

Alteration of Tight Junctional Permeability in the Rat Parotid Gland after Isoproterenol Stimulation

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ABSTRACT The permeability of junctional complexes to ultrastructural tracers of different molecular weight and the freeze-fracture appearance of junctional structure were investigated in the resting and stimulated rat parotid gland. Tracers were administered retrogradely via the main excretory duct, and allowed to flow by gravity (16 mmHg) into the gland for 15–60 min. Secretion was induced in some animals by intraperitoneal injection of isoproterenol. In resting glands, the tracers microperoxidase, cytochrome *c*, myoglobin, tyrosinase (subunits), and hemoglobin were restricted to the luminal space of the acini and ducts. In glands stimulated 1–4 h before tracer administration, reaction product for microperoxidase, cytochrome *c*, myoglobin, and tyrosinase was found in the intercellular and interstitial spaces, whereas hemoglobin was usually retained in the lumina. In contrast, horseradish peroxidase and lactoperoxidase appeared to penetrate the tight junctions and reaction product was localized in the extracellular spaces in both resting and stimulated glands. Diffuse cytoplasmic staining for horseradish peroxidase and lactoperoxidase was frequently observed in acinar and duct cells. The distribution of horseradish peroxidase was similar in both Sprague-Dawley and Wistar-Furth rats, and at concentrations of 0.1–10 mg/ml in the tracer solution. Freeze-fracture replicas of stimulated acinar cells revealed an increased irregularity of the tight junction meshwork, but no obvious gaps or discontinuities were observed. These findings indicate that (a) tight junctions in the resting rat parotid gland are impermeable to tracers of molecular weight $\geq 1,900$; (b) stimulation with isoproterenol results in a transient increase in junctional permeability allowing passage of tracers of molecular weight $\leq 34,500$; (c) junctional permeability cannot be directly correlated with junctional structure; and (d) the behavior of horseradish peroxidase and lactoperoxidase in the rat parotid gland is inconsistent with their molecular weights. Cell membrane damage due to the enzymatic activity or binding of these two tracers may account for the observed distribution.

Regulation of the local environment at various body surfaces is an important homeostatic function of epithelial tissues. The ability of epithelial cells to perform this function depends in part upon the integrity of the barrier they form between the surface and the underlying connective tissue. Intercellular junctions play an important role in the maintenance of this barrier. In glandular tissues a junctional complex consisting of a tight junction (zonula occludens), adhering junction (zonula adherens), and one or more desmosomes (maculae adherens), joins adjacent cells and separates the luminal space from the intercellular and interstitial spaces (1). The tight junction, a continuous, belt-like structure appearing in thin

sections as one or more fusions of adjacent cell membranes (1, 2) and in freeze fracture replicas as a system of anastomosing ridges and grooves (2, 3), has been identified as the anatomic structure that limits epithelial permeability through the intercellular spaces.

Previous studies of mammalian salivary glands have suggested that under certain conditions the tight junctions may become permeable to various organic substances (4–7). This altered permeability might be expected to have significant physiological, pharmacological, and pathological consequences. We undertook the present study to further characterize the permeability of the junctional complexes in the rat

parotid gland. Tracers of various molecular weights were administered by retrograde infusion in both resting glands and glands stimulated by the β -adrenergic agonist isoproterenol, and localized by ultrastructural cytochemistry. Freeze-fracture electron microscopy was employed to examine potential alterations in the structure of tight junctions. The results indicate the tight junctions are normally impermeable to tracers $\geq 1,900$ daltons, but that permeability increases markedly after secretory stimulation with isoproterenol, without concomitant changes in junctional structure.

MATERIALS AND METHODS

Animals: Male Sprague-Dawley and Wistar-Furth rats weighing between 250 and 450 g were used. Secretion was stimulated in some animals by (\pm)-isoproterenol (Sigma Chemical Co., St. Louis, MO; 30 mg/kg, intraperitoneally) 1–24 h before cannulation of the duct. Anesthesia was obtained with chloral hydrate (400 mg/kg body weight) administered intraperitoneally and a patent airway was maintained by a tracheostomy tube.

Tracer Administration: The tracers were dissolved in normal saline and placed in a syringe suspended at a height of 20 cm above the animal. The main excretory duct of one parotid gland was cannulated extraorally using a 30 gauge needle that was connected by polyethylene tubing (PE-10) to the syringe containing the tracer solution. The tracer was allowed to flow by gravity (~ 16 mmHg) into the gland for 15 min–1 h. The various tracers employed and the cytochemical incubation conditions utilized are described in Table I.

Tissue Preparation for Cytochemistry: The animals were sacrificed immediately after tracer administration by vascular perfusion with a modified Karnovsky fixative (13) containing 2% glutaraldehyde (Ladd Research Industries, Burlington, VT) and 2% formaldehyde (Ladd Research Industries) in 0.1 M sodium phosphate buffer, pH 7.4. After perfusion for ~ 10 min the parotid glands were removed, immersed in fresh fixative for a total of 1–2 h, then rinsed in 0.1 M sodium phosphate buffer, pH 7.4, containing 5% sucrose, cut into strips $1 \times 1 \times 5$ mm, and stored in buffer overnight at 4°C. The tissue was then chopped into 75- μ m sections on a Sorvall TC-2 tissue sectioner and incubated in the appropriate cytochemical medium to demonstrate the tracers (Table I). Controls included sections from the contralateral glands that had not received tracer, but were incubated in the complete cytochemical medium. After two rinses in sodium phosphate buffer, the sections were postfixed in 1% osmium tetroxide in Veronal acetate buffer, pH 7.4, rinsed in distilled water and stained en bloc with 0.5% uranyl acetate in distilled water, dehydrated in ethanol, and embedded in Spurr's resin (14). Thick (1 μ m) sections of embedded tissue, unstained or stained with toluidine blue, were examined by light microscopy. Thin sections of selected areas were cut with a diamond knife and mounted on bare copper grids. The sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM-10A electron microscope.

Tissue Preparation for Freeze-Fracture: Parotid glands of control or isoproterenol-stimulated animals were fixed by vascular perfusion with 2% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, with 0.05% CaCl_2 . After fixation and a brief rinse in buffer small pieces of tissues were infiltrated in 30% glycerin, frozen in Freon 22 at liquid nitrogen temperature, and freeze-fractured and platinum-carbon shadowed in a Balzers freeze-fracture apparatus (Balzers, Hudson, NH). Replicas were cleaned in bleach and examined in a Philips 400 electron microscope. Observations were

limited to tight junctions whose position in the gland could clearly be determined, with particular attention to those in the acini.

RESULTS

Controls

The structure of the acinar, intercalated duct, and striated duct cells of unstimulated parotid glands was similar to that described previously (15, 16). Adjacent parenchymal cells were joined at their apical ends by junctional complexes consisting of a tight junction, an adhering junction, and one or more desmosomes, which separated the luminal space from the intercellular and interstitial spaces of the glands. In the acini, intercellular canaliculi, finger-like projections of the lumen that extended toward the base of the acinus were located between adjacent cells and were also separated from the intercellular spaces by a continuous junctional complex.

1 h after isoproterenol stimulation, as previously shown by Amsterdam et al. (17) and Simson (18), the acinar cells were devoid of mature secretory granules. The lumina and intercellular canaliculi were still enlarged by secretory granule membranes added during exocytosis. Small smooth surfaced vesicles, present in the cytoplasm around the lumen, presumably represented endocytic retrieval of granule membrane. No detectable alterations in the thin section appearance of the junctional complexes were evident in any portion of the gland.

In general, the control glands were unreactive after incubation in the various cytochemical media. The secretory peroxidase present in the acinar cells was effectively inhibited by fixation (19). Although acinar secretory granules in preparations incubated for microperoxidase activity sometimes appeared weakly reactive in thick sections examined by light microscopy, no reaction product could be discerned by electron microscopy. Erythrocytes remaining in capillaries and peroxisomes in the parenchymal cells were stained after incubation in diaminobenzidine-containing media, due to their content of hemoglobin and catalase, respectively.

Tracer Infusion in Resting Glands

After infusion of microperoxidase, cytochrome *c*, myoglobin, tyrosinase (subunits), or hemoglobin into resting glands for 1 h, reaction product was primarily localized in the lumina of the ducts and acini (Figs. 1, *a-c*, 2, and 3). The tight junctions appeared to prevent the passage of the tracers from the acinar lumina and intercellular canaliculi into the intercellular spaces. Only small amounts of tracer were present

TABLE I
Ultrastructural Tracers Employed for Retrograde Infusion into the Rat Parotid Gland

| Tracer* | Mol wt | Concentration mg/ml | Cytochemistry | Reference |
|--|----------------------|------------------------|---|-----------|
| Lactoperoxidase | 82,000 | 30 | DAB-H ₂ O ₂ [‡] 1 h, RT [§] | (8) |
| Hemoglobin | 66,000 | 100 | DAB-H ₂ O ₂ 1 h, RT | (8) |
| Horseradish peroxidase (types II & VI) | 40,000 | 0.1–10 | DAB-H ₂ O ₂ 1 h, RT | (8) |
| Tyrosinase | 34,500 | 10 | L-Dopa [¶] 3 h, 37°C | (9) |
| Myoglobin | 17,800 | 40–50 | DAB-H ₂ O ₂ 1 h, 37°C | (10) |
| Cytochrome <i>c</i> | 12,000 | 40 | DAB-H ₂ O ₂ 20 min, 37°C | (11) |
| Microperoxidase | 1,900 | 2.5–5 | DAB-H ₂ O ₂ 1 h, RT | (12) |

* Obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification.

[‡] 3,3'-Diaminobenzidine-hydrogen peroxide.

[§] Room temperature.

^{||} Subunit molecular weight.

[¶] L- β -3,4-Dihydroxy phenylalanine.

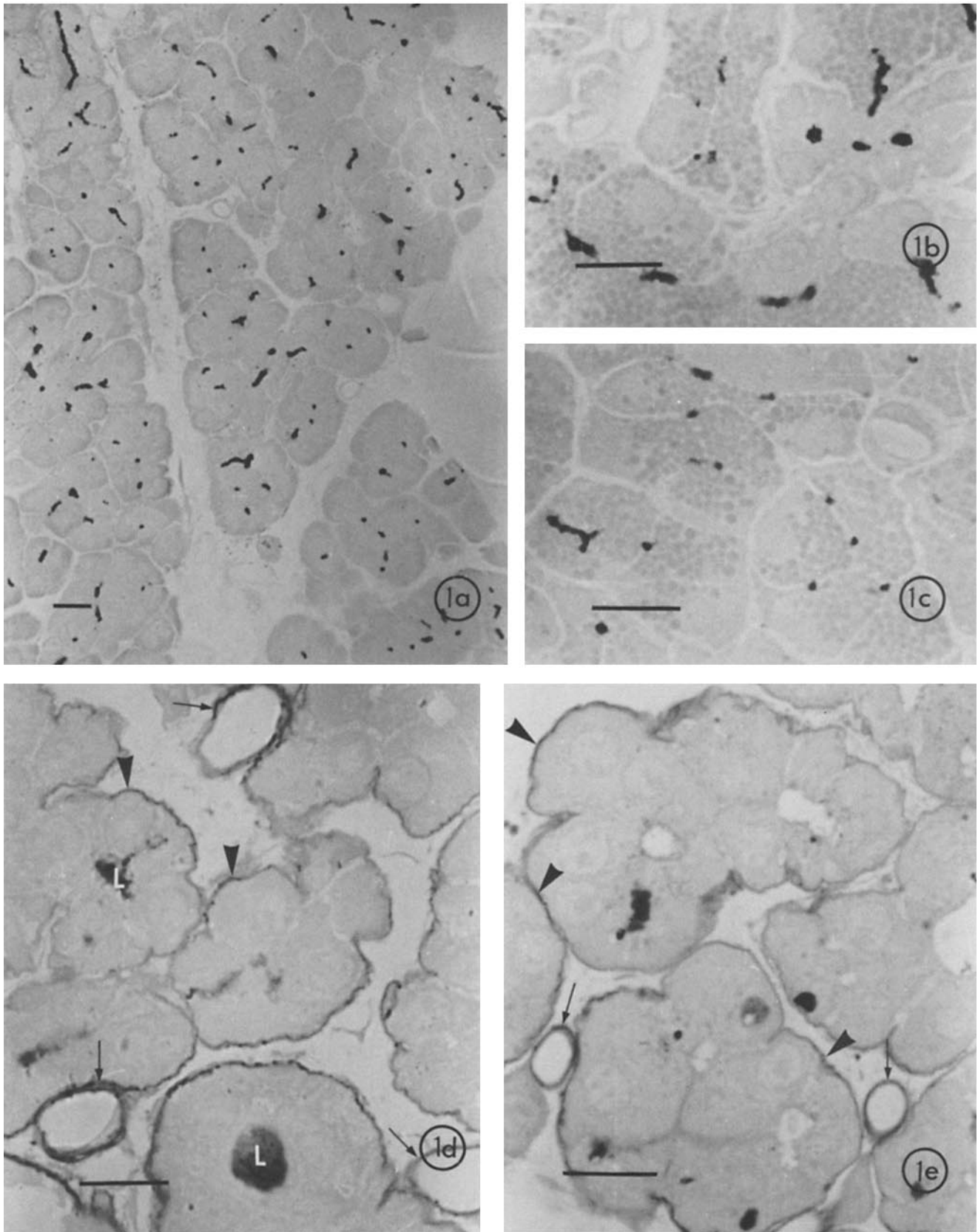


FIGURE 1 Light micrographs of resting (a–c) and isoproterenol-stimulated (d and e) rat parotid glands infused with tracer solution for 1 h. (a) Cytochrome c (10 mg/ml) and (b–c) myoglobin (40 mg/ml); resting glands. Reaction product is present in the lumina of the acini and ducts. (d) Cytochrome c (10 mg/ml) and (e) myoglobin (40 mg/ml); 1 h isoproterenol stimulation. Reaction product is present in some of the lumina (L), at the basal surfaces of the acinar cells (arrowheads), and around the blood vessels (arrows). Bars, 10 μ m. \times 675 (a); \times 1,550 (b and c); \times 1,600 (d and e).

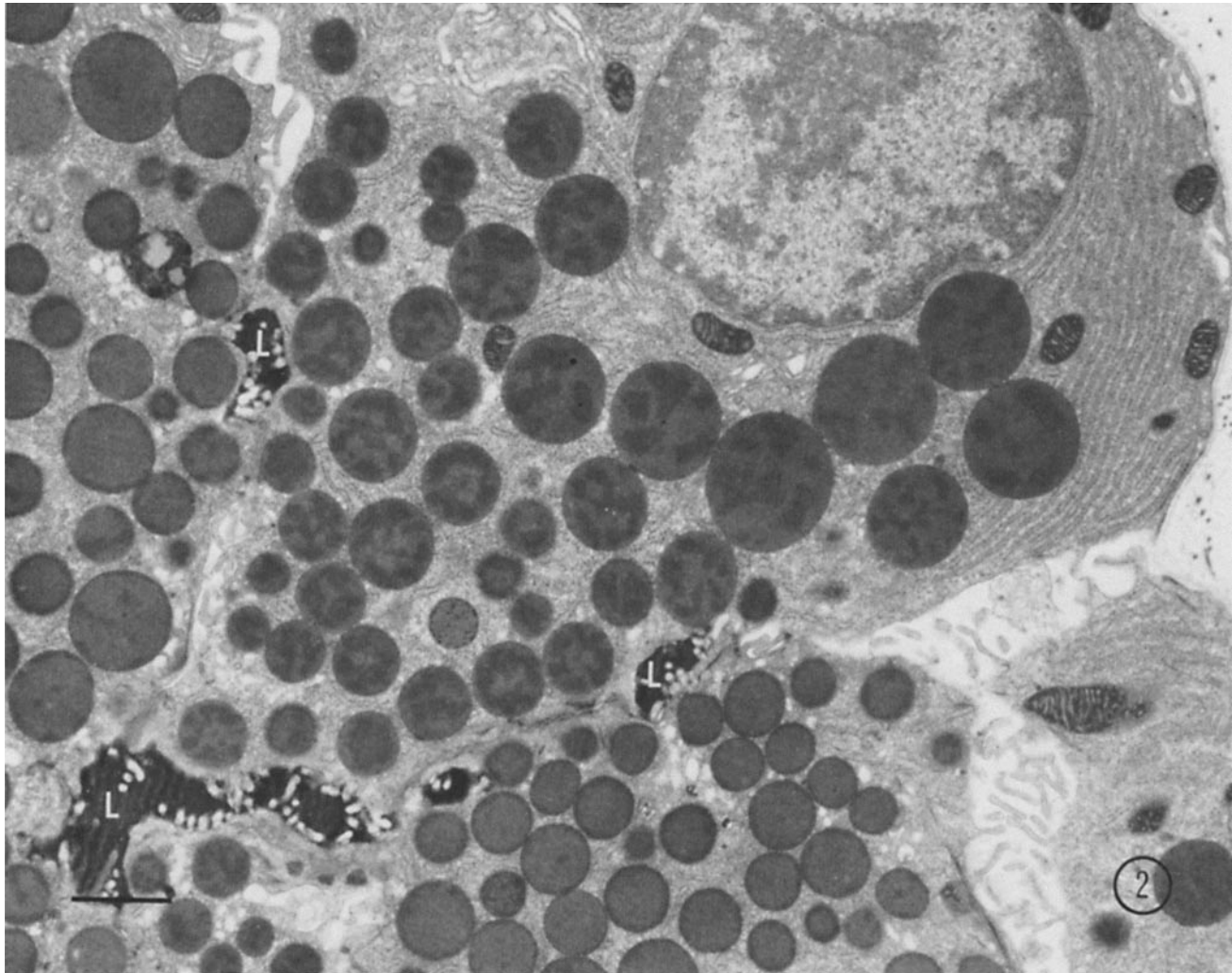


FIGURE 2 Electron micrograph of acinar cells of a resting gland infused with myoglobin (50 mg/ml) for 1 h. The tracer is restricted to the lumen (L) of the acinus. No tracer can be detected in the intercellular spaces or at the base of the acinar cells. Bar, 1 μ m. \times 13,600.

intracellularly, predominantly in small vesicles and various types of lysosomes. Rarely, scattered acinar and duct cells exhibiting a diffuse cytoplasmic stain were encountered. In a few instances, most notably with hemoglobin and myoglobin, but also occasionally with cytochrome *c* and microperoxidase, the lateral intercellular spaces were dilated and filled with reaction product. However, movement of the tracer into the interstitial spaces appeared to be limited by the basement membrane around the acinus.

The overall distribution of the tracer in the gland was rather uneven. Lobules or portions of lobules with abundant luminal reaction product were adjacent to areas with little or no reaction product.

Tracer Infusion after Isoproterenol Stimulation

When the main excretory duct was cannulated 1 h after isoproterenol injection and tracer was infused for an additional hour, reaction product for microperoxidase, cytochrome *c*, myoglobin, and tyrosinase was present in the intercellular and interstitial spaces in addition to the acinar and ductal lumina (Figs. 1, *d* and *e* and 4). Reaction product

was particularly prominent in the basement membranes surrounding the acini. Lesser amounts were usually present in the lateral intercellular spaces of the acini. Occasionally, little or no reaction product was present in the lumina, despite abundant extraluminal deposits. In some regions extraluminal reaction product filled the interstitial spaces and was present around blood vessels, nerves, and ducts. In the intralobular (intercalated and striated) ducts, reaction product often extended into the intercellular spaces, reaching the level of the tight junctions at the apical ends of the cells.

As in resting glands, the distribution of tracer throughout the gland was uneven. Furthermore, in some areas extraluminal reaction product could not be detected, even though the lumina were filled with tracer.

The permeability of the tight junctions was also assessed at later times after stimulation with isoproterenol. When the main excretory duct was cannulated 2 or 3 h after stimulation and tracer was allowed to flow into the gland for an additional hour, reaction product was present in the intercellular and interstitial spaces as well as the lumina (Figs. 5 and 6). When the duct was cannulated 4 h after stimulation, less extralu-

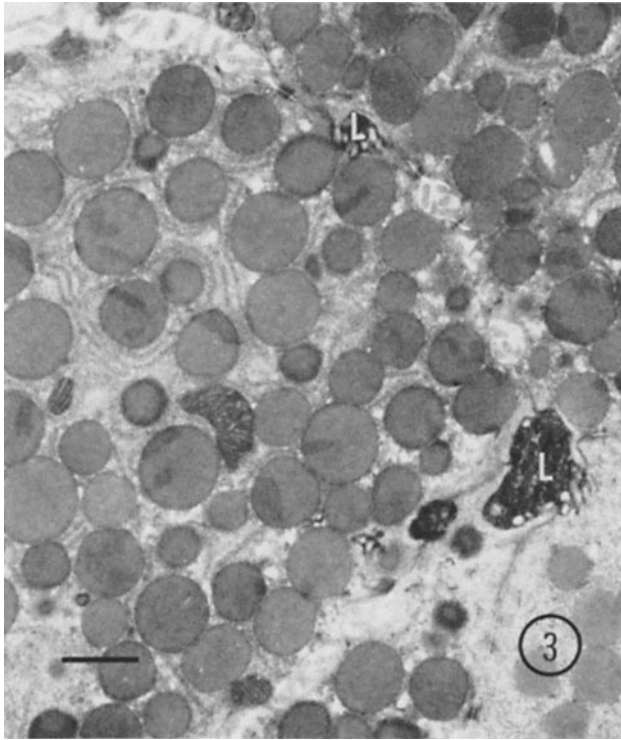


FIGURE 3 Acinar cells of a resting gland infused with microperoxidase (3.3 mg/ml) for 1 h. Reaction product is restricted to the lumen (L). Bar, 1 μ m. \times 9,900.

minimal reaction product was present than at earlier times, and at 6, 9, 12, and 24 h after isoproterenol, the tracer again was restricted to the acinar and ductal lumina (Fig. 7).

In contrast to the lower molecular weight tracers, hemoglobin remained in the lumina and did not reach the intercellular or interstitial spaces (Fig. 8).

Distribution of Horseradish Peroxidase and Lactoperoxidase

When solutions of horseradish peroxidase or lactoperoxidase were administered by retrograde infusion to either resting or isoproterenol-stimulated glands, reaction product was consistently present in the intercellular and interstitial spaces in addition to the lumina of the ducts and acini (Figs. 9, *a* and *c*, 10, and 11). Many acinar and duct cells also contained diffuse reaction product distributed throughout their cytoplasm (Figs. 9, *b* and *c*, 10 and 12). When these tracers were administered for shorter periods of time, e.g., 15–20 min, cytoplasmic reaction product was often restricted to the region immediately adjacent to the lumen or intercellular canaliculus (Fig. 11). Varying the concentration of horseradish peroxidase from 0.1–10 mg/ml did not alter the distribution of reaction product, although the density of both extracellular and intracellular reaction product was reduced when lower concentrations of tracer were used. No difference in the distribution of the reaction product was observed between Sigma type II and type VI horseradish peroxidase. Diffuse cytoplasmic staining and the presence of extracellular reaction product were also observed in resting glands of Wistar-Furth rats (Fig. 9*a*).

The distribution of reaction product of the various tracers in resting and isoproterenol-stimulated parotid glands is summarized in Table II.

Freeze-Fracture Analysis of Junctional Structure

The structure of tight junctions of intercellular canaliculi (Fig. 13) and acinar lumina (Fig. 14) were essentially identical and consisted of a simple meshwork of one to four elements connected by fairly regularly spaced elements. Free ends often extended basally from this meshwork. Doubled elements were occasionally observed. Particle chains or incompletely fused ridges were common on the P face, but particles lying in E face furrows were much less frequently observed. The organization and structure of tight junctions between intercalated duct cells were similar to those of the acinar cells. Adjacent striated duct cells were joined by much more elaborate junctions (see Fig. 17); the elements had a more regular arrangement and they varied in number from 3–9.

No obvious changes interpreted as indicating increased permeability were observed in the junctional meshwork of the acinar cells after stimulation of secretion (Figs. 15 and 16). However, irregularities of all varieties increased, particularly doubled elements. Complete gaps in the junctional meshwork were not found despite an exhaustive search. Particle chains, regions in the meshwork of ridges where incomplete fusion of elements had occurred, were present in approximately equal frequency in both stimulated and resting glands. Changes of tight junction structure in the ducts were not observed.

DISCUSSION

The results of this study indicate that the permeability of the tight junctions of the rat parotid gland is altered during secretory stimulation with the β -adrenergic agonist isoproterenol. In resting glands, retrogradely administered ultrastructural tracers as small as microperoxidase (1,900 daltons) are retained in the lumina of the ducts and acini, whereas in stimulated glands, reaction product of tracers as large as tyrosinase subunits (34,500 daltons) is found in the intercellular and interstitial spaces. Tracers of still larger size, such as hemoglobin (66,000 daltons), are retained in the lumina under both resting and stimulated conditions, suggesting that there is an upper limit on the size of molecules to which the junctions become permeable. The alteration in junctional permeability appears to be a transient phenomenon. When administered 1–4 h after isoproterenol, myoglobin leaks out of the lumina into the interstitial spaces, but extraluminal reaction product is not detectable when tracer is infused 6–24 h after isoproterenol. Despite the evidence from tracer studies for enhanced junctional permeability, no clear-cut structural alterations indicative of increased permeability were ever observed in freeze-fracture replicas.

Both physiological and morphological studies have suggested that secretory stimulation may produce changes in junctional permeability in the salivary glands and exocrine pancreas. Martin and Burgen (4) and Martin (5) observed an increase in salivary concentrations of glucose and other small organic molecules after sympathetic nerve stimulation or administration of catecholamines. This was interpreted as indicating an enhanced tight junctional permeability and paracellular movement of tracers, rather than transcellular transport, since the rates of permeation of several different substances were consistent with their rates of free diffusion, and the quantity of fluid transferred was insufficient for a vesicular transport process. Similarly, Jansen et al. (20) observed an increase in the permeability of the exocrine pancreas

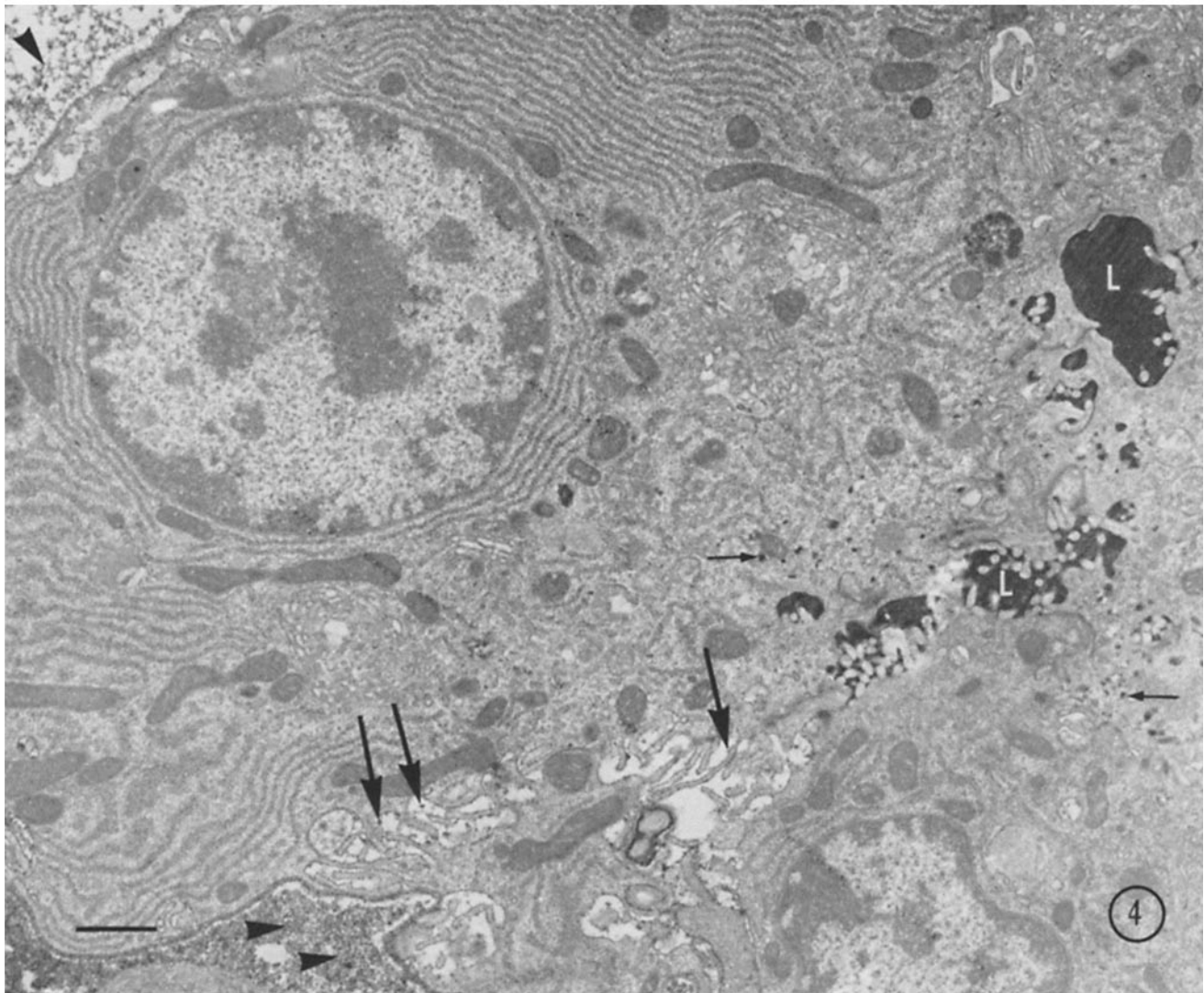


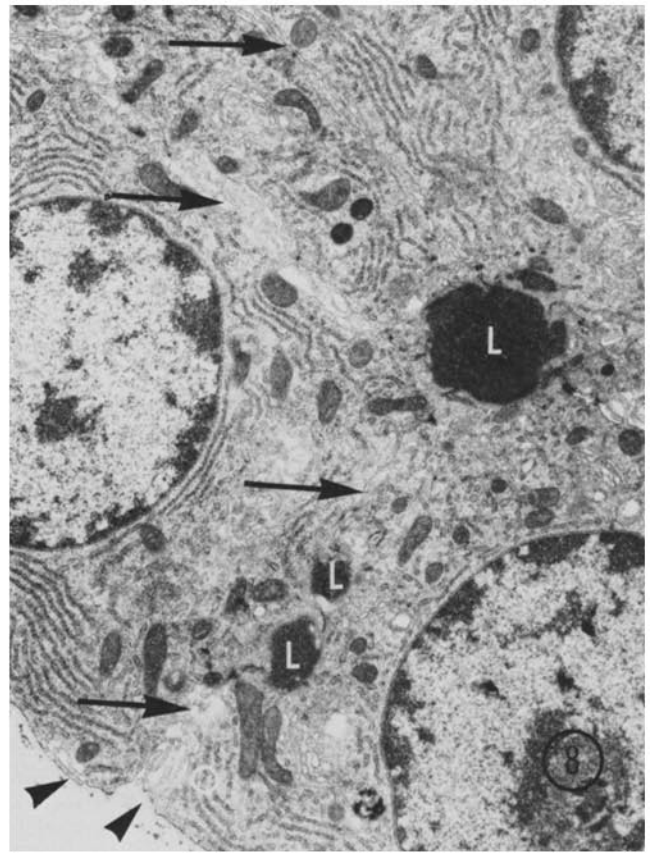
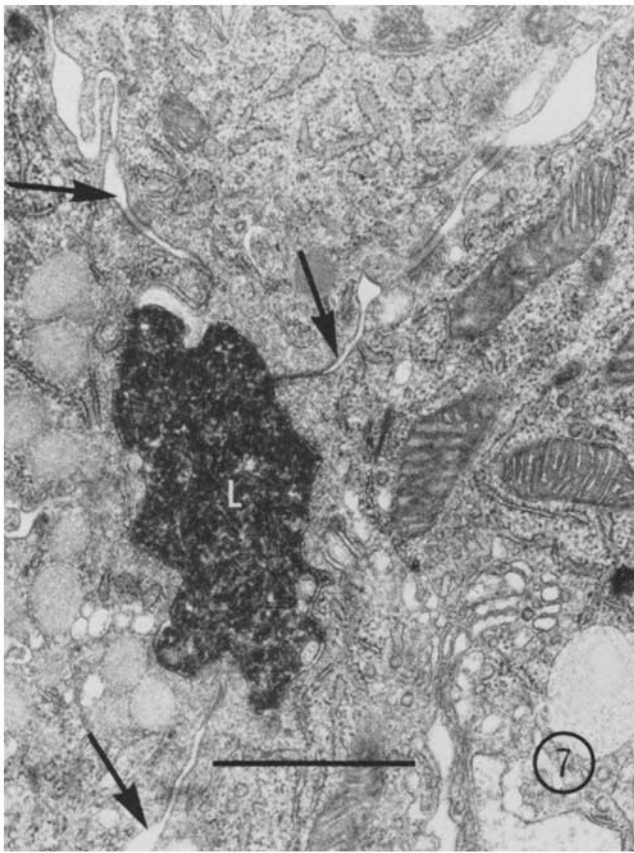
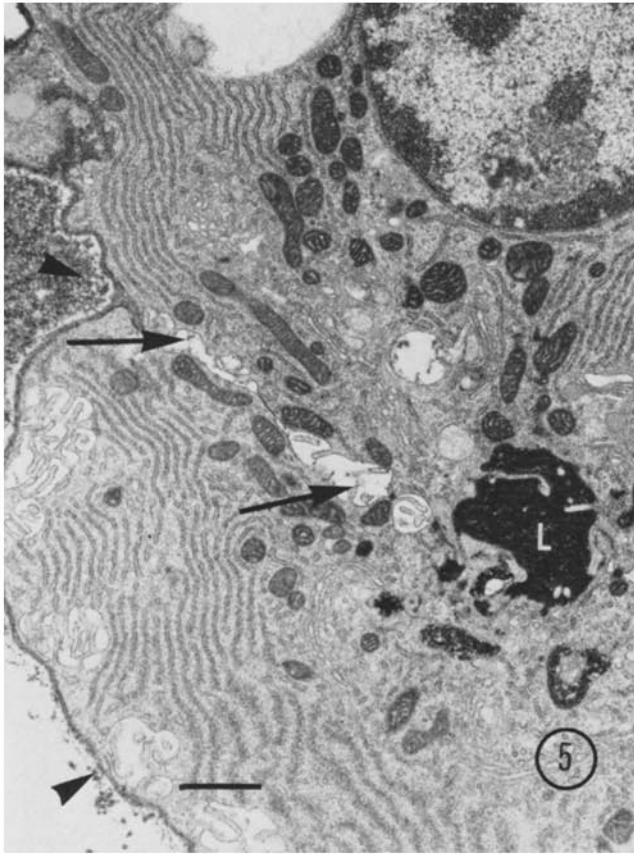
FIGURE 4 Acinar cells of a parotid gland stimulated with isoproterenol 1 h prior to infusion of myoglobin (40 mg/ml). The stimulus has resulted in complete degranulation of the cells. The tracer is present in the lumen (L), the intercellular spaces (large arrows), and at the base of the cells (arrowheads). Endocytic vesicles near the lumen also contain tracer (small arrows). Bar, 1 μ m. \times 11,500.

to sucrose and other small molecules following stimulation with carbachol.

Horseradish peroxidase has been used as an ultrastructural tracer in several studies of exocrine glands. Yoshida et al. (21) detected intravenously administered horseradish peroxidase in rat submandibular saliva after pilocarpine stimulation, but attributed its presence in saliva to transcellular transport by the gland cells (22). Oliver and Hand (7) noted an apparent increase in junctional permeability to retrogradely infused

horseradish peroxidase after isoproterenol stimulation of the rat parotid gland. Garrett and colleagues have studied permeability in the submandibular gland of rabbits (23–25) and dogs (26–28) using horseradish peroxidase administered both retrogradely and perarterially. Their results suggested that tight junctions are permeable to horseradish peroxidase in both resting and stimulated conditions, but that junctions of the acini and ducts may show different properties in the two species.

FIGURES 5–8 Acinar cells of parotid glands stimulated with isoproterenol prior to infusion of tracer. Fig. 5: myoglobin (40 mg/ml); 2 h after isoproterenol. Tracer is localized in the lumen (L) and intercellular spaces (arrows). Note the accumulation of tracer in the basement membrane and interstitial spaces (arrowheads). Bar, 1 μ m. \times 10,500. Fig. 6: tyrosinase (20 mg/ml); 2 h after isoproterenol. Tracer is present in the basement membrane (arrowheads). A small amount of tracer is present in the lumen (L) and intercellular space. Cellular uptake of the tracer is limited to endocytic vesicles (arrows) and multivesicular bodies (MVB). Bar, 1 μ m. \times 19,500. Fig. 7: myoglobin (40 mg/ml); 6 h after isoproterenol. Tracer is localized in the lumen (L) of the acinus; no reaction product is present in the intercellular spaces (arrows). Bar, 1 μ m. \times 26,500. Fig. 8: hemoglobin (100 mg/ml); 2 h after isoproterenol. Hemoglobin is restricted to the acinar lumen (L). No tracer is present in the intercellular spaces (arrows) or at the base of the cells (arrowheads). Bar, 1 μ m. \times 9,000.



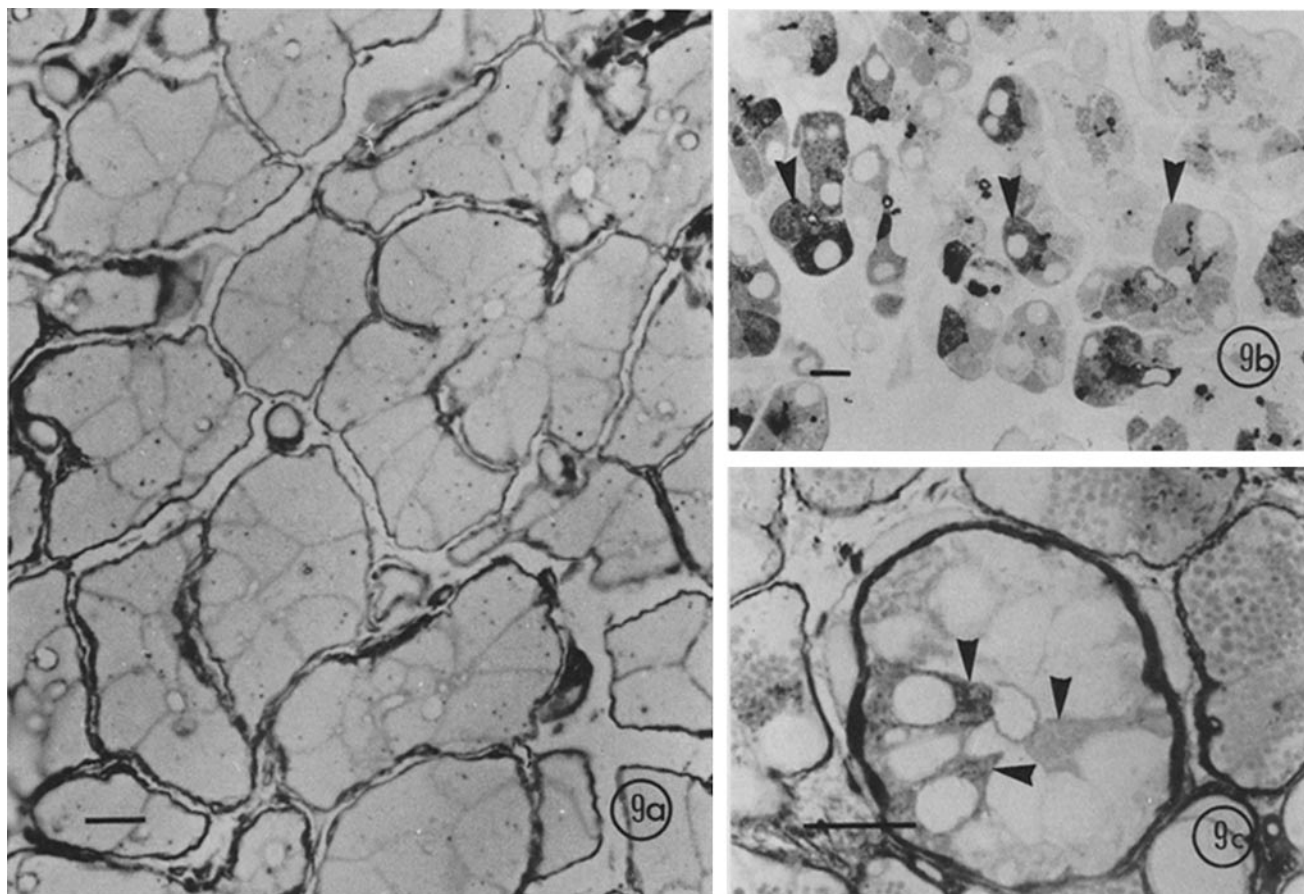


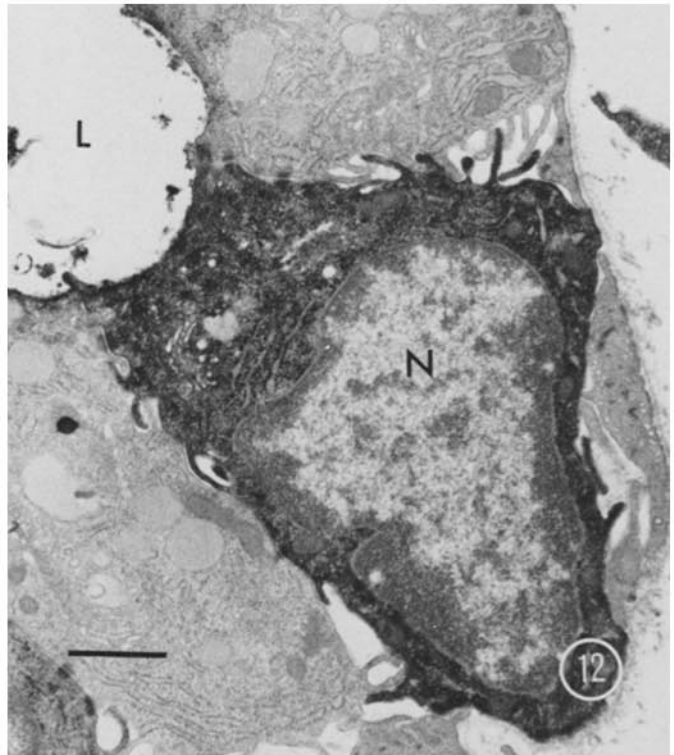
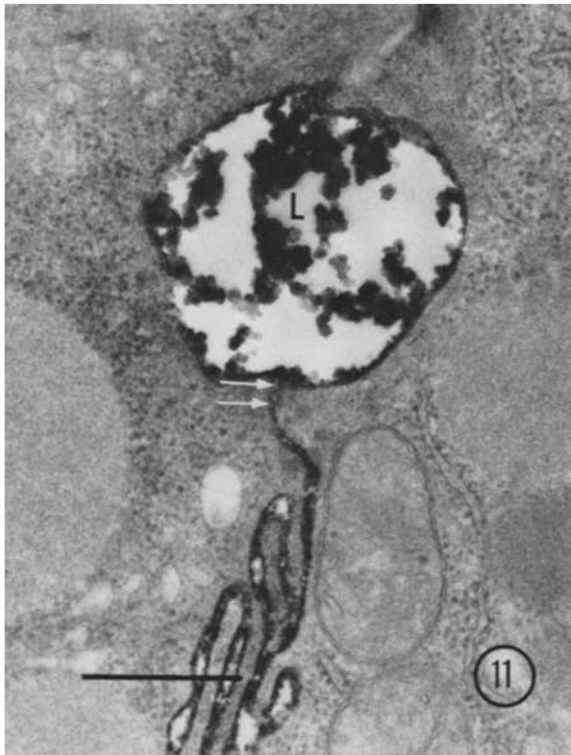
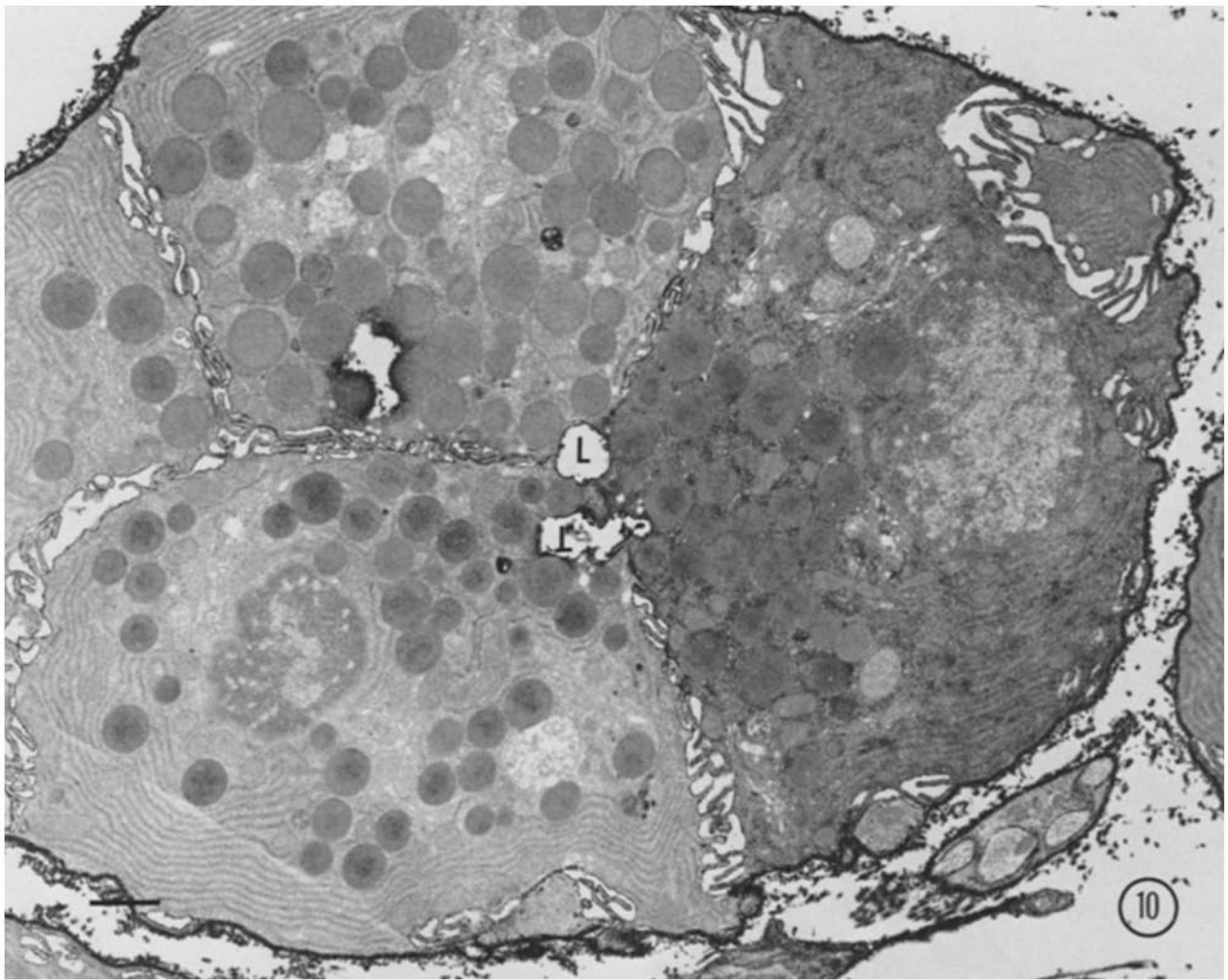
FIGURE 9 Light micrographs of resting parotid glands infused with horseradish peroxidase (10 mg/ml). (a) Wistar-Furth rat, 1 h infusion. Tracer is abundant around the acini; lesser amounts are present in the lumina and between adjacent cells. (b and c) Sprague-Dawley rats infused for 30 min. Numerous acinar (b) and duct (c) cells contain diffuse cytoplasmic reaction product (arrowheads). Tracer is present in the lumina (b and c) and is especially prominent at the basal surfaces of the acinar and duct cells. Bars, 10 μm . (a) \times 750; (b) \times 500; (c) \times 1,500.

Our findings with horseradish peroxidase and lactoperoxidase suggest that caution must be exercised in interpreting results with these tracers, at least after retrograde infusion in the rat parotid gland. Both horseradish peroxidase and lactoperoxidase exhibited anomalous behavior relative to their molecular sizes. Under resting conditions reaction product of horseradish peroxidase and lactoperoxidase was found in the intercellular and interstitial spaces, whereas tracers of smaller size were retained in the lumina. Diffuse cytoplasmic staining, although variable in distribution and intensity, was consistently seen in acinar and duct cells with horseradish peroxidase and lactoperoxidase. Diffuse cytoplasmic staining after horseradish peroxidase has also been noted by other investigators studying a variety of tissues (23–27, 29–34), suggesting that damage to the cell membrane may have allowed the tracer to enter the cell. Although many mechanisms have been postu-

lated to explain this phenomenon, in our system a direct effect of horseradish peroxidase and lactoperoxidase on the cell membrane is the most likely explanation, since other tracers employed under the same conditions did not exhibit significant cytoplasmic staining. Both horseradish peroxidase and lactoperoxidase are glycoproteins and may bind to the cell membrane (24, 26, 29, 35); such binding, possibly in combination with the peroxidatic activity of these tracers, may damage the membrane. It is conceivable that a similar mechanism may alter the permeability of the tight junctions in the rat parotid gland to horseradish peroxidase and lactoperoxidase. Clementi (36) also observed a dose-dependent increase in mouse lung capillary permeability after intravenous administration of horseradish peroxidase.

We cannot entirely rule out the possibility that the extraluminal reaction product in resting glands infused with horse-

FIGURES 10–12 Electron micrographs of resting glands infused with horseradish peroxidase (10 mg/ml). Fig. 10: 20-min infusion. Reaction product is present in the lumen (L), intercellular spaces and at the basal surfaces of the acinar cells. Note the diffuse cytoplasmic staining in the acinar cell at the right. Bar, 1 μm . \times 9,000. Fig. 11: 20-min infusion. Reaction product is present in the lumen (L) and intercellular spaces. Tracer also appears to be located in the region of the tight junction (arrows). Diffuse cytoplasmic reaction product is present near the lumen in the cell on the left. Bar, 0.5 μm . \times 42,000. Fig. 12: 15-min infusion. Diffuse staining occurs throughout the cytoplasm of an intercalated duct cell. No reaction product is present in the nucleus (N). L, lumen. Bar, 1 μm . \times 13,000.



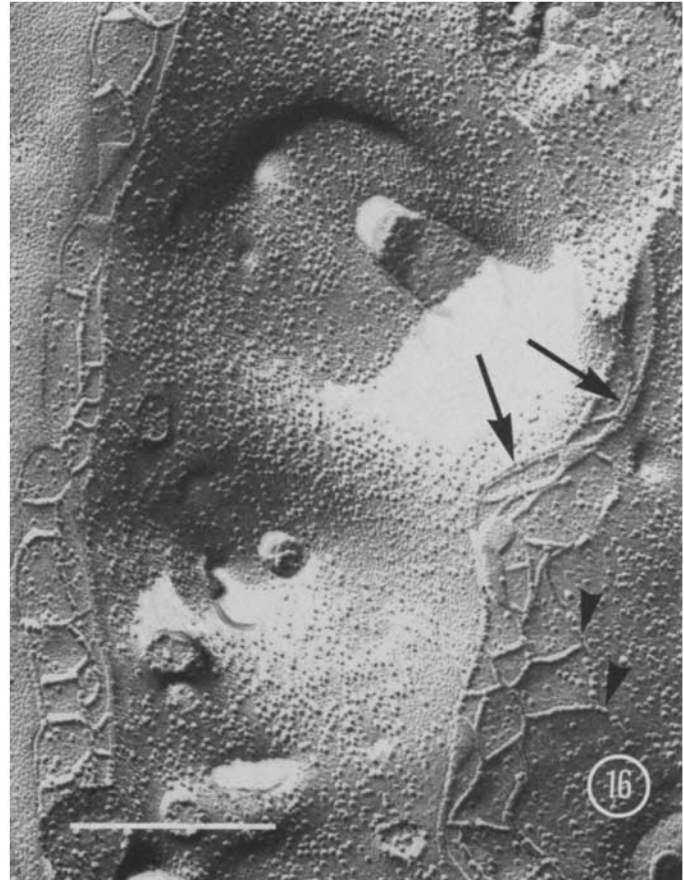
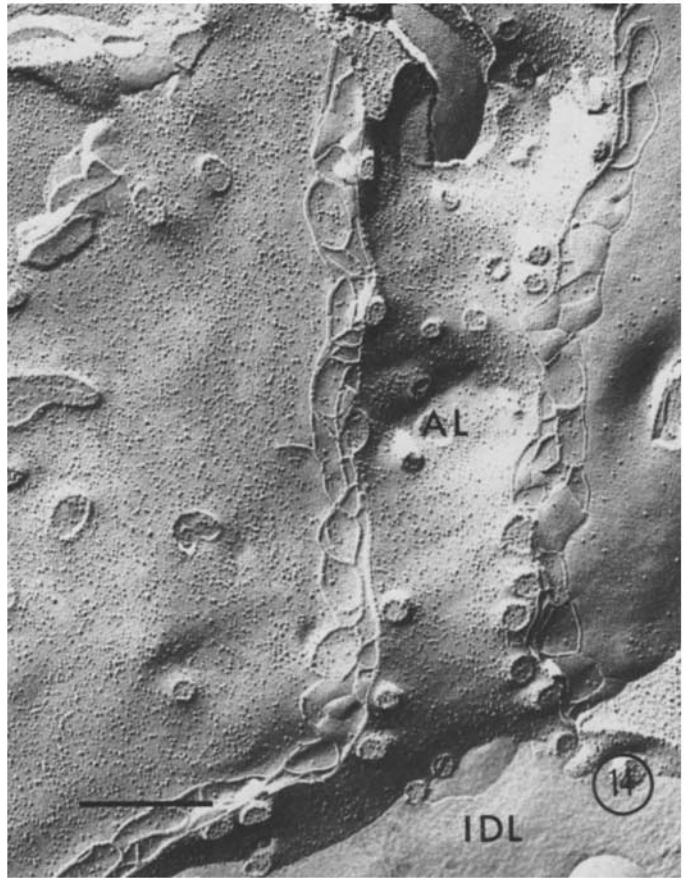


TABLE II
Distribution of Reaction Product in the Rat Parotid Gland after
Retrograde Administration of Tracers

| Tracer | Resting gland | | Isoproterenol-stimulated gland | |
|------------------------|---------------|-------------------|--------------------------------|-------------------|
| | Lu- minal | Inter- stitial | Lu- minal | Inter- stitial |
| Lactoperoxidase | + | + | + | + |
| Hemoglobin | + | - | + | - |
| Horseradish peroxidase | + | + | + | + |
| Tyrosinase | + | - | + | + |
| Myoglobin | + | - | + | + |
| Cytochrome c | + | - | + | + |
| Microperoxidase | + | - | + | + |

radish peroxidase or lactoperoxidase is an artifact generated by our techniques or inherent in our model system. Horseradish peroxidase is known to alter vascular permeability in Sprague-Dawley rats due to histamine release from mast cells (37). However, an identical distribution of reaction product was obtained in Wistar-Furth rats. It is possible that there is some redistribution of tracer during fixation, but this was not seen with the other tracers. Finally, because of the amplification afforded by the enzymatic activity of horseradish peroxidase and lactoperoxidase, we may be able to detect them in the intercellular and interstitial spaces in smaller amounts than the other tracers. After isoproterenol stimulation, however, there was no difficulty in demonstrating extraluminal reaction product of the other tracers.

The possible effect on junctional permeability of the pressure induced by retrograde infusion in isoproterenol-stimulated animals is difficult to address experimentally. However, the following points suggest that the alteration in permeability is a specific cellular response unrelated to the pressure. The infusions were not begun until one hour after isoproterenol administration, a time when secretion is essentially complete (17, 18). By adjusting the height of the reservoir containing the tracer solution, the pressure was maintained at a level (16 mmHg) which is lower than that (20–30 mmHg) developed in the duct system of the rat parotid gland during secretion induced by stimulation of the sympathetic and parasympathetic nerves (38). The studies of Martin and Bergen (4), Martin (5), and Junqueira et al. (6) demonstrate that sympathetic stimulation or administration of adrenalin increases the permeability of salivary glands to blood-borne substances. Similarly, recent experiments in our laboratory indicate that the alteration in permeability is specific for β -adrenergic stimulation and does not occur after administration of α -adrenergic or cholinergic agonists (39).

Despite some notable exceptions (40, 41), a general correlation has been established between the structure of tight

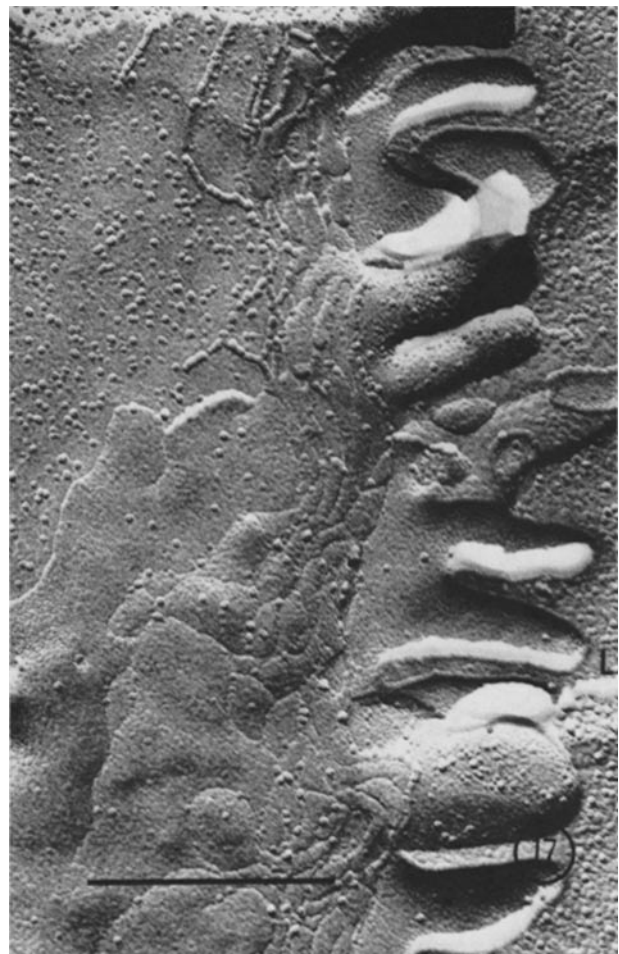


FIGURE 17 Freeze-fracture micrograph showing the P face (upper part) and E face (lower part) appearance of a tight junction from a striated duct cell of a resting parotid gland. Junctions between cells are much more highly developed than in the acini, with 3–9 elements. L, lumen. Bar, 0.5 μ m. \times 64,000.

junctions (i.e., number of elements and apical-basal depth in freeze-fracture replicas) and their “tightness” (transepithelial electrical resistance) (3). Our freeze-fracture results and previously published work by others (42, 43) suggest that the correlation may also hold for the salivary glands. The junctions in the striated and excretory ducts are well developed, with 3–9 elements; these ducts are relatively impermeable to water (44) and their electrical properties are inconsistent with a paracellular pathway for electrolyte transport (45). In contrast, the acinar junctional meshwork is less regular and typically has 1–4 elements. Recent ionic tracer studies by Simson and Dom (46) support the hypothesis that one site of

FIGURES 13–16 Freeze-fracture micrographs of the P face structure of tight junctions in resting and isoproterenol-stimulated parotid acini. Bars, 0.5 μ m. Fig. 13: resting gland. A tight junction surrounds the base of an intercellular canaliculus. In this location 2–3 elements parallel to the lumen (L) are interconnected at fairly regular intervals. Occasional free ends (arrowheads) and doubled elements (arrows) are present. \times 30,800. Fig. 14: resting gland. A tight junction at the juncture of an acinar lumen (AL) and intercalated duct lumen (IDL) exhibits a simple meshwork with few irregularities in the spacing of interconnecting elements. \times 35,900. Fig. 15: 2 h after isoproterenol. \times 52,100. Fig. 16: 1 h after isoproterenol. \times 54,000. In secreting acini the arrangement of the junctional elements sometimes appears disorganized (Fig. 15), with free ends (arrowheads), doubled elements (arrows), and less regular spacing of interconnecting elements. No obvious gaps or discontinuities can be discerned. In other junctional meshworks (Fig. 16) the configuration of elements is indistinguishable from that seen in the resting gland.

fluid and electrolyte entry into rat parotid saliva is the acinar tight junction. Our tracer studies also implicate the acinar junctions as the site of leakiness. Some tracers occasionally appeared to accumulate in the intercellular spaces of the acini with no detectable spread beyond the basement membranes. If the junctions between duct cells were permeable to the tracers, we would have expected to find tracer between adjacent duct cells and in the interstitial spaces in addition to (or instead of) the acinar intercellular spaces.

Less certain is the extent to which changes in junctional permeability are reflected in the structure and organization of the junctional elements. Although tight junctions are disrupted in some systems during experimental manipulations of permeability (47), in others, no change (40, 41) or only a reorganization (48) of the junctional elements can be detected. The apparent lack of correlation between the results of our tracer studies and our freeze-fracture observations again raises serious questions about the interpretation of tight junction images seen in freeze-fracture replicas. To account for tracer penetration in the absence of obvious gaps in the junctional meshwork one must assume that (a) the configuration of tight junction elements is altered during fixation and does not always accurately represent the *in vivo* state; (b) all elements seen in replicas do not represent membrane fusion (cf. 49); (c) some of the small gaps between particles might in fact represent incomplete membrane fusions rather than "lost particles" as recently suggested (48); or (d) regional heterogeneities not detected by our sampling procedures occur in junctional structure and permeability (50). Although transcellular transport could account for the presence of extraluminal tracer, no evidence was obtained to suggest this. In view of the altered tight junction morphology shown to occur after aldehyde fixation of fresh tissue (51) and the discrepancies between gap junction structure from material prepared by rapid freezing and aldehyde fixation (52), we are inclined to think that the first of these possibilities is the more likely.

The cellular and molecular mechanisms involved in regulating tight junctional permeability in the rat parotid gland are unknown. Stimulation of exocytosis in parotid acinar cells by isoproterenol is a multi-stage process (53, 54) that includes agonist-receptor interaction, activation of adenylate cyclase and generation of cyclic AMP, phosphorylation of specific protein(s), and subsequent intracellular steps that culminate in fusion of secretory granules with the luminal plasma membrane. Presumably, any or all of these steps could affect junctional permeability. Studies in other systems suggest that Ca^{2+} and cyclic AMP interactions with cytoskeletal elements may be involved in maintaining and regulating junctional integrity. Removal of Ca^{2+} decreases transepithelial electrical resistance in Madin-Darby canine kidney cell cultures (55), and causes fragmentation and disassembly of tight junctions in pancreatic acinar cells (56). Cyclic AMP increases epithelial resistance and alters junctional structure in *Necturus* gallbladder (57), but increases paracellular permeability in proximal tubules of rabbit kidney (58). Drugs which disrupt microfilaments affect transepithelial resistance in Madin-Darby canine kidney cells (59) and *Necturus* gallbladder (60), and alter junctional structure and permeability in rat liver (61). Mechanical stress induced by duct ligation alters junctional structure in the liver (47, 48, 62) and pancreas (63). During isoproterenol-stimulated secretion in parotid acinar cells, the luminal cell membrane is highly active and undergoes considerable motion due to the rapid fusion and fission events during

exocytosis and the subsequent endocytic retrieval of granule membrane (64). The resulting stress on the luminal membrane and tight junctions may be sufficient to induce the observed alterations in permeability.

In conclusion, the present work demonstrates that the tight junctions of the rat parotid gland are normally impermeable to retrogradely administered ultrastructural tracers. Stimulation with the β -adrenergic agonist isoproterenol results in a transient increase in junctional permeability, although corresponding changes in junctional structure cannot be detected. Whether similar alterations in permeability occur during physiologically induced secretory activity remains to be established.

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