

FUSION OF THE ENVELOPE OF MUCOUS DROPLETS WITH
THE LUMINAL PLASMA MEMBRANE IN
ACINAR CELLS OF THE CAT SUBMANDIBULAR GLAND

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In broad outline, the cytological events associated with the liberation of secretory products from the cell by a merocrine process are well established (Palade, 1959). Secretory granules approach the cell periphery and their limiting membranes fuse with the plasmalemma. Rupture of this membranous barrier results in the formation of an avenue of variable size through which the granule contents may flow passively or be actively expelled. By means of this exocytotic mechanism, secretory products can pass through the plasma membrane without any breach in this vital structure.

The precise details of the fusion between secretory granules and plasmalemma are not entirely clear. It is the purpose of this report to describe some of the intermediate steps in this process as observed in the mucous acinar cells of the cat submandibular gland.

MATERIALS AND METHODS

Submandibular glands of 11 adult mongrel cats of both sexes were fixed by vascular perfusion via the carotid or lingual artery. One of two fixatives was used on each cat. The first was half-strength Karnovsky's (1965) fixative buffered with phosphate (final osmolality of the complete fixative = 990 mOs/kg H₂O). The second was trialdehyde-DMSO (Kalt and Tandler, 1971) buffered with phosphate (final osmolality = 1,350 mOs/kg H₂O) or cacodylate (final osmolality = 1,420 mOs/kg H₂O). The pH of all fixatives was 7.2–7.3. After ~ 50 ml of fixative was perfused through a gland, the organ was removed from the animal, diced, and fixation continued in the same type of fixative. After a brief rinse in a 1:1 mixture of 0.2 M sucrose and 0.3 M buffer, the

tissue specimens were postfixed in 2% osmium tetroxide in phosphate buffer (Millonig, 1961) (final osmolality = 363 mOs/kg H₂O). The specimens were rinsed in distilled water, and were soaked overnight in an aqueous solution of 0.5% uranyl acetate. After another rinse in distilled water, the tissue was dehydrated in graded ethanol and embedded in Epon (Luft, 1961). Thin sections were sequentially stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and examined in a Philips 300 or a Siemens Elmiskop 1A electron microscope.

RESULTS

Acini of the cat submandibular gland consist of two types of mucous cells—those surrounding a central lumen (hereafter referred to simply as acinar cells), and demilunar cells that secrete a second type of mucus (Shackleford and Wilborn, 1970). The following description applies only to the acinar cells. In the resting gland, the bulk of these cells is occupied by mucous droplets, which displace the nucleus basally. Sparse cytoplasmic organelles are evident in the small cytoplasmic interstices between droplets. Individual droplets are relatively large, measuring up to 2 μ m in diameter.

Mucous droplets at the apical pole of the acinar cells often are very close to (Fig. 1) or are in direct contact with the luminal plasma membrane. Areas of contact appear as five-layered structures (Fig. 2). The central linear density is composed of the inner leaflet of the plasma membrane and the outer leaflet of the droplet membrane, and shows a higher degree of contrast than do the flanking

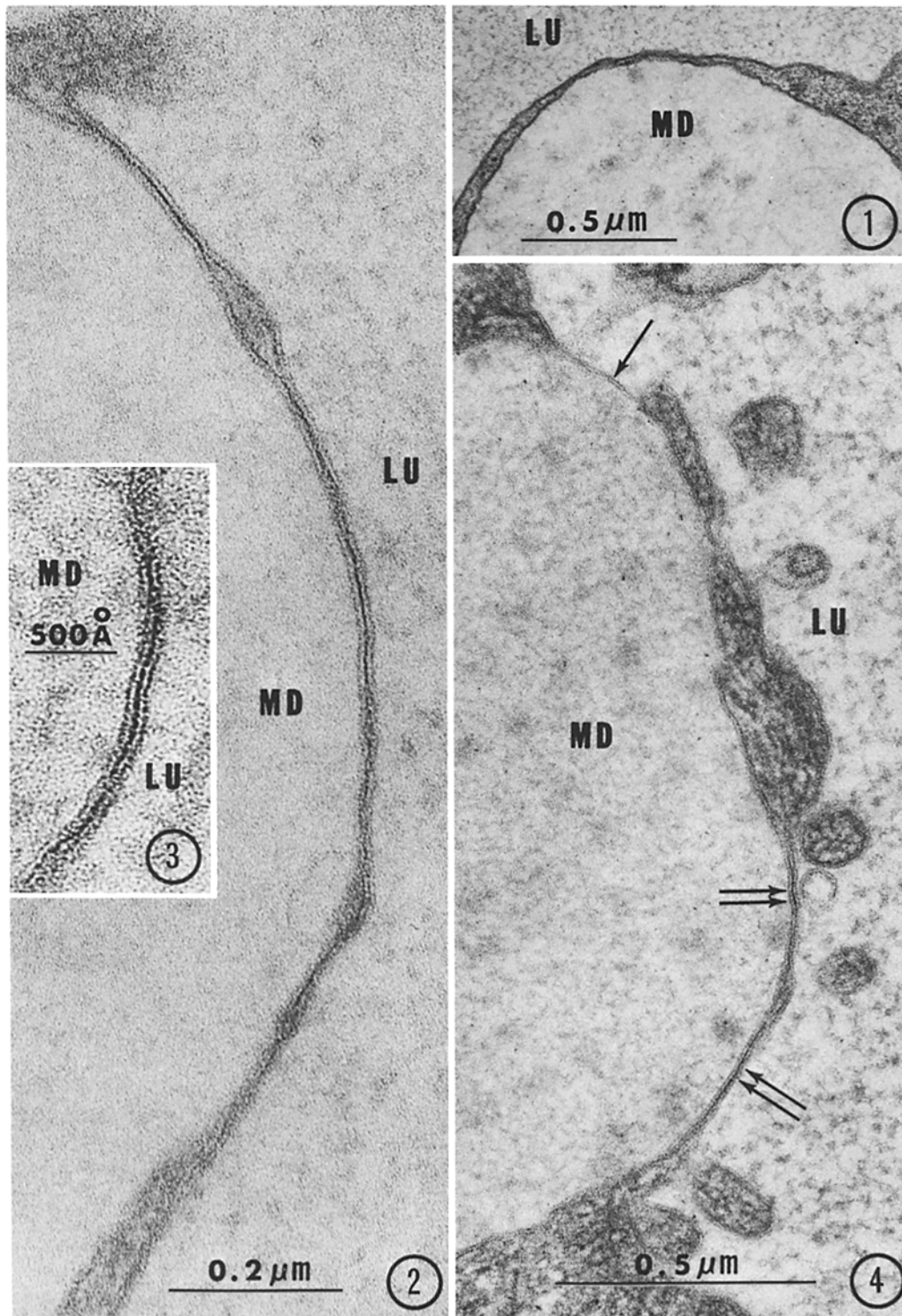


FIGURE 1 A mucous droplet is separated from the luminal plasma membrane by a narrow cytoplasmic interval. $\times 57,000$. Abbreviations used in all figures: LU, lumen; MD, mucous droplet.

FIGURE 2 The membranous envelope of a mucous droplet has fused with the apical plasmalemma to form a five-layered contact. The central dark line is of a higher density than the outer dark lines. $\times 175,000$.

FIGURE 3 A portion of a five-layered contact between droplet and plasmalemma. The central dense line is thicker than the flanking dense lines. $\times 269,000$.

FIGURE 4 A portion of a mucous droplet is shown at the luminal border of an acinar cell. Its contents are separated from the lumen by a five-layered membranous array (double arrows) and by a single unit membrane (single arrow). The two types of contacts are kept apart by some entrapped cytoplasm. $\times 77,500$.

dense lines (Figs. 2 and 3). In a single section, these areas of contact may be quite restricted or may extend fully half-way around the circumference of a droplet. Sections of the same droplet taken some distance apart demonstrate that the membrane contact may be extensive in the third dimension.

Occasionally, a droplet abutting the plasmalemma shows a five-layered contact zone over part of its surface, and a three-layered membrane (unit membrane) bordering the lumen in other places (Fig. 4). The five-layered areas and the three-layered areas are almost never in direct continuity—they are usually separated from each other by a small amount of residual cytoplasm trapped between the envelope of the droplet and the plasmalemma. Such imprisoned cytoplasm exhibits the same electron density and appearance as does the cytosol in the remainder of the mucous cell. In a few instances, the three- and five-layered membranes are almost directly continuous with one another, with virtually no intervening cytoplasm (Fig. 5). In some cases, the five-layered contact shows an area where the plasma membrane has become elevated from the droplet membrane, forming an empty-appearing "blister" (Fig. 6). The formation of these blisters is unrelated to the osmolality of the fixative. The contents of certain other mucous droplets are separated from the lumen solely by a single unit membrane (Figs. 7 and 8). Where this occurs, it is sometimes possible to observe direct continuity of the outer leaflet of the droplet membrane with the inner leaflet of the plasma membrane at the point where the single membrane begins (Fig. 8). In other droplets also separated from the lumen by a single membrane, the membrane appears discontinuous, but these membrane fragments are held in their proper location, indicating that in regions out of the plane of section they still remain in continuity with the cell surface (Fig. 9). Finally, many droplet configurations are noted that show the free passage of mucus into the lumen through a gaping channel. In resting glands, variously shaped membranous structures are present in the lumen. Although they may lie close to the cell, these usually have no obvious continuity with the cell surface.

Not only do the mucous droplets fuse with the plasma membrane, but adjacent droplets often fuse with each other (Figs. 7 and 8). The contacts between limiting membranes may be either five-layered (Fig. 8) or three-layered (Fig. 10). The same droplet may be attached to one neighbor by a

five-layered contact and to another by a three-layered one. The frequency with which three-layered contacts are noted between contiguous droplets appears to be related to the overall quality of cell preservation—the better the fixation, the fewer the three-layered contacts. Disruption of the membranous barrier between abutting droplets occurs only in poorly fixed cells. In contrast, the morphology and incidence of contacts between droplets and luminal plasmalemma are independent of the fixative used or the degree of preservation obtained.

DISCUSSION

That fusion between membrane-bounded vesicles and the plasmalemma actually occurs has been elegantly documented by Palade and Bruns (1968). It was found that the two membranes come into apposition and form a five-layered contact. This contact is converted into a three-layered one, which is further attenuated to form a single-layered diaphragm that separates the interior of the vesicle from the external milieu. The final step is the loss of the diaphragm, thus eliminating the last barrier between the vesicular content and the external medium.

On the basis of the present study, it may be inferred that a similar series of events occurs during liberation of mucus from acinar cells in the submandibular gland of the cat. Apposition of individual mucous droplets to the plasmalemma produces a five-layered membrane structure. Such a configuration has also been observed in the stimulated sublingual gland of the rat (Kim, Nasjleti, and Han, 1972). In the cat, the five-layered contact is succeeded either entirely or piecemeal by a trilaminar membrane; this occurrence was not reported in the rat sublingual gland. The penultimate step, to wit, the conversion of the three-layered membrane to a single-layered diaphragm, has not been observed in this study. Formation of such a diaphragm implies that the single membrane must become progressively thinner, which ultimately may change its permeability characteristics. Increased permeability of such an attenuated membrane would permit an influx of water. Because mucus has a strong tendency to swell as it imbibes water, increased internal pressure would probably result in a rupturing of the thinned unit membrane before it is transmuted into a structurally stable diaphragm.

In mast cells, it has been postulated that focal

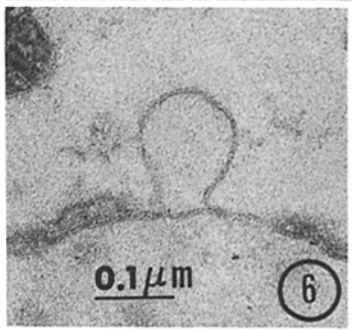
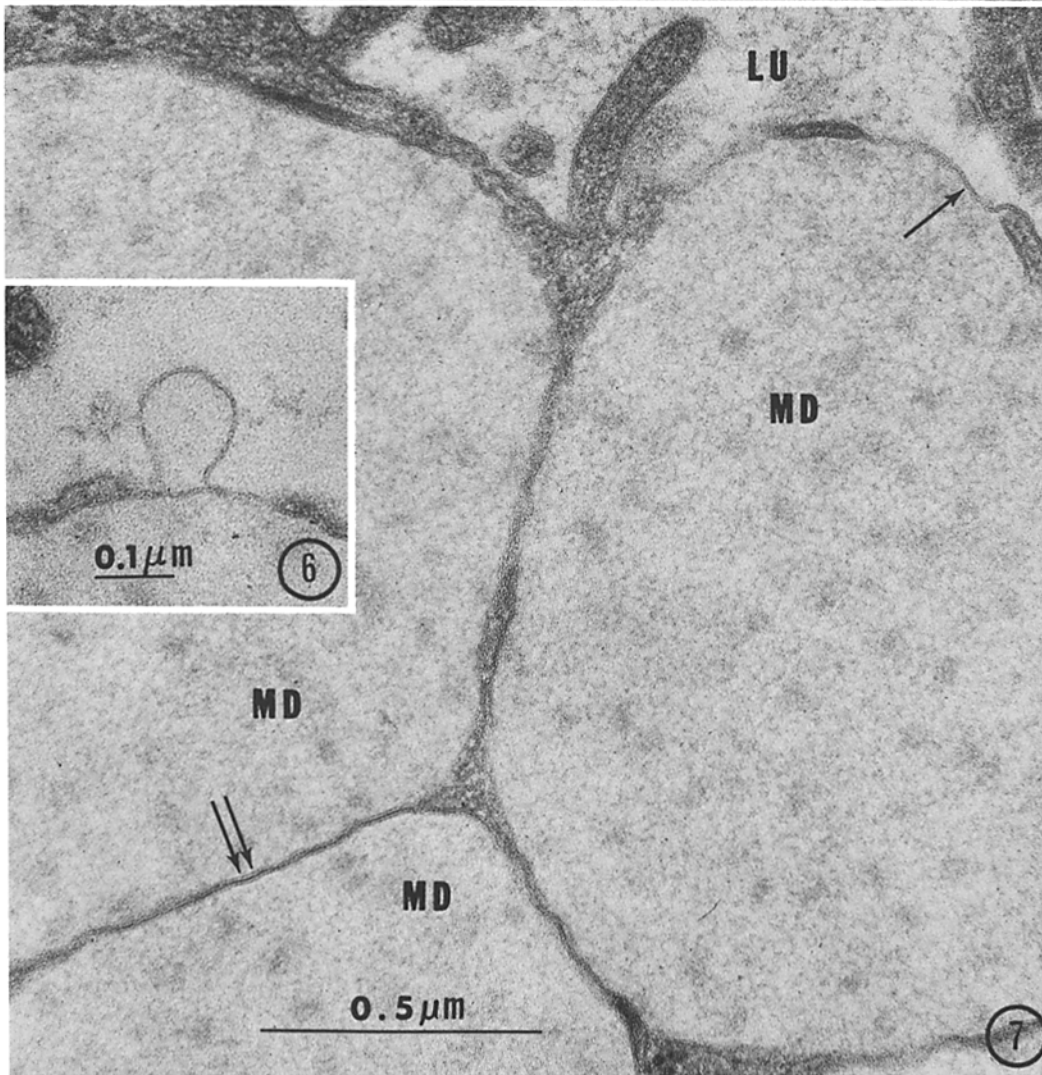
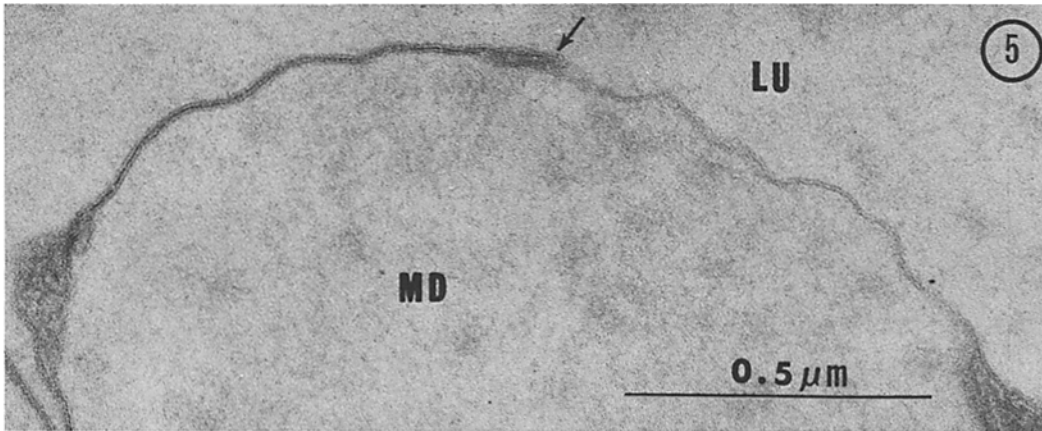


FIGURE 5 A mucous droplet separated from the lumen by a membranous barrier that is partially five-layered and partially three-layered, with no intervening cytoplasm between the two areas. The segment of membrane indicated by the arrow may be a free-ended membrane resulting from the shedding of a portion of the plasmalemma. $\times 81,000$.

FIGURE 6 A "blister" formed by separation of the plasma membrane from the membrane of an underlying mucous droplet. This membranous protuberance is probably shed to yield a three-layered membrane. $\times 104,500$.

FIGURE 7 A mucous droplet is separated from the acinar lumen by a single unit membrane (single arrow). Two adjacent droplets are joined by a five-layered contact (double arrow). $\times 74,000$.

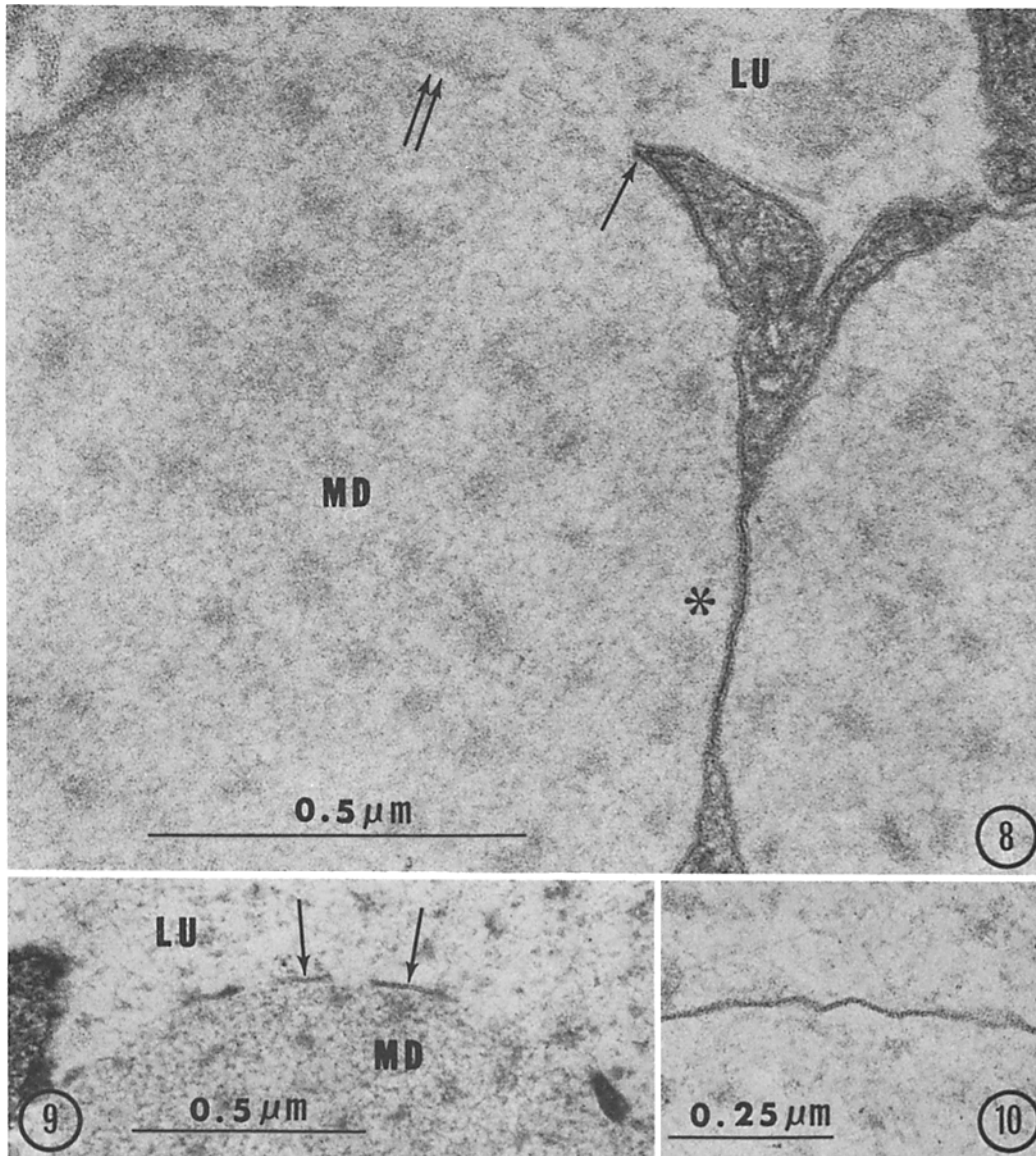


FIGURE 8 A mucous droplet bordering the acinar lumen. At the point where the droplet membrane first contacts the plasma membrane (single arrow), continuity between the outer leaflet of the droplet and the inner leaflet of the plasmalemma is clearly apparent. The barrier (double arrow) between the contents of the droplet and the lumen is probably a single membrane that has been sectioned obliquely; a less likely interpretation is that a single membrane has been transformed into a diaphragmatic structure. Lateral fusion of two adjacent mucous droplets is indicated by the asterisk. $\times 99,000$.

FIGURE 9 A fragmented unit membrane (arrows) at the junction of mucus and lumen. Although disrupted, these membrane remnants are maintained in their correct anatomical position, i.e., between droplet contents and lumen, probably because they are connected to surface membranes outside of the plane of section. $\times 61,000$.

FIGURE 10 A three-layered contact between adjacent mucous droplets. $\times 88,500$.

fusion of granule and plasmalemma results in the formation of a pore through which the granule contents are extruded (Lagunoff, 1973). In the cat submandibular gland, it was shown that the areas of membrane fusion can be quite extensive and that progressive changes in the resultant membrane barrier may be found at different sites on the same droplet. The step that requires further elucidation is the change from a five-layered to a three-layered structure. While it is possible that the two membranes simply mingle by a reshuffling of membrane subunits to form a single trilaminar structure (Satir, 1974), no configurations suggestive of this process were noted. Instead, in a few instances it appeared as if the outer layer, i.e., the plasmalemma, lifts and is peeled from the five-layered membrane (see Fig. 5). In Fig. 6, the portion of the membrane indicated by the arrow can be interpreted as a flaplike remnant of a shed blister. It is possible that the membrane-like structures in the acinar lumina of resting glands may in part represent avulsed plasma membrane. Such membranous formations have been described in the resting sublingual gland of the rat, but they are absent after profound pharmacologic stimulation of the gland (Kim, Nasjleti, and Han, 1972). In the latter case, these membrane-like structures are probably washed out of the acini by the greatly enhanced flow of saliva resulting from stimulation; in keeping with these observations, phospholipids have been found in saliva from human submandibular glands (Mandel and Eisenstein, 1969). In the rat sublingual gland, both the luminal plasmalemma of demilune cells and the myelinlike membranes in the lumina of the demilunes and intercellular canaliculi are stained by a technique that purportedly demonstrates adenyl cyclase activity (S. K. Kim, Veterans Administration Hospital, Ann Arbor, Michigan, personal communication). This observation supports the idea that the myelinlike formations are related to plasma membrane, rather than to secretory material. Similar myelin-type structures are associated with the surface of secreting mast cells (Lagunoff, 1973); here there is no possibility that they represent trapped secretory products that have undergone myelinlike rearrangements. It may eventually be possible to determine unequivocally the fate of the outer ply of the five-layered membrane by the use of immunocytochemistry.

The significance of membrane fusions between adjacent mucous droplets is debatable. Certain serous cells secrete by serial fusion of approxi-

mated granules to form moniliform catenations opening into the acinar lumen (Ichikawa, 1965; Amsterdam, Ohad, and Schramm, 1969; Hand, 1970). This also seems to happen during secretion by mast cells (Röhlich, Anderson, and Uvnäs, 1971; Lagunoff, 1973). In mucous cells of the rat sublingual gland, five-layered membranes are found between tightly packed mucous droplets (Kim, Nasjleti, and Han, 1972). These pentalamellar membranes appear to fragment, allowing the contents of appressed droplets to merge. In the cat, trilaminar membranes as well as five-layered membranes are found between conjoined droplets. The absence of occasional three-layered barriers between droplets and the fragmentation of the five-layered membranes in the rat sublingual gland suggest that in this system less-than-optimum fixation of the mucous secretory products may play a critical role in the observed amalgamation of mucous droplets.

SUMMARY

The release of mucus from acinar cells of the cat submandibular gland was examined by electron microscopy. The limiting membrane of mucous droplets fuses with the luminal plasma membrane to form a five-layered contact. This is converted to a three-layered membrane (unit membrane) by avulsion of the plasmalemma. Attenuation and rupture of this membranous barrier permits the contents of the mucous droplets to flow into the lumen.

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