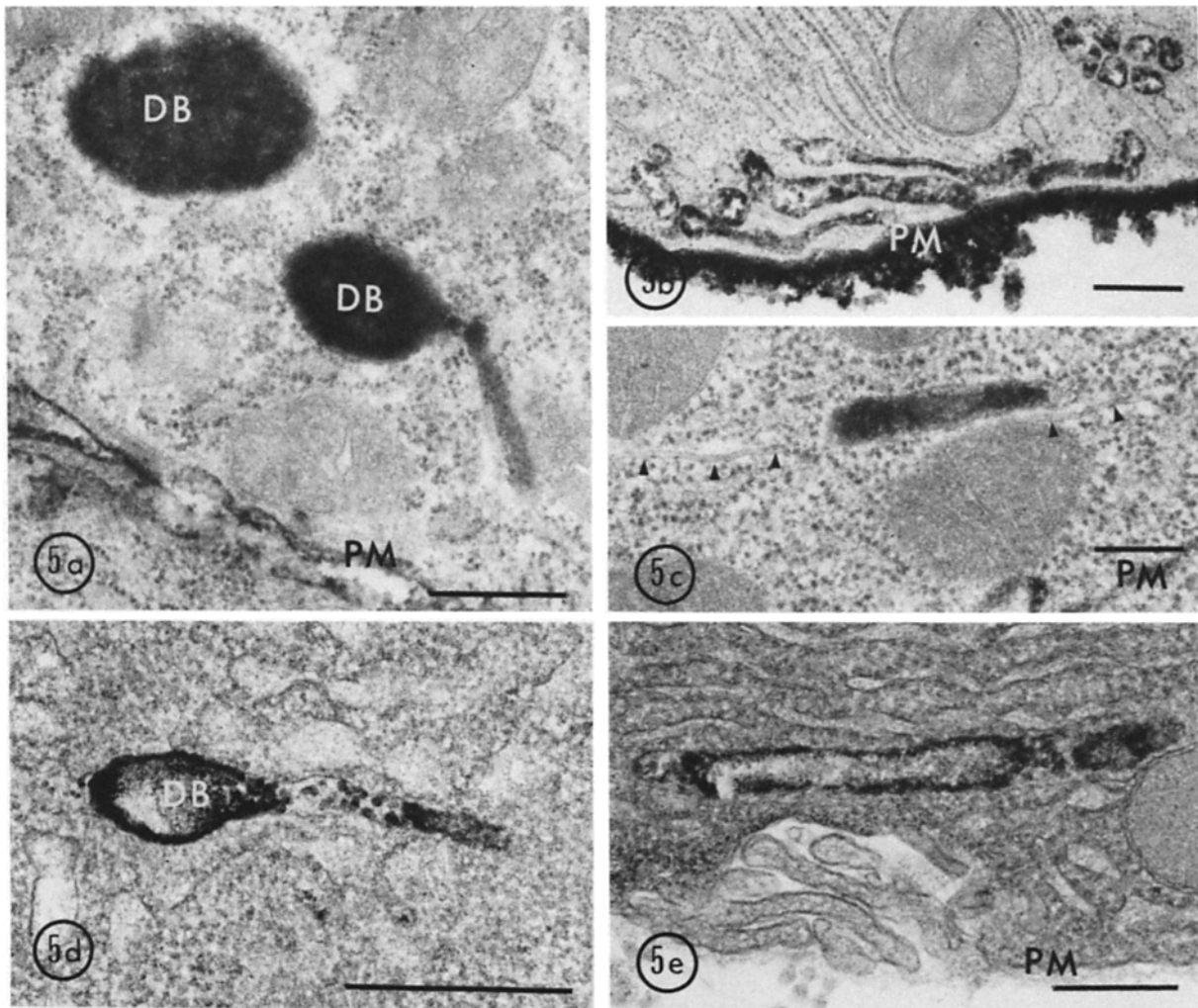
FIGURE 5 Stimulated rat parotid. 6 h after HRP and isoproterenol. (a) HRP reaction product is located in dense bodies (DB) and an associated elongated tubule. (PM, plasma membrane). × 37,000. (b and c) Stimulated rat pancreas. 1 h after HRP and pilocarpine. (b) Elongated cisternae near the basal cell surface contain HRP reaction product. (PM, plasma membrane). × 24,000. (c) An elongated tubule with HRP reaction product lies parallel to cisternae of the rough endoplasmic reticulum and is in close association with cytoplasmic microtubules (arrowheads). (PM, plasma membrane). × 33,000. (d and e) TMPase reaction product is localized in a dense body (DB) connected to an elongated lysosome adjacent to plasma membrane (PM) infoldings. (d) Resting rat parotid. × 60,000; (e) Resting rat pancreas. × 33,000. (a) Stimulated rat parotid. 6 h after HRP and isoproterenol Bars, 0.5 μm.

and vesicles near the Golgi apparatus (Fig. 6 b and c). With time, more HRP accumulated in multivesicular bodies, vesicles, and lysosomes adjacent to the Golgi apparatus (Fig. 7 a), as well as in secretagogue-induced autophagic vacuoles (26, 33). Tubules containing HRP reaction product were occasionally seen near the cis face of the Golgi apparatus (Fig. 7, inset). By 6–7 h after HRP administration, vesicles and tubules in the apical cytoplasm of stimulated cells also contained HRP reaction product (Fig. 8 a and c). However, no evidence of extracellular discharge at the apical cell surface was observed. C- and ring-shaped apical vesicles (29) without reaction product were also present (Fig. 8 a). However, if HRP is administered through the main excretory duct, reaction product is present in these apical vesicles (Fig. 8 b; Fig. 4, reference 29). At no time was HRP reaction product found in Golgi saccules, GERL, immature or mature secretory granules.

The fate of material endocytosed from the apical cell surface with that internalized at the lateral and basal cell surfaces, was compared in animals receiving native ferritin via retrograde ductal administration and HRP by intravenous injection. Im-

mediately following tracer administration, the animals received an IP injection of secretagogue. The ability of the apical junctional complexes to confine the ferritin in the lumen was assessed by examining the lateral and basal extracellular spaces for the presence of ferritin. Because of the possibility that the peroxidase reaction product might obscure individual ferritin particles, both incubated and adjacent unincubated sections were examined. Under the experimental conditions employed, the ferritin remained restricted to the lumen (Fig. 10). By 3–4 h following such treatment both tracers could be localized in the same lysosome near the Golgi apparatus (Fig. 9), thus indicating that mixing of material brought in from all cell surfaces did occur.

## DISCUSSION

The results presented here show that HRP is endocytosed from the lateral and basal cell surfaces primarily in coated vesicles, and that the tracer rapidly gains access to a system of basally located lysosomes. Reaction product is also found in vesicles and multivesicular bodies adjacent to the plasma membrane
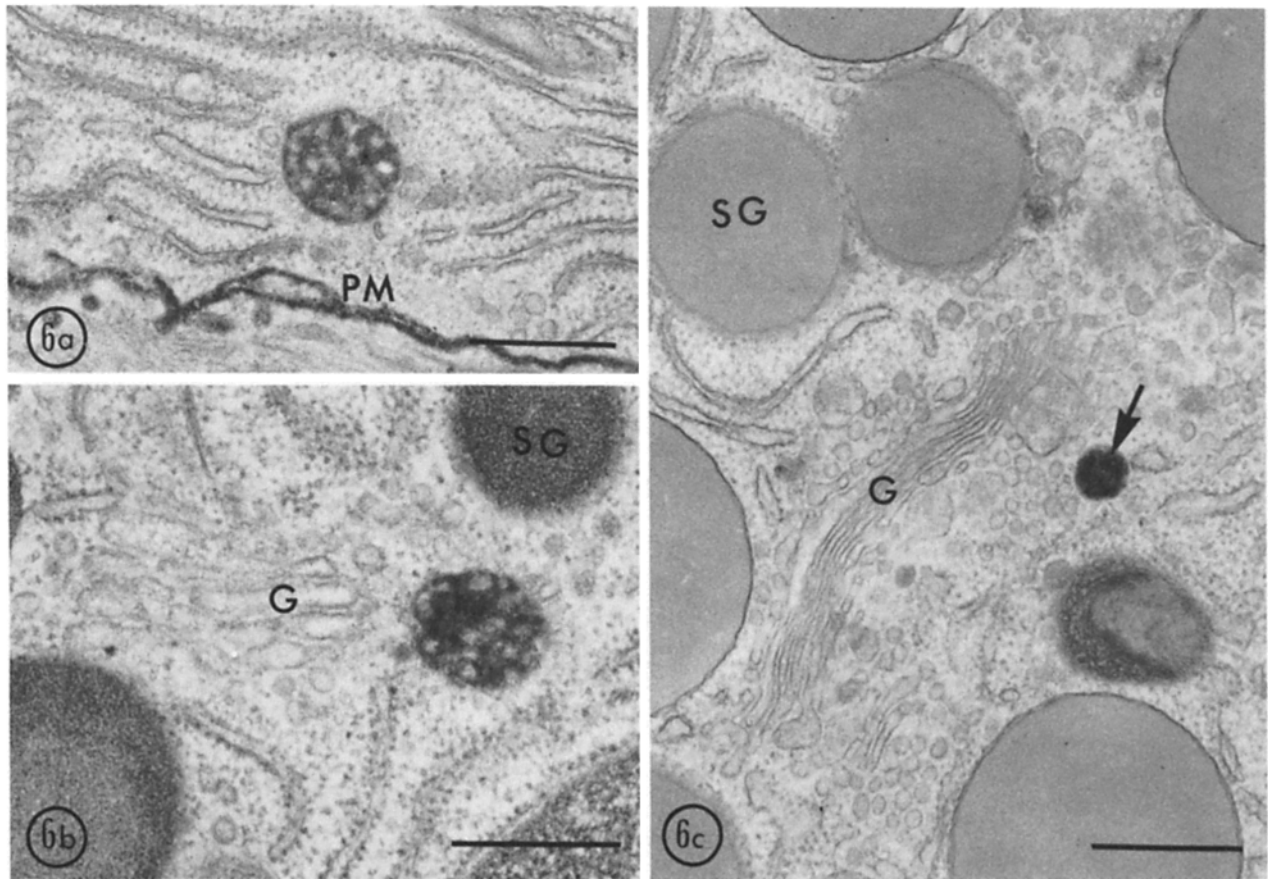
FIGURE 6    (a) Resting rat parotid. 30 min after HRP. Reaction product is localized in a multivesicular body adjacent to the plasma membrane (PM). X 40,000. (b) Rat parotid. 30 min after HRP. A multivesicular body near the Golgi apparatus (G) contains reaction product. (SG, secretory granule). X 45,000. (c) Resting rat pancreas. 30 min after HRP. A vesicle (arrow) near the Golgi apparatus (G) contains reaction product. (SG, secretory granule). X 40,000. Bars, 0.5 μm.

and to the Golgi apparatus. At later times, HRP accumulates in lysosomes near the Golgi apparatus as well as in apically located tubules and vesicles. Additionally, significantly more tracer is internalized following secretagogue administration.

The initial pattern of sequestration of HRP from the lateral and basal cell surfaces is in contrast to that seen at the apical cell surface of pancreatic and parotid acinar cells (13, 15, 29). At the luminal cell surface, although coated vesicules may participate in endocytosis of HRP, especially in resting pancreatic acinar cells, the majority of the HRP and associated membrane appears to be retrieved as smooth-surfaced apical vesicles (2, 19, 29). Once internalized, these vesicles ultimately fuse with lysosomes adjacent to the Golgi apparatus. Tracer internalized from the lateral and basal surfaces is initially taken up by coated vesicles and rapidly localized in a system of basal tubular lysosomes. Apically administered tracer does not gain access to these basal lysosomes, although, as shown in this study, mixing of tracer brought in from the apical cell surface with that endocytosed from the lateral and basal surfaces may occur in lysosomes adjacent to the Golgi apparatus. The manner in which material endocytosed from the lateral and basal cell surface gains access to the Golgi-associated lysosomes is unclear at present. The basal lysosomes may migrate toward the Golgi apparatus and fuse completely with the Golgi-associated lysosomes or the basal lysosomes may be connected to the Golgi-associated lysosomes by tubular extensions. It is also possible that some material is delivered directly to the Golgi-associated lysosomes and bypasses the basal lysosomes entirely.

Furthermore, the uptake of HRP from the lateral and basal cell surfaces appears to be markedly dependent on secretagogue administration. These results suggest that there are two distinct endocytic pathways in exocrine acinar cells, one involving the apical surface and the other, the lateral and basal surfaces.

Lysosomes with the morphological (28), cytochemical (27), and functional characteristics of the basal lysosomes seen here, have not been identified in other cell types. Their position in the cell, the rapid appearance of HRP in these lysosomes following endocytosis in coated vesicles as well as the increased uptake of tracer noted after secretagogue administration suggests that the basal lysosomes may play a role in sequestration of receptor-ligand complexes. These lysosomes may be somewhat analogous to the receptosome in fibroblasts (43) and the peripheral tubules and vesicles in hepatocytes (36, 41), and may serve as the initial repository for internalized receptor-ligand complexes. Besides having some morphological and functional similarities with the receptosome and peripheral tubules, and basal lysosomes in concert with these other structures, also lack cytochemically demonstrable acid phosphatase. The possible role of these basal lysosomes in receptor-ligand processing is currently under investigation.

The fate of the HRP in this study is in agreement with most studies employing soluble phase markers (1, 3, 4, 8, 10, 11, 14–17, 19, 30, 35, 37, 38, 44). As in the other investigations, the tracer is eventually localized in Golgi-associated lysosomes and is not found in Golgi saccules, GERL, immature or mature secretory granules. This indicates that the incoming vesicles
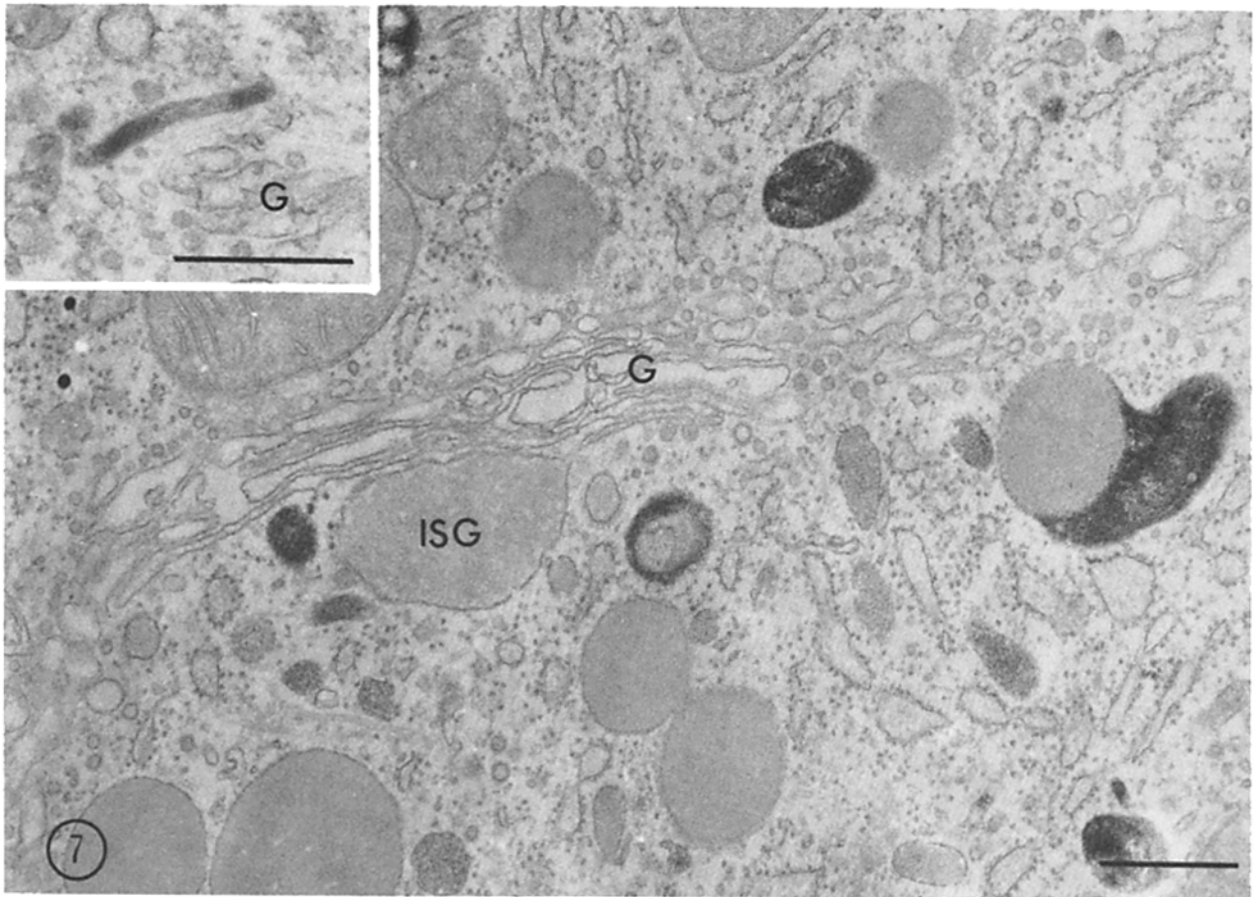
FIGURE 7  Stimulated rat pancreas. 4 h after HRP and pilocarpine. HRP reaction product is localized in lysosomes, vesicles, and an occasional tubule (inset) adjacent to the cis Golgi saccules (G). (ISG, immature secretory granule). Bars, 0.5 μm. × 38,000, inset × 50,000.
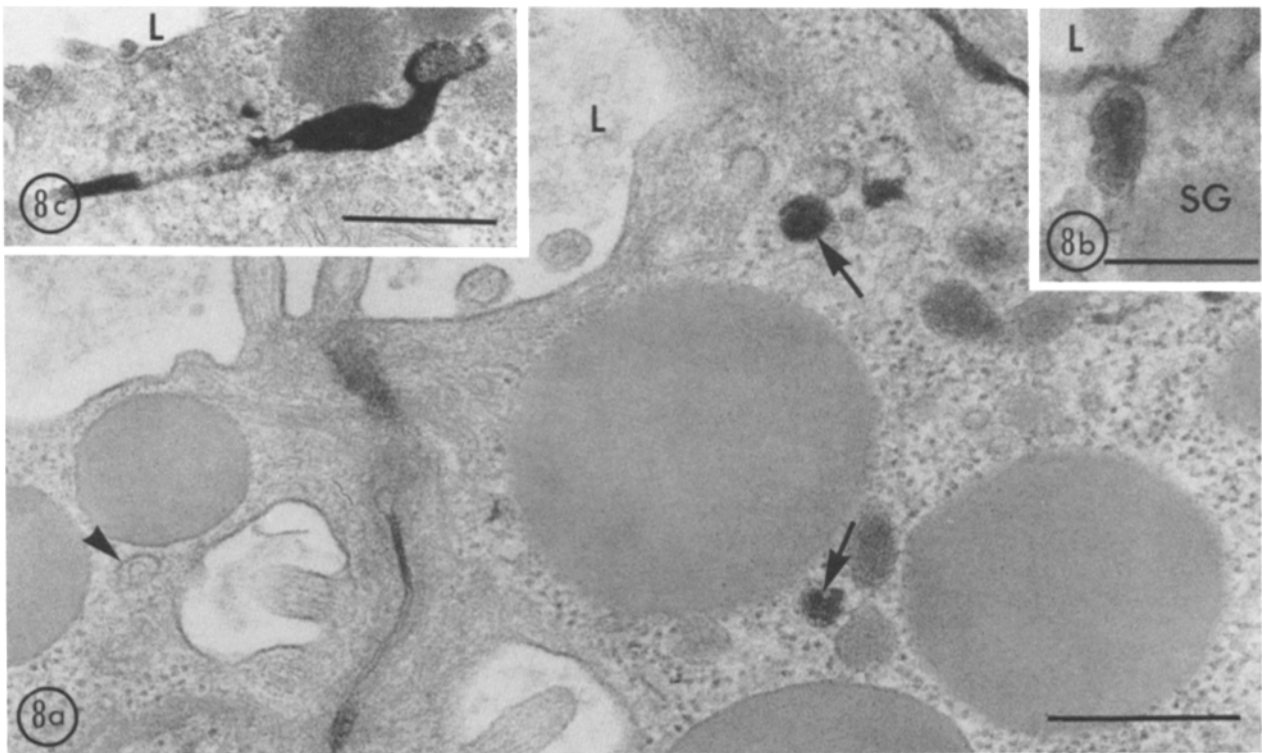


FIGURE 8  (a) Stimulated rat pancreas. 6 h after HRP and pilocarpine. Vesicles (arrows) in the apical cytoplasm contain HRP reaction product. An unreactive c-shaped vesicle (arrowhead) is also present. (L, lumen). × 50,000. Bar, 0.5 μm. (b) Stimulated rat pancreas. 2.5 h after ductal administration of HRP. A forming apical vesicle contains HRP. (L, lumen, SG, secretory granule). × 80,000. Bar, 0.25 μm. (c) Stimulated rat parotid. 6 h after HRP and isoproterenol. An elongated tubule adjacent to the lumen (L) contains reaction product. × 40,000. Bar, 0.5 μm.

FIGURE 9   Stimulated rat pancreas. 4 h after ductal administration of native ferritin, intravenous injection of HRP and intraperitoneal injection of pilocarpine. Both the ferritin (arrows) and HRP are localized in the same lysosome adjacent to the Golgi apparatus (G). Bar, 0.5 μm. × 50,000.
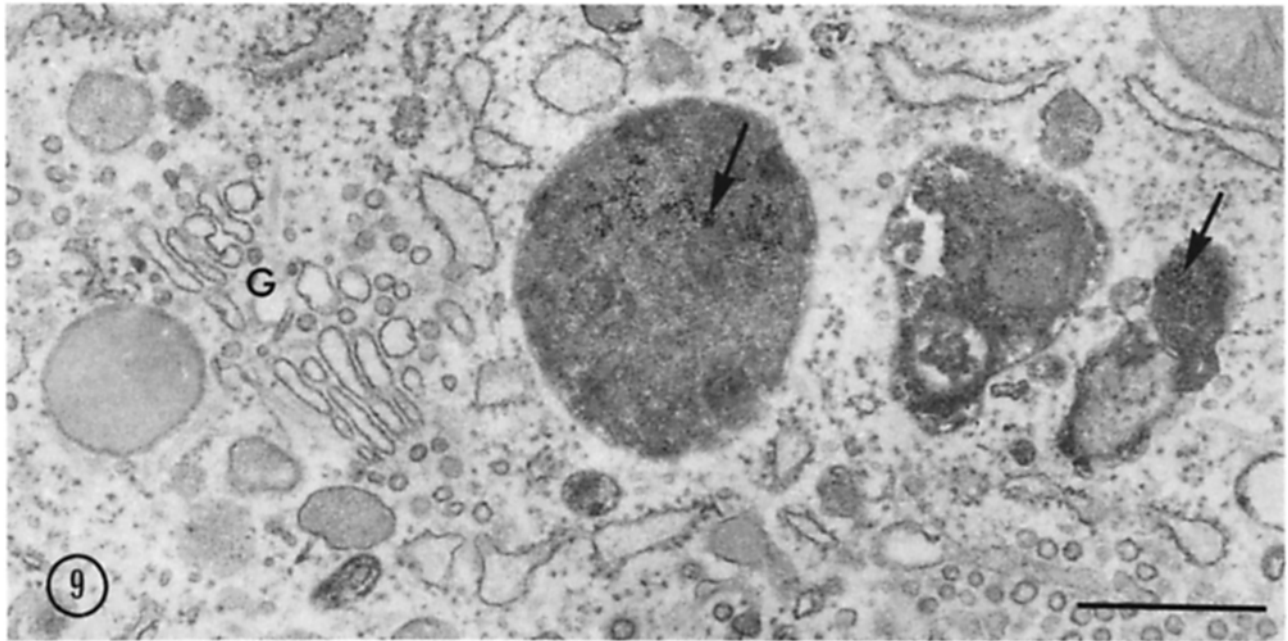


FIGURE 10   Stimulated rat pancreas. 4 h after ductal administration of native ferritin, intravenous injection of HRP, and intraperitoneal injection of pilocarpine. Control section not incubated for peroxidase activity. Ferritin (arrowheads) is restricted to the lumen (L). No ferritin is present in the intracellular spaces (arrow). Bar, 0.5 μm. × 88,000.

fuse with lysosomes rather than other cellular organelles. Because the soluble phase markers label content, not the membrane itself, it is not possible to say with certainty whether the vesicle membrane is degraded by the lysosomes or whether the vesicles discharge their contents into lysosomes and then go on to be reutilized in formation of new secretory granules. In a few cases, such as cells of the small intestine (5), seminal vesicles (9, 21, 22), and somatotrophs (8, 31, 37) of the anterior pituitary, endocytosed HRP has been localized in Golgi saccules, GERL, and secretory granules, suggesting that in some cell types, there may be direct reutilization of membrane. Endocytosis of cationized ferritin (8, 13–15, 30, 39, 40), ricin-HRP (12) and cholera toxin-HRP (18) complexes, tracers known to bind to plasma membranes, have also provided evidence for the direct reutilization of membrane. The reasons for the differences in localizations of the various tracers is not clear. The finding of HRP at sites other than lysosomes in a few cell types may be due to basic physiologic differences among cell types, whereas the localization of membrane-bound tracers in Golgi saccules, GERL, and secretory granules may represent the fate of the endocytic vesicle membrane as opposed to its contents. Conversely, the differences in localization of soluble phase vs. membrane-bound markers could reflect a perturbation of the membrane on the part of the bound markers. It is not known how these markers affect the membrane or whether by binding, they may alter the fate of the internalized membrane in a manner similar to concanavalin-A (6).

Although the present study is the first to demonstrate that a cell may internalize the same tracer by more than one pathway, previous studies employing multiple tracers (8, 13, 15, 30) have shown that a cell may possess more than one endocytic pathway. Uptake of different tracers by different endocytic mechanisms may provide an explanation for the differences in localization between HRP and dextran in pancreatic (15) and parotid acinar cells (13) as well as between native and cationized ferritin in mammotrophs (8) and thyroid follicular cells (14). The uptake of HRP from the lateral and basal cell surfaces observed in this study may be a combination of fluid phase pinocytosis in smooth-surfaced vesicles and receptor-mediated endocytosis, with the HRP either being passively carried in by

the coated vesicles or being bound to the plasma membrane by its carbohydrate moieties. Because both the number of coated vesicles and the amount of HRP internalized increase dramatically following secretagogue administration, the major pathway at the lateral and basal cell surfaces in stimulated cells is probably receptor-mediated. The endocytosis of membrane seen at the apical cell surface may then be primarily involved with secretory granule membrane retrieval and recycling.

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