THE ULTRASTRUCTURE AND HISTOPHYSIOLOGY OF HUMAN ECCRINE SWEAT GLANDS

BRYCE L. MUNGER, M.D.

From the Department of Pathology, The Johns Hopkins Hospital, Baltimore, and the Armed Forces Institute of Pathology, Washington. Dr. Munger's present address is Department of Anatomy, Washington University School of Medicine, St. Louis

ABSTRACT

The electron microscopy of human eccrine sweat glands has been studied before and after stimulation by pilocarpine iontophoresis. The identity of the dark and clear cells in the secretory segment as defined by Montagna *et al.* (23) was determined by studying serial sections, thin for electron microscopy and thick for light microscopy. Cells with numerous apical secretory vacuoles are termed mucoid (dark) cells, since these vacuoles stain positively for acid mucopolysaccharide. Clear cells are intimately associated with intercellular canaliculi. The "cuticular border" of surface cells of the duct is a condensation of tonofilaments and granules. Numerous mitochondria are concentrated in basal cells of the duct. The presence of mucoid cells in the secretory segment may bear on the interpretation of the pathologic findings in the disease cystic fibrosis of the pancreas, and suggests that this disease may be due to a basic disorder of mucopolysaccharide production. The possible roles of the various cellular components in the elaboration of sweat are discussed.

INTRODUCTION

The histology of human eccrine sweat glands has recently been summarized by Montagna (22), and the secretory products of these glands have been characterized chemically (32) and physiologically (33) in other recent reviews. One of the problems confronting any attempt to correlate the structure of eccrine sweat glands with the chemistry and physiology of sweat has been the inability to establish the functional role of cells of the secretory segment by light microscopy. Two different cell types have been postulated to be present in the secretory segment, based on light microscopy (literature reviewed by Montagna (22)). Electron microscopic studies published to date (4, 13) have not demonstrated significant differences within cells of the secretory coil of human eccrine sweat glands. The present study was undertaken in order to correlate the light microscopy, ultrastructure, chemistry, and physiology of these glands.

MATERIALS AND METHODS

Two techniques were used to obtain samples of skin for examination: (*a*) The volar aspect of the forearm of unanesthetized volunteers was cleansed with 70 per cent alcohol and a 3 or 5 mm disc of skin was excised, using the high speed rotary punch biopsy (36); and (*b*) the skin of patients under general anesthesia for surgery was cleansed with soap, alcohol, and iodine, and an ellipse of skin was excised adjacent to the original surgical incision.

Sweat secretion was stimulated by pilocarpine iontophoresis according to the method of Gibson and Cooke (12). Iontophoresis avoids the injection of solutions into the dermis and provides adequate stimulation for the determination of sweat sodium and chloride in clinial application (12). Two-

tenths per cent pilocarpine nitrate and, as a control, 0.9 per cent sodium chloride were used under the electrode, and the current passed for 5 minutes. The areas of skin thus stimulated were biopsied at 5, 15, 30, and 60 minutes after completion of stimulation. Two or three samples for each time period were examined by electron microscopy.

Following excision, the skin specimen was cut into small cylinders perpendicular to the skin surface by slicing with a sharp blade and placed in Dalton's chrome-osmium fixative (6) for 1 hour at room temperature. Other blocks were fixed in neutral formol or Zenker-formol and embedded in paraffin. The osmium-fixed specimens were dehydrated through graded alcohols, embedded in a mixture of butyl-methyl methacrylate (7:1), and polymerized at 60°C, using benzoyl peroxide as a catalyst, or in Epon 812 according to the method developed by Luft (18). Thick sections from the blocks were cut on a Porter-Blum microtome and examined in a phase microscope for localization of sweat glands. Thin sections of appropriate areas were cut and mounted on grids coated with collodion or Formvar, examined with and without staining by uranyl acetate (37) in either an RCA EMU 3B or 3D electron microscope, and photographed at initial magnifications of 1400 to 20,000.

For the demonstration of cytoplasmic basophilia in 1 to 2 micron sections from the methacrylate-embedded tissue, numerous techniques were tried; the most successful method was the gallocyanin chrome alum stain of Einarson (10) prepared as described by him and used fresh. Sections 1 to 2 μ thick were mounted on glass slides by floating on 10 per cent acetone and flattening with the aid of mild heat. The slides were treated with benzene for 2 hours to remove the methacrylate, then with xylene and alcohols of descending strength to water, and stained with gallocyanin chrome alum for 4 to 5 days. Staining for the usual period of 24 to 48 hours produced only faint staining in osmium-fixed tissue. After completion of staining, the sections were rinsed in water, dehydrated in graded alcohols, cleared in xylene, and mounted in Permount.

For the study of various carbohydrate components in sections of sweat glands several techniques were employed. Metachromasia in both osmium- and formol-fixed tissue was studied by staining sections with buffered toluidine blue at pH 5.0, using McIlvane's buffer as recommended by Montagna *et al.* (23). The periodic acid–Schiff technique was used as described by McManus (19). The Alcian blue technique for acid mucopolysaccharides (24) was found to be applicable only on formol-fixed tissue, since the osmium fixative used contained chromate which precipitated the dye. Superb results were obtained by use of the modified Hale colloidal iron stain (25) on osmium-fixed tissue. The intensity of staining is greatly increased with no loss of specificity when 0.5 or 1 cc of concentrated nitric acid is substituted for acetic acid in the staining solution. The washing was also changed, the entire procedure being as follows: (*a*) Place in xylene or benzene to remove methacrylate, 1 to 2 hours. (*b*) Hydrate sections with graded alcohols. (*c*) Stain 1 hour in colloidal iron made as follows: stock colloidal iron (29), 22 cc; distilled water, 17 cc; nitric acid, 1 cc. (*d*) Wash in running tap water for 5 minutes. (*e*) Place in potassium ferrocyanide and hydrochloric acid, equal parts of 2 per cent solutions of each, for 20 minutes. (*f*) Wash in running tap water for 5 minutes. (*g*) Dehydrate, clear, and mount.

Control sections were always stained with the ferrocyanide step alone to determine whether any staining was due to iron present in the tissue section.

OBSERVATIONS

Identification of Cell Types

Human eccrine sweat glands are simple tubular glands composed of two distinct parts: a duct lined by a double layer of cuboidal cells, and a secretory segment composed of a layer of columnar or pyramidal cells. The greater part of the gland is coiled into a loose "glomerulus" at the dermo-subcutaneous junction, with the duct extending from the skin surface into the coil itself; the remainder of the coiled part is the secretory segment, in which two cell types are present. Montagna *et al.* (23) termed them dark cells and clear cells, referring to their affinity for basic dyes. The basophilia of dark cells is abolished by ribonuclease (23), suggesting that the material which is responsible for the basophilia is ribonucleic acid. The dark cells appear to cap or cover clear cells, and this overlapping results in an apparent stratification.

To identify dark and clear cells in electron micrographs, serial sections were cut, the first one thin for examination in the electron microscope, and the adjacent section 1 μ thick for examination in the light microscope after staining with gallocyanin chrome alum as described above.

Paired serial sections, thin for electron microscopy and thick for gallocyanin staining and light microscopy, are illustrated in Figs. 1 and 2. In the light micrograph (Fig. 2) cells with basophilic cytoplasm appear dark grey. These "dark cells" alternate with cells having only the nuclei stained,

i.e., "clear cells." Cytoplasmic basophilia is reticulated, and numerous small unstained vacuoles are present in the apical cytoplasm. Two types of cells can also be seen in the electron micrograph of the adjacent section (Fig. 1). One type, containing numerous apical vacuoles, corresponds to the dark cells of the light micrograph, and the second, showing a very electron opaque cytoplasm, corresponds to clear cells in the light micrograph. In addition, the clear cells in the electron micrograph (Fig. 1) are associated with tubular channels into which microvilli project; these channels are intercellular canaliculi (described in detail later). Thus cells with numerous apical secretory vacuoles seen in phase and electron micrographs are the dark cells of light microscopy, while cells with very electron opaque cytoplasm associated with intercellular canaliculi are the clear cells of light microscopy.

Mucopolysaccharide Content

The modified Hale reaction applied to 1 μ sections of osmium-fixed tissue stains specifically 1 to 2 μ round bodies in the apex of dark cells (Fig. 3). These darkly stained bodies correspond exactly in size and distribution to the secretory vacuoles seen in electron micrographs (Fig. 1) and to the clear areas of cytoplasm seen in gallocyanin-stained sections (Fig. 2). In addition to staining the secretory vacuoles, the Hale method stains the contents of the glandular lumen and also the small spaces between adjacent clear cells (Fig. 3) that are identical in size and shape with intercellular canaliculi seen in Fig. 1. A very faint staining of nuclei and a stippling of the cytoplasm of duct cells are also seen after staining by the Hale method. The stain is also localized in the connective tissue surrounding the gland, where it is present in the material around collagen fibers but not within the fibers themselves. The cytoplasm of clear cells and myoepithelial cells fails to show any coloration. Control sections stained with ferrocyanide were negative for intrinsic iron.

A very faint but definite metachromasia is present in the zone of secretory vacuoles in the dark cells of the secretory segment in osmium-fixed tissue sections. After PAS staining, the glycogen in clear cells could be beautifully demonstrated, but only questionable staining could be seen in the secretory vacuoles of dark cells. Both the PAS and Alcian blue stains demonstrated some positive-staining material in the secretory segment in paraffin sections of formol-fixed tissues, but the exact cytologic localization could not be determined.

Since the so-called dark cells (23) contain secretory vacuoles which stain positively for acid mucopolysaccharide, these cells are considered to be a type of mucus-producing cell. The terminology of light microscopy is confusing when electron micrographs are considered, for the dark cell looks clear in the electron micrograph owing to the numerous secretory vacuoles. Thus to avoid confusion these cells will be designated in the present report as mucoid (dark) cells. The term "clear cell" as defined by Montagna *et al.* (23) will be used for cells with electron dense cytoplasm.

Ultrastructure of Normal Eccrine Gland Secretory Segment

Mucoid (dark) cells are tall columnar cells with irregularly shaped nuclei situated beneath apical secretory vacuoles that measure 1 to 2 μ in diameter (Fig. 1). Secretory vacuoles have a limiting membrane that surrounds the greater part of the vacuole, but the extent of this limiting membrane varies (Fig. 4). Confined within the limiting membrane of secretory vacuoles is a relatively scant amount of faintly fibrillar and granular material which cannot be resolved as any organized structure. In occasional mucoid cells secretory vacuoles appear to be in the process of being liberated from the cytoplasm into the gland lumen (Fig. 5). Secretory vacuoles being extruded from the cell are more commonly seen following stimulation.

The cytoplasm surrounding the secretory vacuoles of mucoid (dark) cells is finely granular; many of the granules are dense and discrete and measure about 150 A, resembling the particles of ribonucleoprotein associated with the ergastoplasmic membranes of protein-synthesizing cells (30; Figs. 4 and 5). Most of these granules are free in the cytoplasm and not associated with membranes, but occasionally ergastoplasmic sacs are present.

A well defined Golgi apparatus is present in mucoid cells and consists of a loose array of flattened agranular membranous sacs, vacuoles, and vesicles (Fig. 4). These agranular membranous sacs are interspersed with small secretory vacuoles

containing fibrillar and granular material indentical with that in the apical secretory vacuoles (Fig. 4). These small vacuoles appear to be analogous to prozymogen granules (26) in pancreatic acinar tissue and are termed prosecretory vacuoles. A continuity can be traced between prosecretory vacuoles and apical secretory vacuoles, suggesting that secretory vacuoles are formed in the Golgi zone and migrate to the apical cytoplasm.

Rod-shaped mitochondria with tightly packed cristae are sparsely scattered throughout the apical cytoplasm of mucoid cells (Fig. 4). Only rarely do mitochondria contain dense mitochondrial granules.

Occasionally the luminal surface of mucoid cells is thrown into irregularly disposed microvilli, but most of the free surfaces of these cells are relatively smooth (Fig. 4). Terminal bars are present between adjacent cells as they abut on the lumen (Fig. 5).

A few mucoid cells have large globules of homogeneous material of moderate density (Figs. 1 and 12). This material is presumed to be some form of lipid and most likely corresponds to the solvent-resistant, fluorescent lipid reported to be found in cells of the secretory segment (22).

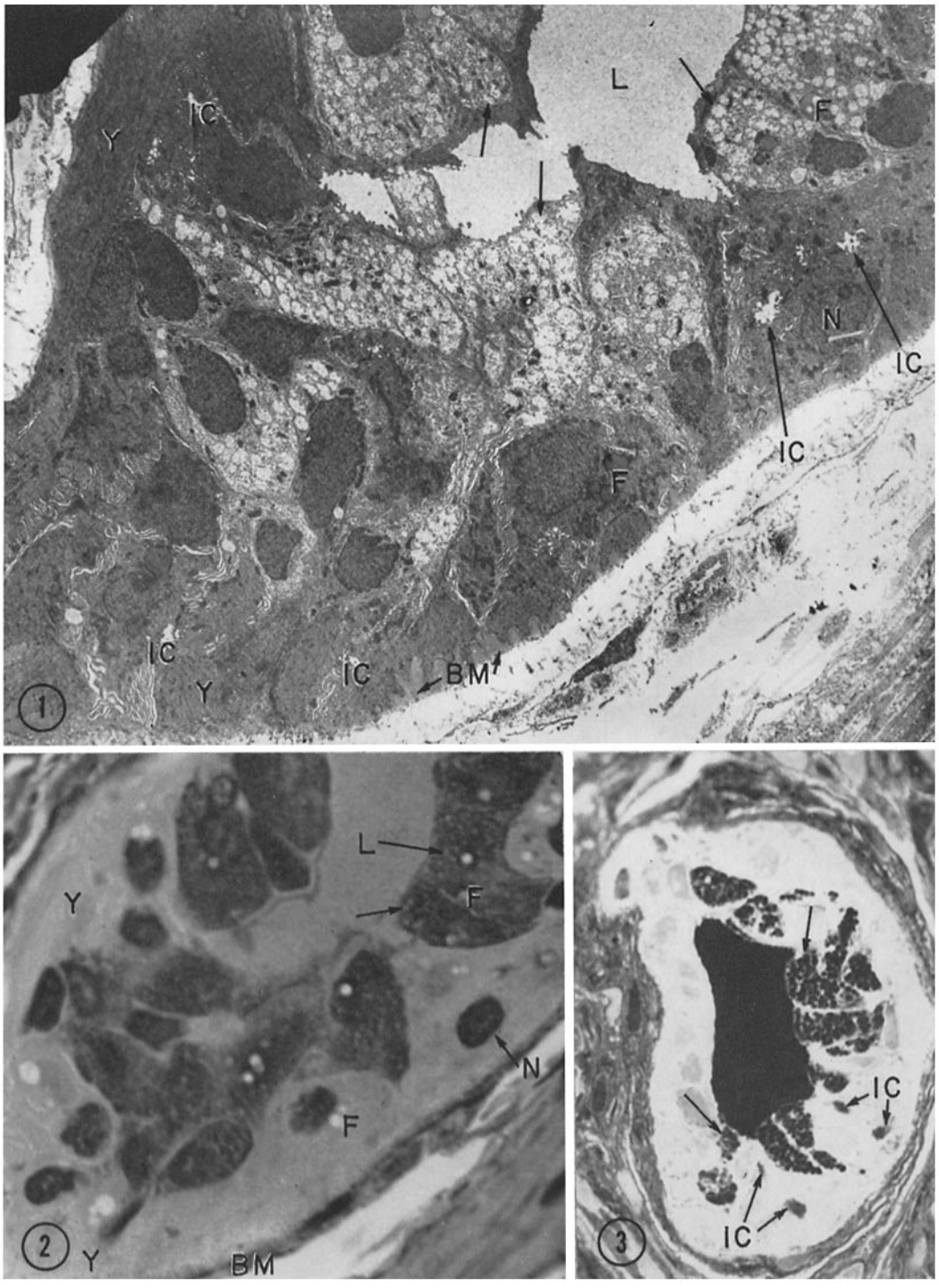
The shape of mucoid (dark) cells is that of an inverted pyramid, the apical end of the cell being larger than the base. The basal part of these cells

FIGURES 1 AND 2

These micrographs are from serial sections of the secretory segment of a human eccrine sweat gland. Fig. 1 is an electron micrograph at a magnification of 3000, and Fig. 2 is a light micrograph, at a magnification of 1500, of an adjacent 1 μ section stained with gallocyanin chrome alum. Comparable areas of the two sections are illustrated. The lumen (*L*) in Fig. 1 is filled with granular material, and in Fig. 2 contains no stainable material. The arrows in Fig. 1 indicate secretory vacuoles that fill the apex of certain cells, and in Fig. 2 indicate the secretory vacuoles which appear as clear areas in the midst of heavily stained cytoplasm. The cells containing secretory vacuoles in Fig. 1 are stained by gallocyanin in Fig. 2; these are the dark cells as defined by light microscopists. The cells with uniform electron opaque cytoplasm in Fig. 1 appear as unstained cells in Fig. 2, with only the nucleus (*N*) staining with the gallocyanin. These cells lack cytoplasmic basophilia in Fig. 2 and are thus the clear cells. Adjacent to the (right) clear cell in Fig. 1 are several intercellular canaliculi (*IC*). These canaliculi are not visible in Fig. 2 owing to lack of coloration of the cytoplasm of clear cells. Near the myoepithelial cells labeled *Y* in Fig. 1 are intercellular canaliculi between other clear cells; corresonding areas of myoepithelial cells are also indicated by (*Y*) in Fig. 2. A prominent basement membrane (*BM*) surrounds the secretory coil in Fig. 1, and its contour is scalloped by projections of myoepithelial cell cytoplasm. At the periphery of the gland, both clear and mucoid (dark) cells can be observed to be in contact with either the basement membrane or myoepithelial cells. The round clear areas (*F*) in Fig. 2 are unstained lipid accumulations of undetermined nature, and the corresponding areas in Fig. 1 also contain lipid globules, although the same globules are probably not present in both sections. The connective tissue investment surrounding the gland in both sections has been disrupted during fixation or embedding.

FIGURE 3

Light micrograph of a 1 μ section of a secretory segment stained with the modified Hale colloidal iron stain, osmium-fixed, methacrylate-embedded; black areas in the micrograph are dark blue under the microscope. Numerous small, round bodies fill the apical part of certain cells (arrows). These intensely stained bodies correspond exactly in location, distribution, and size to the secretory vacuoles seen in Fig. 1 and to their negative image in Fig. 2. Thus these cells with apical secretory vacuoles are mucoid (dark) cells. The nuclei of some cells are very faintly stained. The contents of the lumen of the gland and of intercellular canaliculi (*IC*) are also stained intensely. The connective tissue around the gland stains positively for acid mucopolysaccharides in this preparation. × 975.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rests on either a myoepithelial cell or the basement membrane of the gland (Fig. 1). It is impossible to determine whether every dark cell makes contact with the basement membrane or a myoepithelial cell in the present study, since serial reconstructions were not done. These areas of contact, however, are so frequently seen that they probably exist for every cell.

Clear cells (as defined by light microscopy) are cells that have large irregular nuclei and a cytoplasm that is electron opaque and filled with numerous small, closely packed granules and small vesicles of varying size and density (Fig. 6). The 150 A granules seen in mucoid (dark) cells that presumably represent RNP particles are only rarely seen in clear cells. Glycogen and protein are present in the cytoplasm of clear cells (22), however, and a considerable part of the granularity is probably due to these components. Irregular masses of moderately electron opaque material thought to represent glycogen are scattered throughout the cytoplasm of some clear cells, especially near intercellular canaliculi. Organized intracytoplasmic membrane systems are absent in clear cells as in mucoid (dark) cells. No ergastoplasmic sacs are seen and only a small Golgi apparatus is present in these cells. Globules of lipid material similar to that seen in mucoid cells are also present in clear cells (Figs. 1, 6, and 12).

Intercellular canaliculi are a remarkable specialization of these cells. Canaliculi originate 1 to 2 μ above the bases of the clear cells and course between adjacent cells to empty into the lumen of the secretory coil (Fig. 6). The canalicular lumen measures 1 to 2 μ in diameter, and numerous closely packed microvilli project into it. Extensive and complex interdigitations of the membranes of adjacent cells form a massive double membrane system surrounding the canaliculi (Fig. 6). Terminal bars are frequently seen between adjacent cells as they abut on the canaliculus. Thus, more than one cell participates in the formation of a canaliculus, and the canaliculi are indeed *inter-* and not *intra*cellular (Fig. 6). This participation of cells to form intercellular canaliculi explains the peculiar distribution of clear cells into groups. The cytoplasm of clear cells, though very attenuated, surround the canaliculus to its junction with the gland lumen.

The basal plasma membranes of clear cells are folded in a complex manner as they rest on myoepithelial cells or basement membrane (Fig. 1). The membranes of adjoining mucoid (dark) and clear cells are interdigitated, but these interdigitations are not so extensive as those between adjacent clear cells surrounding the intercellular canaliculi. Occasionally desmosomes are seen between adjacent clear and mucoid cells, and between clear cells and myoepithelial cells. No desmosomes (or terminal bars) have been observed between mucoid cells and myoepithelial cells.

Both types of secretory cells rest on myoepithelial cells or, in areas where there are no myoepithelial cells, on a basement membrane. A description of the myoepithelial cell of eccrine glands and its changes with contraction will be presented as a separate report.

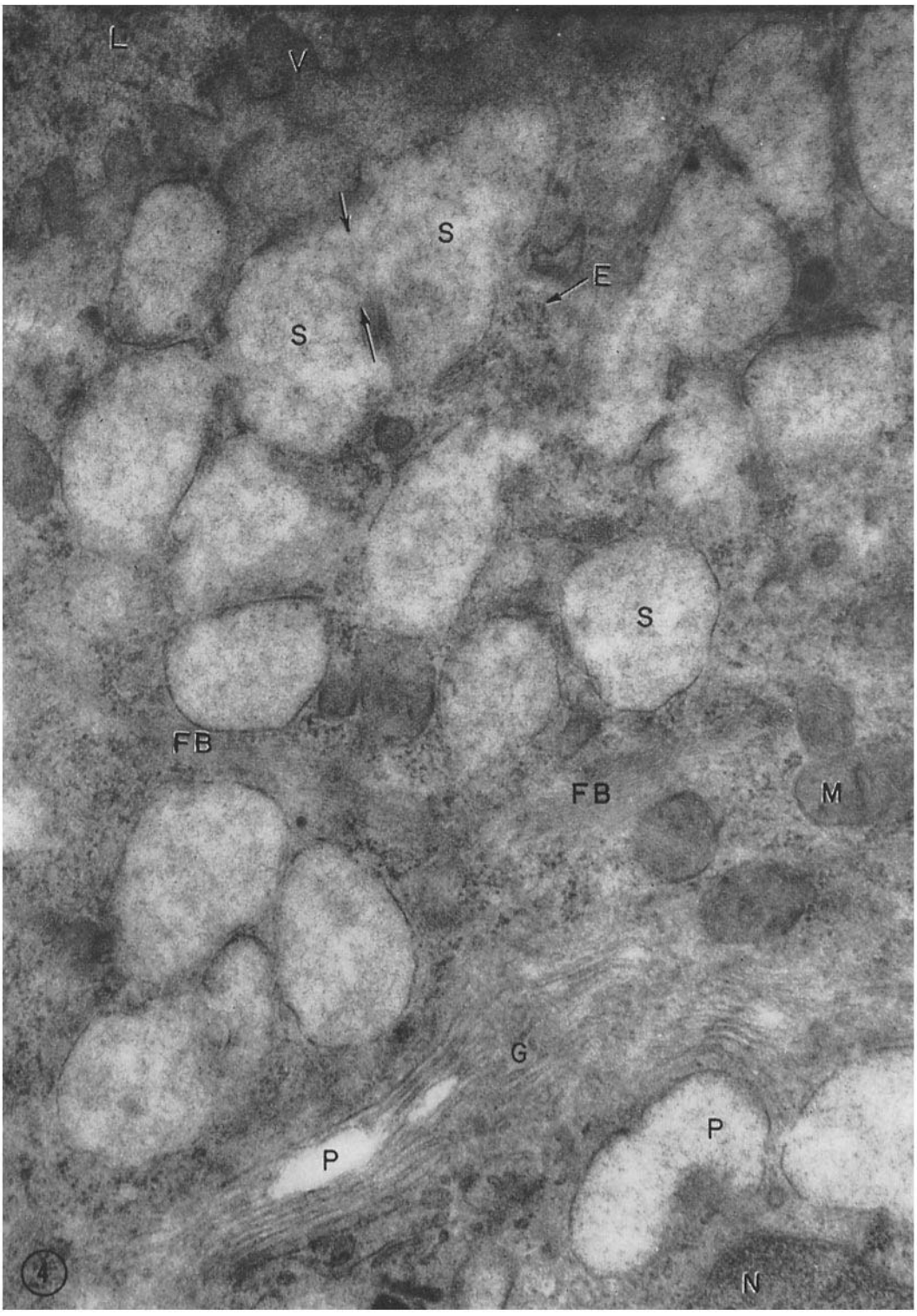
Ultrastructure of Normal Eccrine Duct

The epithelium of the duct of eccrine sweat glands consists of a double layer of cuboidal cells; the two cell layers are designated as surface cells (in contact with the lumen) and basal cells (resting on the basement membrane).

Surface cells have large, irregular nuclei and relatively scant cytoplasm that is largely granular and devoid of intracytoplasmic membranes. The

FIGURE 4

Apical cytoplasm of mucoid cell, Epon-embedded, uranyl acetate–stained. Immediately above the nucleus (*N*) is a large Golgi apparatus (*G*) associated with small vacuoles (*P*) similar in appearance to secretory vacuoles (*S*), and thought to be secretory vacuoles in the process of formation, or prosecretory vacuoles. A definite limiting membrane usually can be seen surrounding secretory vacuoles, but in some areas the vacuoles appear to fuse with one another (arrows). Small ergastoplasmic granules (*E*) are free in the cytoplasm. Occasional bundles of fibrillar material (*FB*) course through the cytoplasm. The contents of the lumen (*L*) are stained intensely with the uranyl acetate. Small microvilli (*V*) project into the lumen from the apical part of the cell. Mitochondria (*M*) are scattered throughout the cytoplasm. × 54,000.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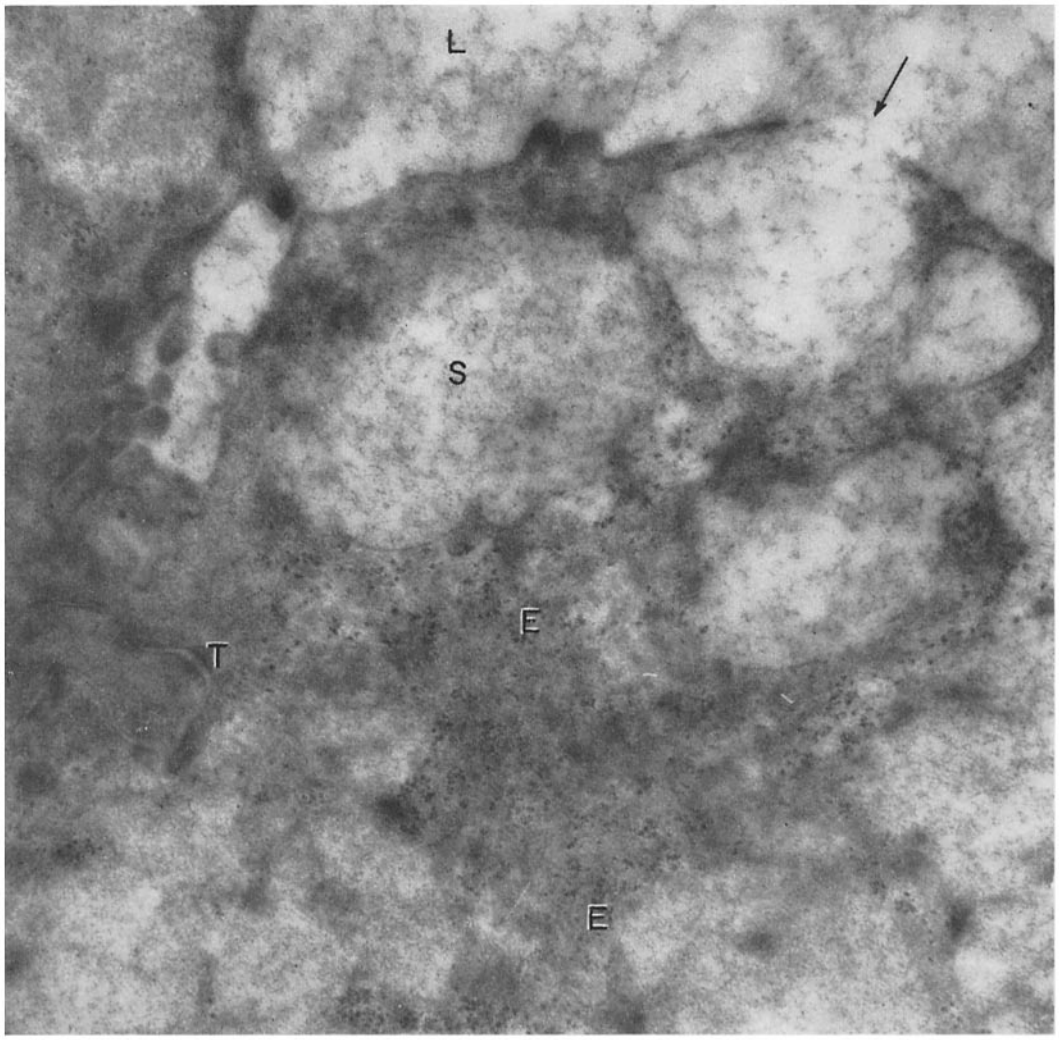


FIGURE 5

Apical cytoplasm of a mucoid cell, Epon-embedded, uranyl acetate–stained. A secretory vacuole (at the arrow) appears to be emptying into the lumen (*L*). Other secretory vacuoles (*S*) appear normal. Terminal bars (*T*) are seen between adjacent mucoid cells as they abut on the lumen. Numerous ergastoplasmic granules (*E*) are present between secretory vacuoles. × 40,000.

cytoplasmic granules are of varying size and density (Figs. 7 and 8). Numerous rod-shaped mitochondria having rare mitochondrial granules are scattered throughout the cytoplasm. A few agranular membranous vesicles are present that might represent a Golgi apparatus, but no discrete Golgi apparatus is identified. The luminal portion of surface cells is specialized into what has been termed the "cuticular border" in light microscopic studies (22). The "cuticular border" appears hyaline and eosinophilic in sections stained with hematoxylin and eosin. By electron microscopy the "cuticular border" is seen to be a dense condensation of small tonofilaments, granules, and small vesicles (Fig. 8) containing occasional mitochondria. The individual filaments are approximately 60 A in diameter and are connected to the numerous desmosomes between adjacent surface cells (Fig. 8). Thus, these filaments are tonofilaments (a terminal web). The structure of

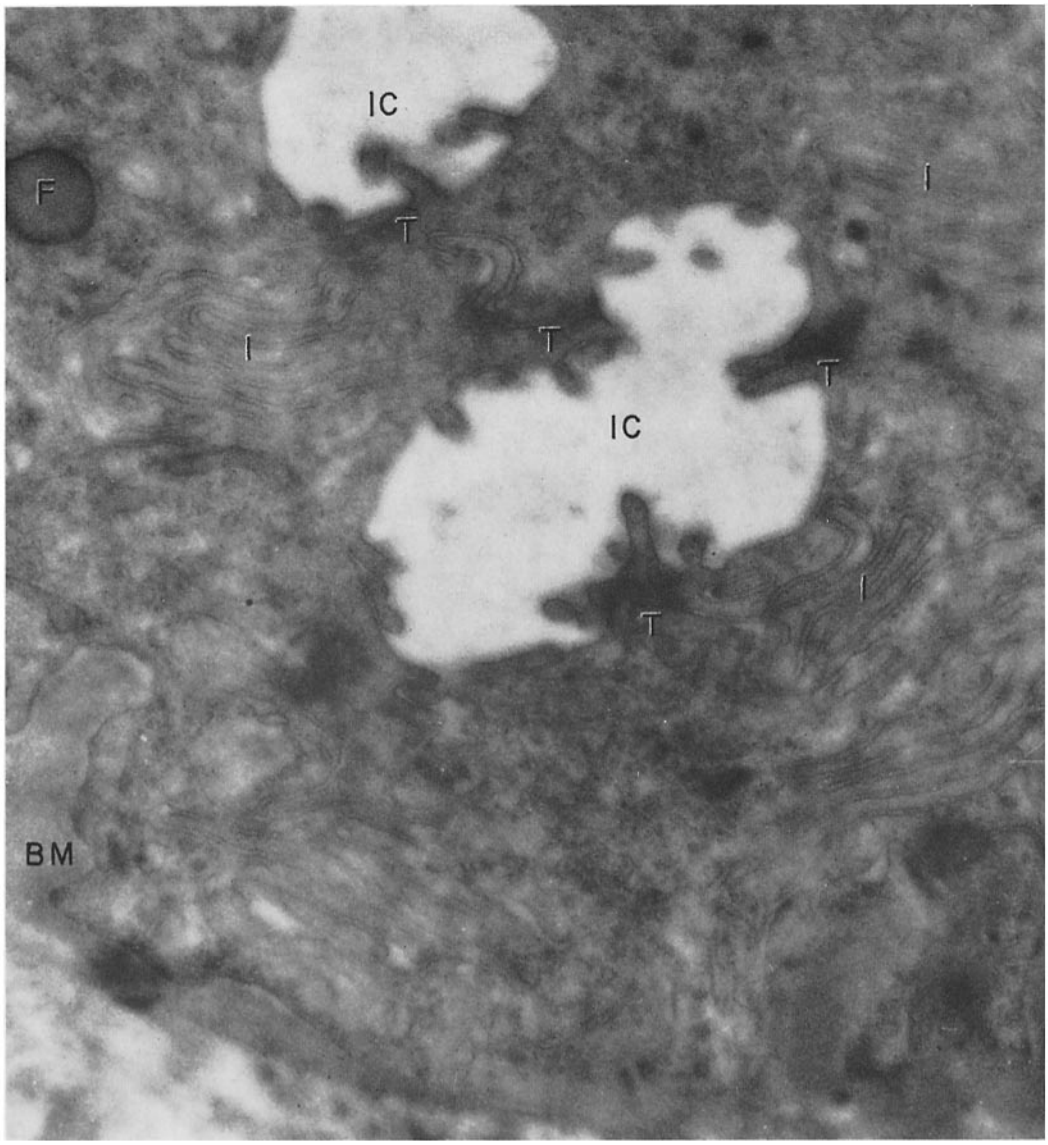


FIGURE 6

Intercellular canaliculus between clear cells in a methacrylate-embedded section. Two portions of the intercellular canaliculus (*IC*) are present and they are surrounded by numerous interdigitations (*I*) of the membranes of adjacent cells. Where these membranes abut on the canaliculus, they appear to have terminal bars (*T*). The background cytoplasm of the clear cell is granular and contains small vesicles. The basement membrane (*BM*) surrounding the gland is prominent and appears to be scalloped. Occasional lipoid droplets (*F*) are present. × 32,800.

the desmosomes of these cells is identical with that of the attachment plaques described by Odland (29) for squamous epithelia (Fig. 8).

The basal cells of the ducts have large, irregular nuclei, and their cytoplasm resembles that of the surface cells in that it is largely granular and devoid of membrane elements. A few vesicular profiles are present which are agranular, but no discrete Golgi apparatus. The mitochondria are more numerous than in surface cells and are

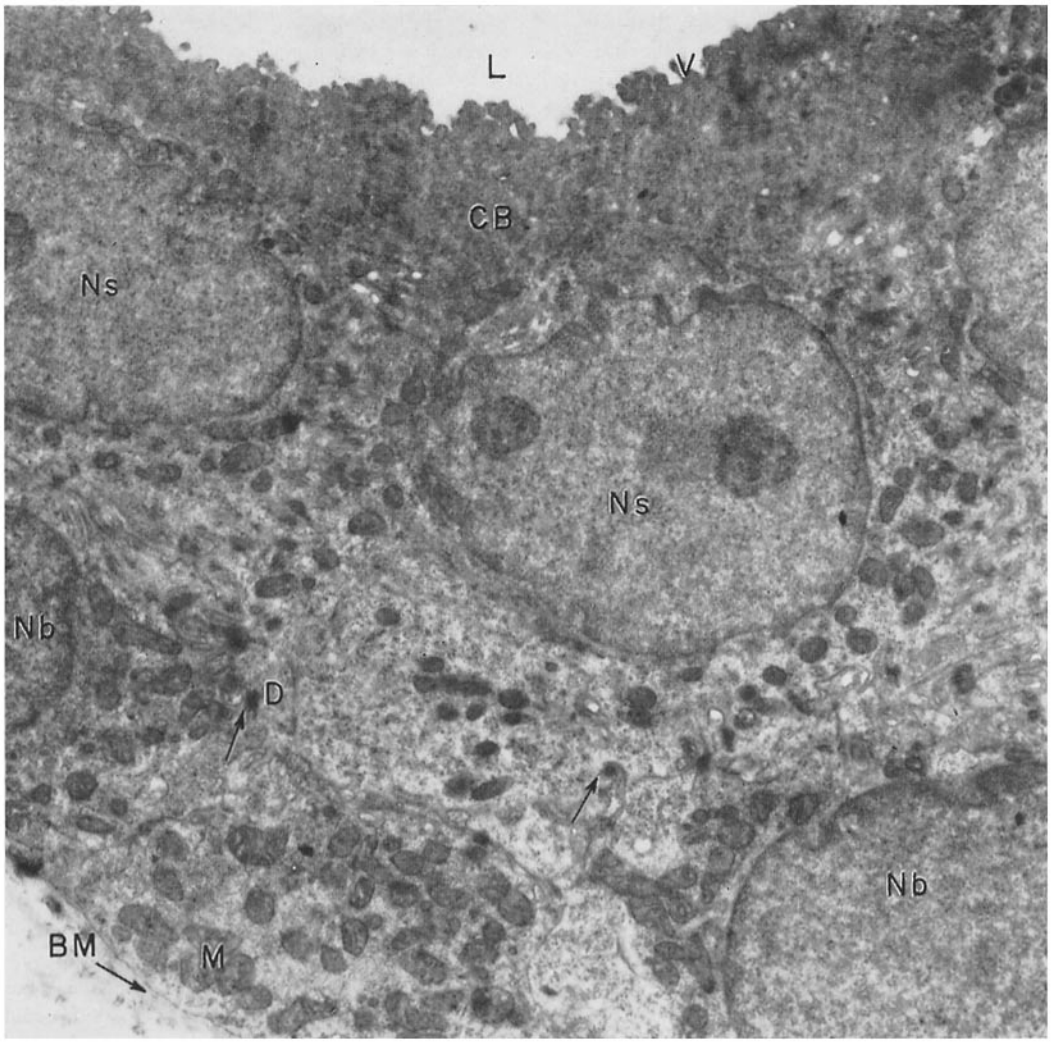


FIGURE 7

Eccrine duct in a methacrylate-embedded section. The wall of the duct is sectioned perpendicularly so as to show the double layer of cuboidal cells that composes its wall. The lumen of the duct (*L*) is at the top, and the basement membrane (*BM*) surrounding the duct is a thin, diffuse line of low density seen at the lower left. The nuclei of surface cells (*Ns*) and basal cells (*Nb*) indicate the extent of the cell layers. The "cuticular border" (*CB*) of surface cells is a prominent, dense band of cytoplasm adjacent to the lumen. Small microvilli (*V*) project from the cell surface into the lumen. Numerous mitochondria (*M*) are present throughout the cytoplasm of both cell types, but they are especially prominent in the basal cells. Desmosomes (*D*) are seen between adjacent cells in the midst of interdigitations of cell membranes. Extending from the cell membranes in the area of desmosomes are dense masses of material similar to tonofibrils in desmosomes of squamous epithelia (arrows). × 13,400.

closely packed, especially at the base of the cell (Fig. 9). Many of these contain mitochondrial granules in section. In no other cells of the entire gland are mitochondrial granules so prominent. Although there are scattered desmosomes between surface cells and basal cells, only rarely do they appear between adjacent basal cells. Basal cells rest on a delicate basement membrane (Fig. 7).

Dermal attachment plaques are occasionally seen on the plasma membrane of basal duct cells, but these are much more prominent in the cat (27).

Around the coiled part of the sweat gland are numerous capillaries, nerves, fibroblasts, and mast cells. Immediately adjacent to the basement membrane surrounding the sweat gland are numerous collagen fibers and elongated cytoplasmic processes of fibroblasts (Fig. 1). Small groups of unmyelinated nerves invested by Schwann cells are found in close apposition to the sweat gland, but nerves have never been identified within the basement membrane. Nerves lacking a Schwann cell investment are difficult to identify with certainty, but suggestive forms have been seen around the sweat glands. A moderately rich network of capillaries surrounds the gland, but capillary endothelium is not intimately associated with glandular epithelium.

Five and Fifteen Minutes after Stimulation

Mucoid (dark) cells show signs of active extrusion of secretory vacuoles, a decrease in the number of apical vacuoles, and contraction of the apical cytoplasm. The limiting membrane of secretory vacuoles frequently appears to have fused with the cell membrane at the luminal border, and in some areas the vacuoles protrude into the lumen, suggesting their extrusion into it (Fig. 10). Often bits of cytoplasmic and membranous debris are seen in the lumen of the gland. The degree to which these changes are visible varies from cell to cell.

In clear cells the major alteration is vacuolization of mitochondria. The cristae of mitochondria appear normal, but "holes" are present between groups of cristae, as though the mitochondrial matrix had dropped out at these points (Fig. 11). Virtually every mitochondrion examined in clear cells demonstrates this change, in both methacrylate- and Epon-embedded material, but differences in the extent of the alteration do exist.

No changes have been seen in the intercellular canaliculi or in the cells of the duct of the sweat gland.

Thirty Minutes and One Hour after Stimulation

The most striking change in the secretory coil is the decrease of density of the cytoplasm of clear cells. The general configuration of the cytoplasmic contents is identical with that of unstimulated clear cells, but the granules that comprise the bulk of the cytoplasm appear to be diminished in number or are separated from one another (Fig. 12). Mitochondrial vacuolization in clear cells is more pronounced at 30 minutes than at 5 minutes after stimulation, and in some cells scant cristae are arranged only around the margin of mitochondria. At this stage the mitochondria appear to be swollen and there is an extreme loss of matrix. Intercellular canaliculi between adjacent clear cells appear occasionally to be dilated. In general, these three changes (decrease of cytoplasmic density, vacuolization and swelling of mitochondria, and dilatation of intercellular canaliculi) occur together in a given clear cell, but they occasionally exist singly. One hour after stimulation, mitochondrial vacuolization is much less.

Mucoid (dark) cells 30 minutes after stimulation show a marked decrease in the number of secretory vacuoles (Fig. 12). Secretory vacuoles and prosecretory vacuoles are still prominent in the Golgi zone, although fewer secretory vacuoles are present in the apical cytoplasm.

DISCUSSION

Electron microscopy reveals that two completely different cell types are present in the secretory segment of human eccrine sweat glands. Ito and Iwashige (15) observed two cell types by light microscopy, but they believed that mucoid (dark) cells were actually superficial and rested upon clear cells. By using toluidine blue to distinguish the two cell types, Montagna *et al.* (23) demonstrated that the mucoid (dark) cells did indeed make contact with the basement membrane and that clear cells did make contact with the lumen. The conclusion reached by Cormia and Kuykendall (5), however, that these cell types were merely stages of the same cell could not be disproved.

The basic difference between these two cells, as described by Montagna *et al.* (23), was a considerable amount of cytoplasmic basophilia in mucoid cells which was removed by ribonuclease and thus considered to be ribonucleic acid. The identity of the two cell types was established by comparing serial thin sections for electron microscopy and thick (1 μ) sections for light microscopy after staining with gallocyanin. In electron micrographs, the cells containing numerous apical

secretory vacuoles are mucoid (dark) cells, and the cells between which intercellular canaliculi course are clear cells.

The nature of the secretory products of these two cell types in humans has long been debated (22). Dobson *et al.* (8) have described diastase-resistant, PAS-positive material released from the apical cytoplasm of mucoid (dark) cells during secretion. Lee (17) has described Alcian blue–positive material in the apical cytoplasm of some cells in the secretory segment (24). The ultrastructure of mucoid cells resembles in general that of goblet cells as described by Palay (31). The present study has localized the mucopolysaccharide to the contents of secretory vacuoles and gland lumen. Jirka and Kotas (16) have identified glucosamine as a component of human sweat (23 to 30 μg/ml in stimulated secretion), and the histologic observations are consistent with this finding.

The presence of mucoid cells in the secretory segment of human sweat glands is also consistent with the theory that in the disease cystic fibrosis of the pancreas, which is characterized by pathologic alterations of the mucus-producing glands (pancreatic, bronchial, salivary, lachrymal, Brunner's, etc.) (3), a basic defect of mucopolysaccharide production is present (11). The sweat of patients affected with this condition contains an abnormally high concentration of NaCl and the total osmolarity of the sweat is nearly isotonic, whereas normal sweat is hypotonic (7). A detailed account of the ultrastructure of the eccrine sweat glands in cystic fibrosis of the pancreas will be presented elsewhere.

Clear cells are characterized by their association with intercellular canaliculi. *Inter*cellular canaliculi and *intra*cellular canaliculi have been described in sweat glands (22), but the cellular relationships and the existence of such small channels are difficult to determine by light microscopy. By electron microscopy these channels are found to be definitely intercellular. The evidence for the intercellular nature of these canaliculi is that terminal bars connect the areas of apposition of adjacent cell membranes as they abut on the canaliculus. At no time has a canaliculus been seen without this distinguishing feature.

Both types of cells of the secretory coil demonstrate morphologic alterations accompanying active secretion. The mucoid cells liberate secretory vacuoles into the lumen, as evidenced by a decrease in the number of secretory vacuoles in the cytoplasm, fusion of secretory vacuoles with the cell membrane at the luminal border, and bulging of the vacuoles into the lumen. Thus, the mucopolysaccharide contained within the secretory vacuoles, in addition to other unidentified components, is liberated into the lumen following stimulation (16).

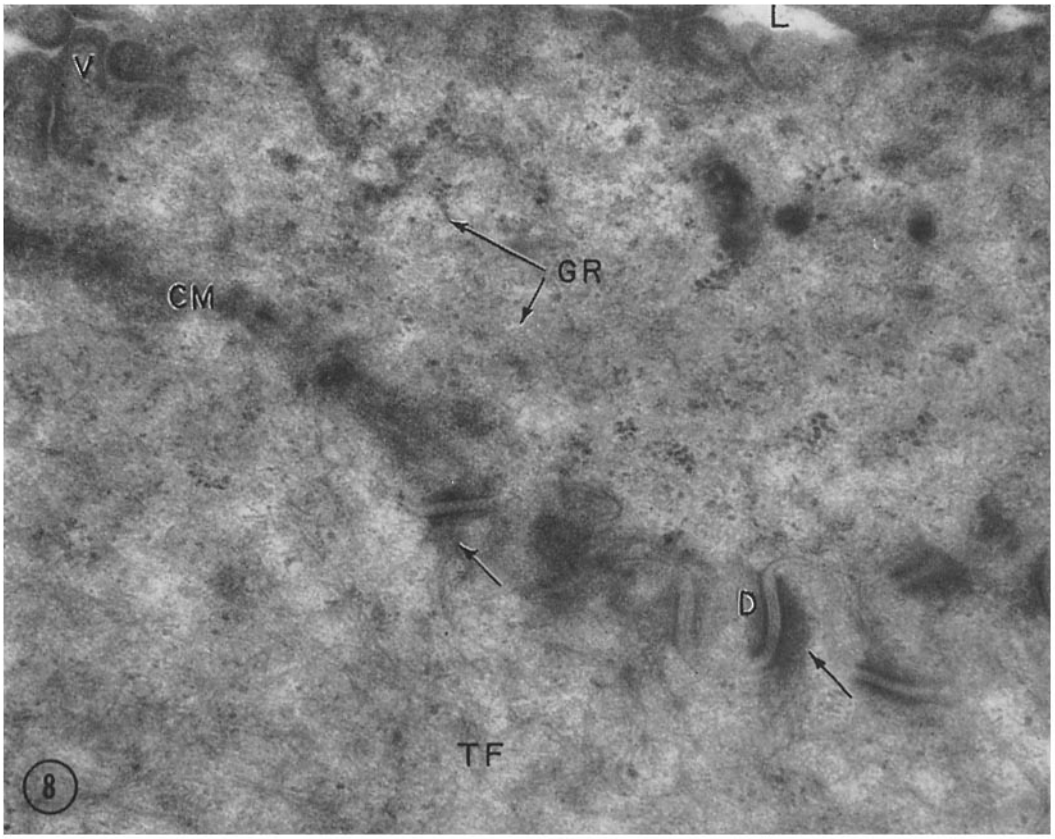
Clear cells also demonstrate morphologic changes that can be correlated with functional alterations accompanying the state of active secretion. Mitochondrial vacuolization in clear cells is the earliest change detected after stimulation, occurring in material biopsied 5 minutes after stimulation and still being evident 30 minutes after stimulation. Mitochondrial vacuolization is seen in diverse circumstances (20, 21, 28), however; and at this time no conclusion can be reached

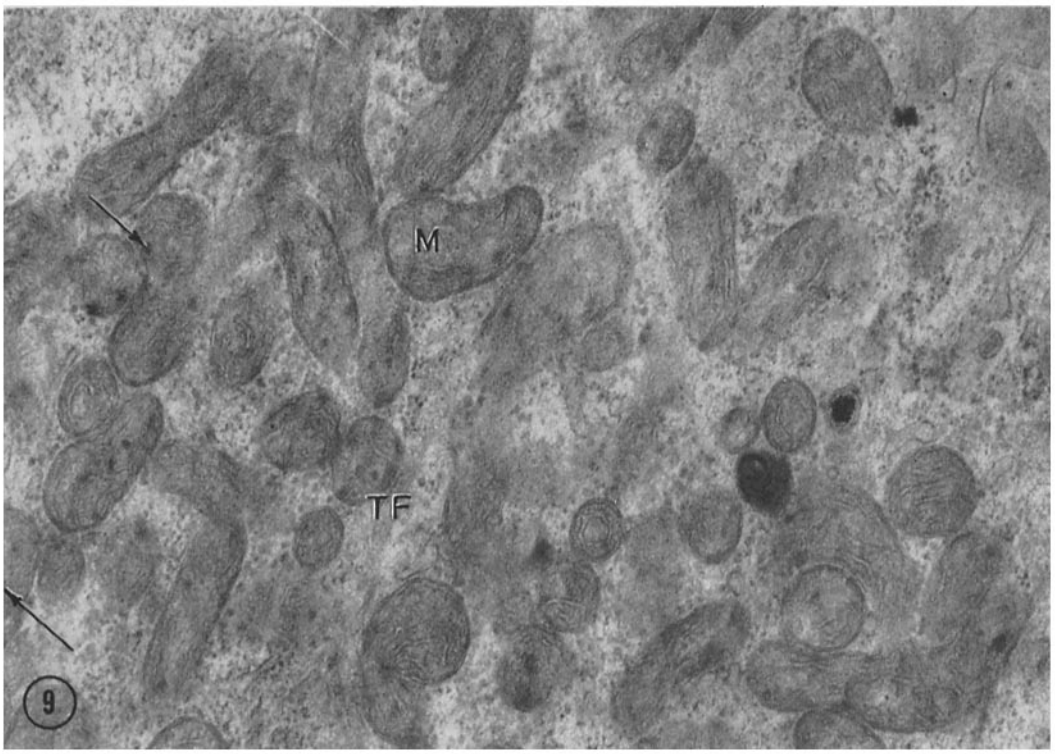
FIGURE 8

"Cuticular border" of surface cells of the duct in an Epon-embedded, uranyl acetate–stained section. The lumen (*L*) is cut tangentially at the upper part of the micrograph. Numerous desmosomes (*D*) connect adjacent surface cells, and inserting into the area of the desmosome are masses of tonofilaments (arrows). Tonofilaments (*TF*) are present in the apical cytoplasm in addition to numerous granules (*GR*) of varying size and appearance. The cell membranes (*CM*) of the adjacent surface cells are cut tangentially. Small blunt microvilli (*V*) project into the lumen. × 52,000.

FIGURE 9

Basal cell of duct in an Epon-embedded, uranyl acetate–stained section. The numerous mitochondria (*M*) with many mitochondrial granules (arrows) are present in the basal cytoplasm of these cells. Small bands of tonofilaments (*TF*) course through the cytoplasm. The background cytoplasm is filled with small granules. × 42,000.


L
V
GR
CM
D
TF
8


M
TF
9

as to what it represents in the eccrine sweat gland other than indicating that stimulation has occurred.

The lessening of the density of the cytoplasm of clear cells which accompanies glandular secretion can be accounted for if it is assumed that the clear cells imbibe water during states of active transport of fluid. If they do transport water and solutes, these clear cells could be either pouring fluid into the lumen of the intercellular canaliculus or actively resorbing components secreted by mucoid cells. The direction of fluid transport cannot be determined on the basis of the present study. Examination of numerous sections of sweat glands, however, gives the impression that an insufficient number of secretory vacuoles exist in mucoid cells to explain the volume of secretion produced by a single gland, which has been estimated to be from 5×10^{-9} to 4×10^{-6} cm^3 in 15 minutes (9). Thus, the most likely conclusion is that clear cells secrete into the intercellular canaliculi a product containing water and various solutes. The function of the intercellular canaliculi would thus be to increase the surface area available for secretion without using the luminal surface of the cell.

Normal human sweat is chemically a hypotonic solution of NaCl containing small amounts of potassium, glucose, amino acids, etc. (32, 33), and it has recently been demonstrated to contain glucosamine (16). Upon stimulation, the concentration of sodium increases as the rate of secretion increases (32). Adams *et al.* (2) have studied the relationship between rate of sweat secretion and the concentration of chemical constituents. On the basis of this study they postulated that two different kinds of sweat exist, one kind predominating at low rates of secretion, and the other kind at high rates. The presence of two different cell types in the secretory coil of eccrine sweat glands could support the thesis of Adams *et al.* (2) that two distinct secretory mechanisms exist, each producing sweat of characteristic composition.

The chemical alterations accompanying secretion can also be considered to be due to active reabsorption of a precursor solution by the eccrine duct. Schwartz and Thaysen (34) came to such a conclusion on the basis of the fact that the initial sample of sweat collected after stimulation always contains a lower concentration of sodium than any subsequent sample. To explain the steadily increasing concentration of sodium achieved after several minutes of secretion, they considered the reabsorptive capacity of the duct to be limited. Basal cells of the duct have large numbers of mitochondria with prominent mitochondrial granules, as compared with other cells of the sweat gland. The unproved hypothesis of Weiss (38) that the mitochondrial granules represent cation exchange resins would certainly be intriguing to invoke in the present case, since sodium reabsorption by the duct would explain the observed chemical changes. A consideration of the ultrastructure and of the chemistry of the secretion of the eccrine sweat glands of the foot pad in cats provides evidence in favor of ductal reabsorption of ions (27).

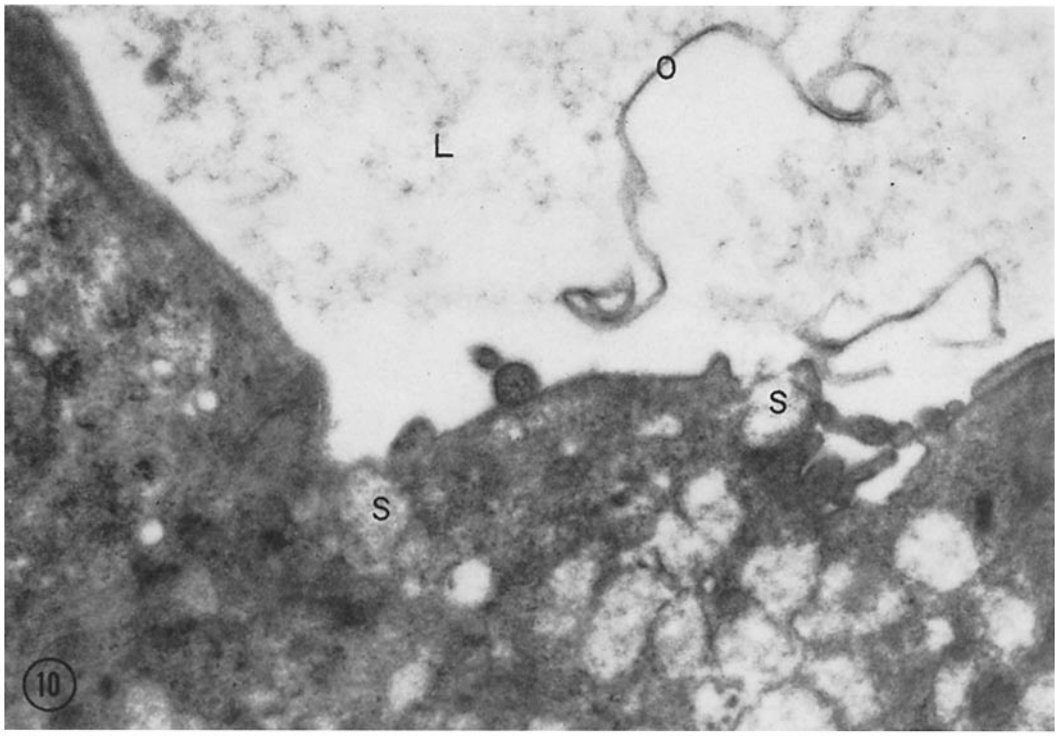
The eccrine sweat glands are seen to be richly innervated when examined by classical silver techniques (22), and cholinesterase is specifically found to be present in a net surrounding the gland (1, 14). As seen in the electron microscope, however, the only identifiable nerves are those which

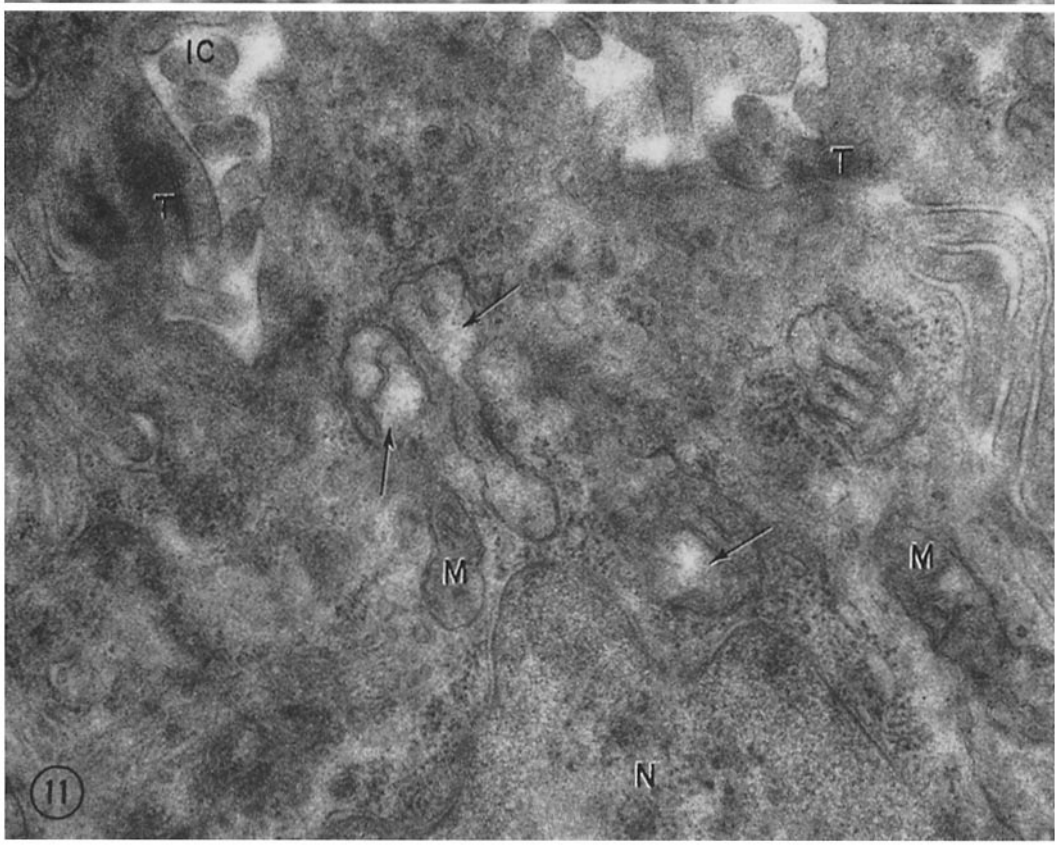
FIGURE 10

Secretory segment 5 minutes after stimulation, methacrylate-embedded section. The lumen (*L*), adjacent to several mucoid cells, contains membranous debris (*O*) in addition to the usual granular and filamentous material. The apical part of the central mucoid cell contains secretory vacuoles (*S*) which appear to be bursting into the lumen. × 30,000.

FIGURE 11

Secretory segment 15 minutes after stimulation, Epon-embedded, uranyl acetate–stained section. The nucleus (*N*) of one clear cell appears in the lower part of the micrograph. Surrounding the nucleus are numerous mitochondria (*M*), the matrix of which has areas of decreased density (arrows). A part of an intercellular canaliculus (*IC*) and the terminal bars (*T*) between adjacent cells as they abut on the canaliculus are present in the upper part of the micrograph. × 42,000.


O
L
S
S
10


IC
T
T
M
M
N
11

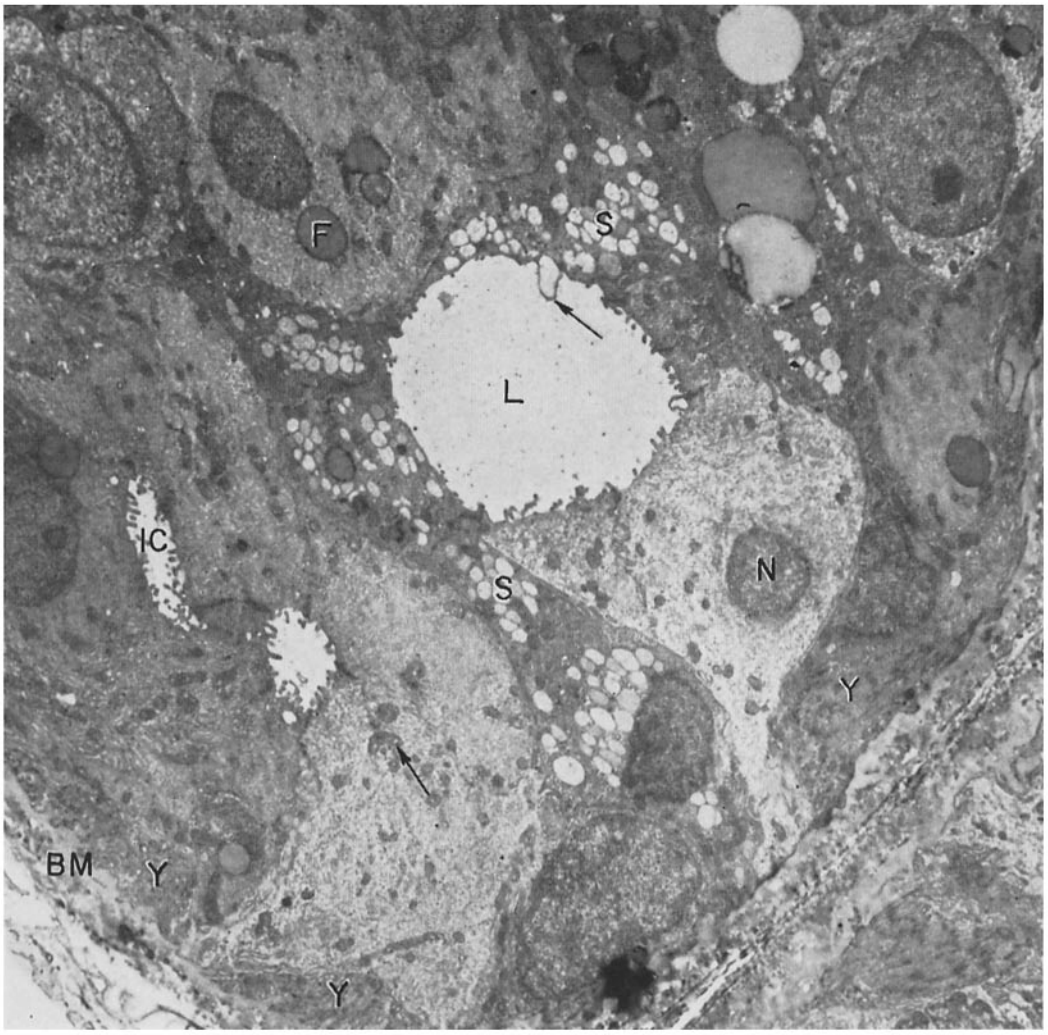


FIGURE 12

Secretory segment 30 minutes after stimulation, methacrylate-embedded section. Secretory vacuoles (*S*) are concentrated in a supranuclear (Golgi zone) part of the mucoid cells. Intercellular canaliculi (*IC*) appear to be dilated. The decrease in density of the cytoplasm of the clear cells that surround the intercellular canaliculi is evident when Fig. 12 is compared with Figs. 1, 6, and 11, and is especially marked in the clear cell with the nucleus labeled (*N*). The mitochondrial vacuolization in clear cells is visible even at this magnification (lower arrow). The lumen (*L*) contains granular material, and a secretory vacuole (upper arrow) is being liberated into the lumen. Lipid material (*F*) is present in both mucoid and clear cells. Myoepithelial cells (*Y*) or secretory cells are in contact with the basement membrane (*BM*). × 6100.

surround the gland; no nerves have ever been seen within the basement membrane. Among glandular tissues, only the salivary and lacrimal glands have been described by electron microscopy as having intimate contacts between nerves and secretory cells (35). Nerves have never been seen in contact with the pancreatic acinar tissue (26), yet the pancreas responds to vagal stimulation. Since the eccrine sweat glands show definite physiological evidence of innervation (33), the

possibility exists that the nerves surrounding the gland release an excitatory substance which diffuses through the connective tissue and basement membrane to the secretory cells without a direct synapse between neuron and secretory cell.

The author wishes to thank Dr. David Bodian, Department of Anatomy, The Johns Hopkins University School of Medicine, for the use of the electron microscope laboratory facilities during a part of this investigation. Assistance in the procurement of human specimens and techniques for stimulation was graciously given by Dr. Robert E. Cooke and Dr. L. E. Gibson, Department of Pediatrics, The Johns Hopkins Hospital.

Received for publication, October 7, 1960.

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