# MICROPLICAE: CHARACTERISTIC RIDGE-LIKE FOLDS OF THE PLASMALEMMA

PETER M. ANDREWS

From the Department of Cell Biology, University of Texas Health Science Center, Southwestern Medical Center, Dallas, Texas 75235

ABSTRACT

Scanning electron microscopy reveals that the free surfaces of stratified squamous epithelial cells lining the alimentary tract, cornea, and conjunctiva exhibit characteristic ridge-like folds of plasmalemma. These microplicae are approximately 0.1–0.2 $\mu$m in width, of variable height (0.2–0.8 $\mu$m) and length, may follow straight or winding paths, often branch, and exhibit a wide variety of patterns over the surfaces of cells. Transmission electron microscopy reveals that microplicae often have a fine (100–150 Å) electron-dense zone subjacent to their plasmalemma and an intracellular matrix characterized by a disorderly array of fine filaments (40–60 Å in diameter). Microplicae appear to arise from plasmalemmal folds which once provided for intercellular interdigitation and desmosome adhesion between adjacent cells. Ruthenium red staining demonstrates that microplicae and interplical grooves are covered with a polyanionic glycocalyx. Although free surface microplicae may merely represent the remnants of intercellular interdigitations or a modified expression of microvillous-like extensions, it is also possible that they serve another specific function. In this regard it is speculated that microplical and interplical grooves may function to hold a layer of lubricating and cushioning mucin designed to protect the underlying plasmalemma from abrasive abuse.

During the course of studying a variety of mammalian organs by scanning electron microscopy, it became apparent that the free surfaces of many of these organs are characterized by similar winding ridge-like folds of the plasmalemma. These microscopic plasmalemmal folds are quite different in appearance from the familiar finger-like microvillous extensions and are probably best termed microplicae. Surfaces which are characterized by microplicae include those of the conjunctiva, cornea, lining oral mucosa, pharynx, esophagus, intermediate zone of the anal canal, and fungiform and circumvallate papillae of the tongue. Although some of these surfaces represent quite different regions of the body, they all have several characteristics in common. First, all of them are wet surfaces consisting of stratified squamous epithelial cells (mainly only partially keratinized or nonkeratinized). Second, they are surfaces that are subjected to considerable abrasive abuse. The present report describes a scanning and transmission electron microscope investigation of microplicae and some of the surfaces that they characterize. The possible origin and functional significance of these unique microextensions are discussed.

## MATERIALS AND METHODS

### *For Scanning Electron Microscopy*

The organs studied during the course of this investigation were taken from adult male and female Rhesus monkeys, domesticated cats, and albino rats. The free (exposed or luminal) surfaces of the cornea, conjunctiva, lining oral mucosa, pharynx, esophagus, intermediate zone of the anal canal, and fungiform and circumvallate papillae of the tongue were fixed *in situ* by flooding them with fixative. The fixative of choice was cacodylate-buffered 3% glutaraldehyde at a pH of 7.2. Immediately after being flooded with fixative, the organs were excised and fixed by immersion for approximately 6 h at room temperature. The organs were then dehydrated through a graded series of acetones and subjected to critical point drying (1, 2). Dried specimens were mounted on aluminum studs, coated with gold (150 Å) in a vacuum evaporator, and studied in either a JEOL JSM-U3 or JSM-35 scanning electron microscope operating at 25 kV.

### *For Transmission Electron Microscopy*

The esophagus and oral mucosa of two adult male domesticated cats and two adult male albino rats were studied by transmission electron microscopy. The organs were fixed *in situ*, excised, cut into small blocks, and fixed by immersion fixation for 6 h. Again, the fixative of choice was cacodylate-buffered 3% glutaraldehyde at a pH of 7.2. After glutaraldehyde fixation, the samples were rinsed in buffer and postfixed in 1% osmium tetroxide for 1½ h. Some of the material was stained during fixation with ruthenium red according to the method of Luft (8, 9). The fixed tissues not stained with ruthenium red were briefly rinsed in water and stained *en bloc* with 5% uranyl acetate for 12h in the dark. After fixation and treatment with either ruthenium red or uranyl acetate, the tissues were dehydrated through a methanol series to propylene oxide and embedded in a mixture of Epon and Araldite resins. Ultrathin sections (50–80 nm), sometimes poststained with uranyl acetate and lead citrate (14), were studied in a Philips 200 electron microscope operating at 60 kV.

## OBSERVATIONS

### *Scanning Electron Microscopy*

The morphology of microplicae on the free (exposed) surfaces of stratified squamous epithelial cells lining the alimentary tract (oral mucosa, oral and laryngeal pharynx, esophagus, papillae of the tongue, and intermediate zone of the anal canal) varies from organ to organ and species to species. Some general characteristics of these microplicae, however, are as follows. Microplicae lining the alimentary tract are approximately 0.1–0.2 μm in width, 0.3–0.8 μm in height, and exhibit irregular surface outlines (Fig. 2). The lengths, degree of twists and turns and branching of plicae vary considerably. Some plicae, both long and short, follow relatively straight paths. Most, however, follow winding paths and often branch. The latter range from simple U-shaped or circular patterns to very winding configurations with numerous branches and subbranches. Figs. 1, 3, and 4 depict microplicae on the luminal surfaces of the esophagus, inner cheek, and fungiform papillae of the tongue, respectively. In an earlier publication (3), I presented a scanning view of microplicae lining the pharynx. Although quite different patterns of plicae may be observed on adjacent cells (Fig. 1), in general, cells in a given region exhibit similar patterns of plicae. It is interesting to note that many of the patterns of plicae observed in this investigation are not unlike the patterns represented by macroscopic plicae on the palmar surface of the hands and plantar surface of the feet (i.e. finger and foot prints). Like plicae patterns, the densities of microplicae vary over different areas and even on adjacent cells. On some cells, plicae are arranged in tightly knit arrays (Fig. 3), while on others there are relatively wide furrows between adjacent plicae (Fig. 1). The intercellular borders between cells characterized by microplicae are often distinct (usually elevated) and allow one to distinguish irregular polygonal cell surface outlines (Figs. 3 and 4). In areas where old or damaged cells are sloughing off, it is evident that both the underlying surfaces as well as the free surfaces of these cells possess microplicae. It should be noted that occasionally short stubby microvillous projections may also be found intermingled among the microplicae of a given cell. It is also of interest to note that as the lining cells extend into more protected regions (e.g. from the oral to nasopharynx), the microvillous projections become more numerous and longer.

Except for the presence of goblet cells on the bulbar and palpebral conjunctiva, the free surfaces of the cornea and bulbar and palpebral conjunctiva appear very similar in their surface topographies (Figs. 5 and 6). Microplicae characterizing the free surfaces of these organs are not quite so tall (0.2–0.3 μm in height) and do not exhibit the occasional long lengths exhibited by microplicae lining the alimentary tract. Except for these fea-

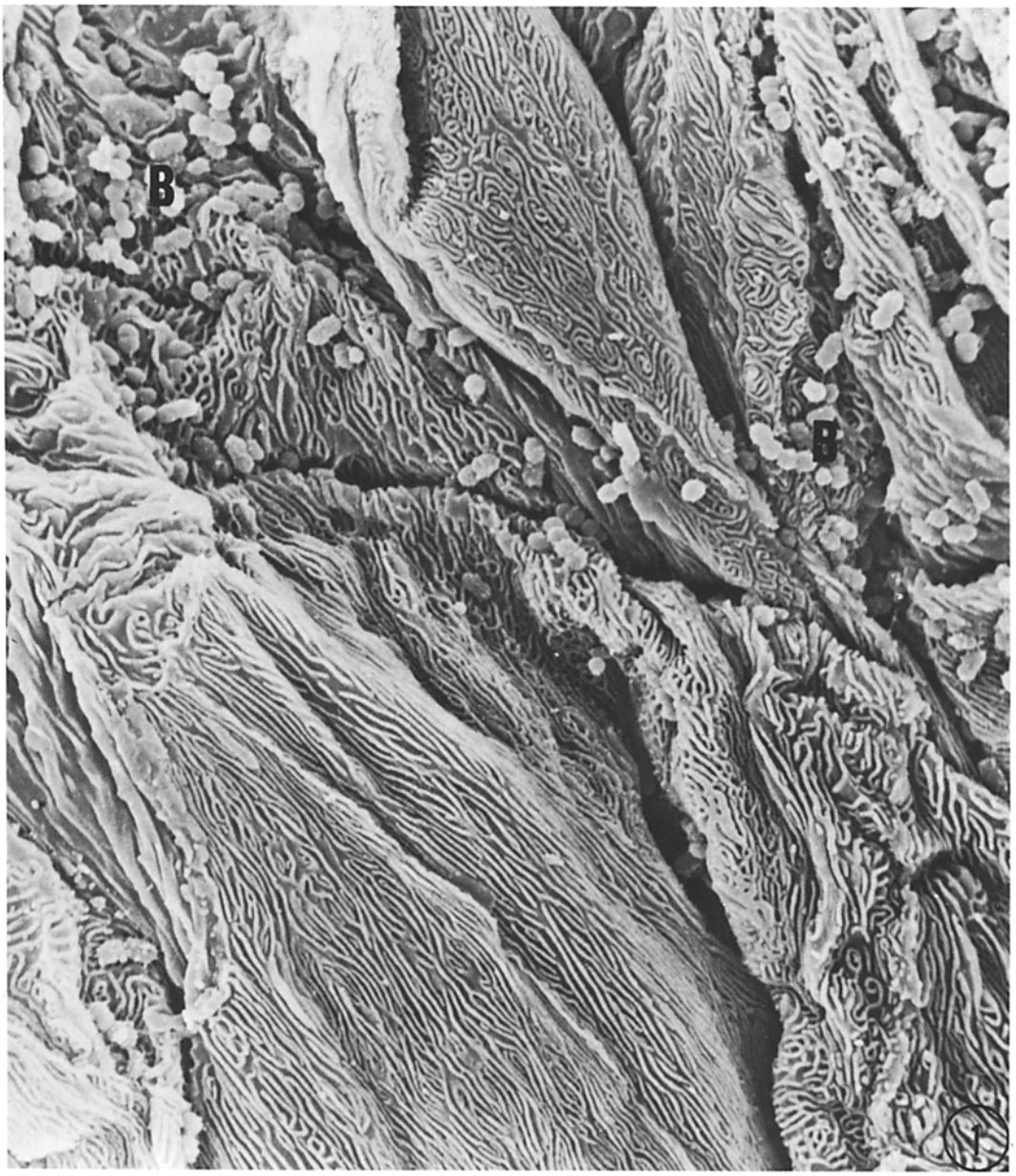



FIGURE 1 Luminal surface of the esophagus (rhesus monkey). Note the variety of plicae configurations, lengths, and densities exhibited by the cells depicted here. Variable numbers of bacteria (*B*) also line the esophageal lumen. × 7,000.

tures, the characteristics of microplicae on these surfaces are very much the same as for the previously described microplicae present on cells lining the alimentary tract. In addition to possessing microplicae, many cells lining the exposed surfaces of the conjunctiva and cornea also possess short (nubby) microvillous projections (Fig. 6).

An interesting feature which is found only on the conjunctival and corneal surfaces is the presence of numerous shallow microcraters (Fig. 5). These craters are usually circular in outline and are confined to the boundaries of a given cell. The lips of the craters are slightly elevated and relatively smooth. The crater's center appears to be the

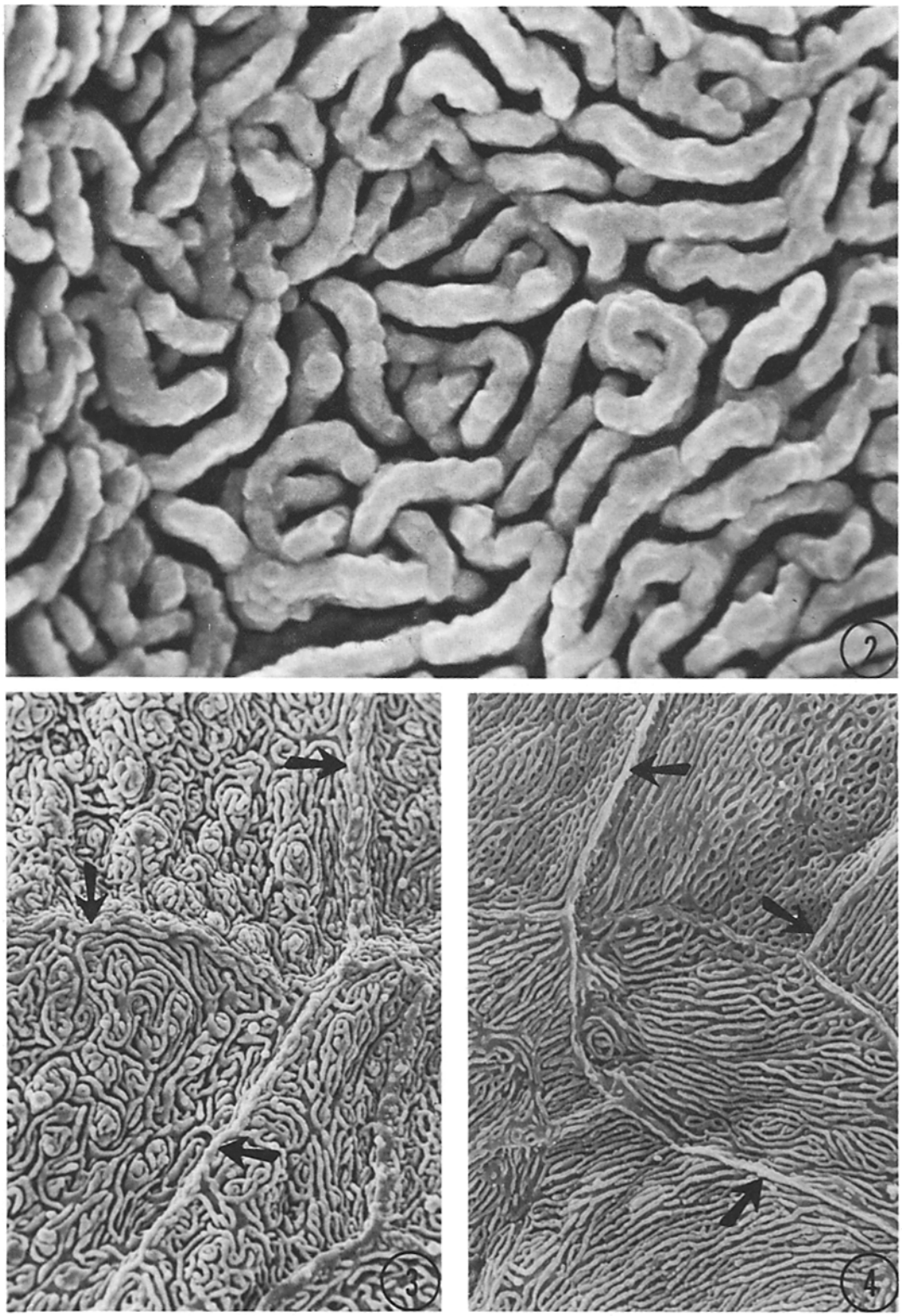


FIGURE 2 High magnification view of microplicae present on the free surface of the inner cheek (rhesus monkey). Note the irregular surface outlines exhibited by plicae (some of the roughness depicted here may have resulted from gold coating the specimen). × 30,000.

FIGURES 3 and 4 The microplicated free surfaces of superficial squamous cells lining the inner cheek (rhesus monkey) (Fig. 3) and fungiform papillae (rhesus monkey) (Fig. 4). Some of the elevated intercellular borders between cells are indicated by arrows. Fig. 3, × 7,500; Fig. 4, × 7,000.

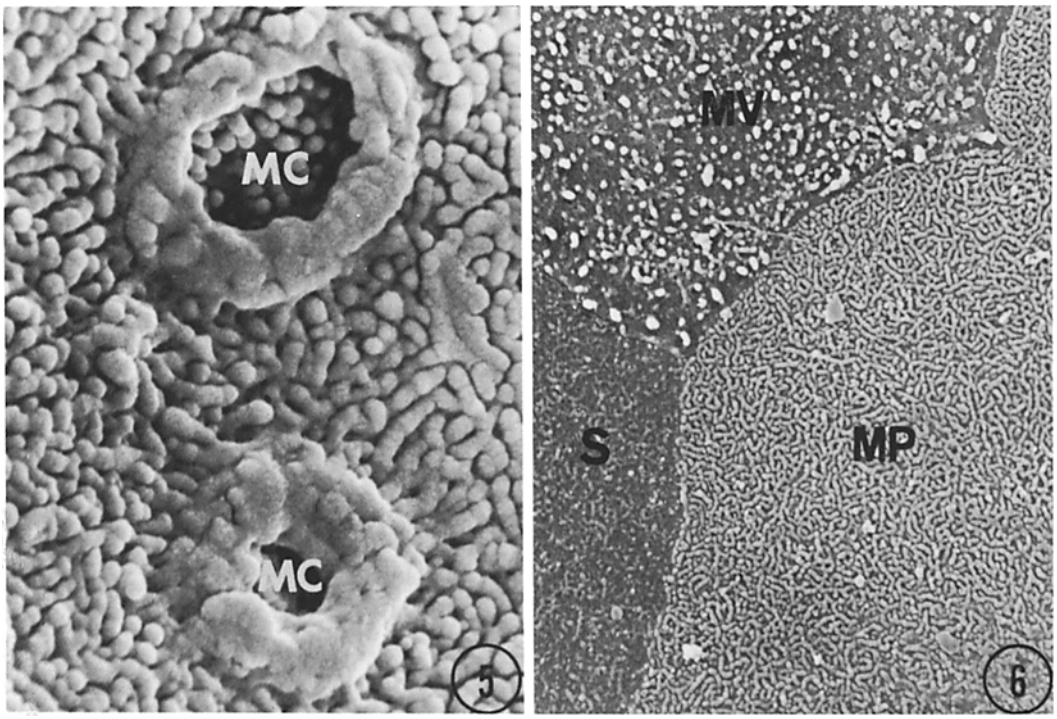



FIGURE 5 Two microcraters (*MC*) on the microplicated free surface of the bulbar conjunctiva (rat). × 22,000.

FIGURE 6 The free surfaces of three adjacent corneal cells (rat). One cell possesses microplicae (*MP*), another short irregular microvilli (*MV*), and a third cell exhibits a nearly smooth free surface (*S*). × 6,000.

microplicated or microvillous surface of an underlying cell. These microcraters may represent a response to damaged regions of a cell or a unique way in which old or damaged corneal and conjunctival cells slough off. In either case, they appear to be an efficient way to repair old or damaged areas without exposing rough cellular edges and thereby disrupting the otherwise smooth integrity of these surfaces. Such integrity is probably necessary when one considers that these delicate surfaces are constantly rubbing over one another.

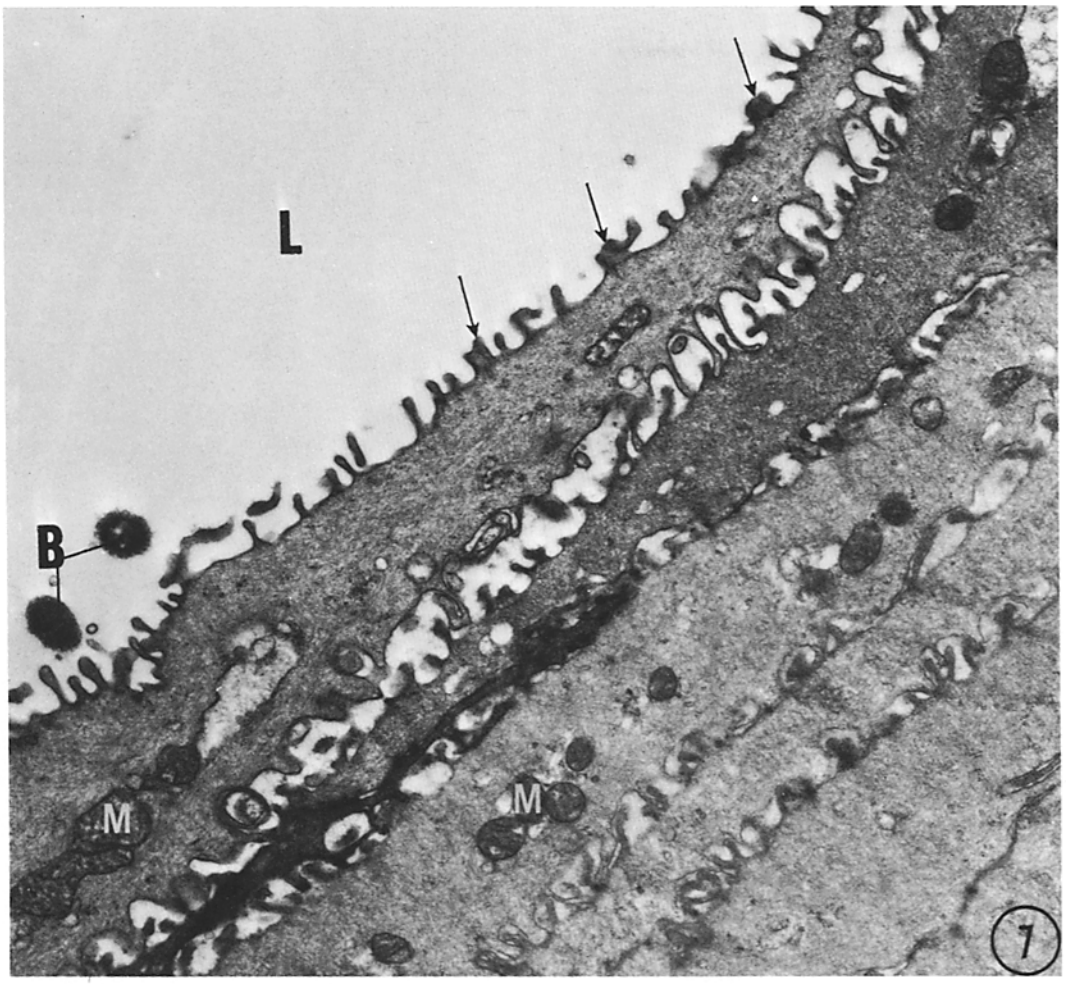
### *Transmission Electron Microscopy*

In thin section, most microplicae appear to be short, irregularly shaped microvillous projections (Fig. 7). This is due to the fact that, when sectioned, microplicae are usually cut in cross or tangential section with respect to their long axis.

FIGURE 7 Thin section through squamous cells lining the esophagus (cat). Although most free surface microplicae appear like irregular microvillous projections in thin section, some (arrows) have been sectioned tangentially enough to give some impression of their lengths. Note that microplicae present on the basal aspect of superficial cells interdigitate with plicae present on underlying cells. The cytoplasm of these lining squamous cells exhibit dense arrays of tonofilaments, and occasional mitochondria (*M*) and cytoplasmic vesicles. *B*, bacteria; *L*, esophageal lumen. × 26,000.

FIGURE 8 Thin section through microplicae lining the esophagus (cat). Sometimes plicae exhibit a filamentous surface coat as depicted in this micrograph. Plicae are also characterized by an electron-dense region (arrows) subjacent to their plasmalemma and by disorderly arrays of fine filaments in a cytoplasmic matrix of variable electron density. × 70,000.

FIGURE 9 A thin section through esophageal free surface microplicae which were treated with ruthenium red (cat). Note the densely staining glycocalyx that coats the plical and interplical surfaces. *B*, bacterium, × 65,000.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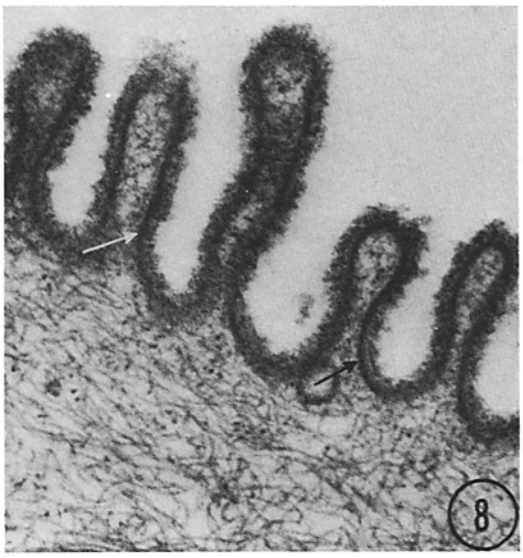
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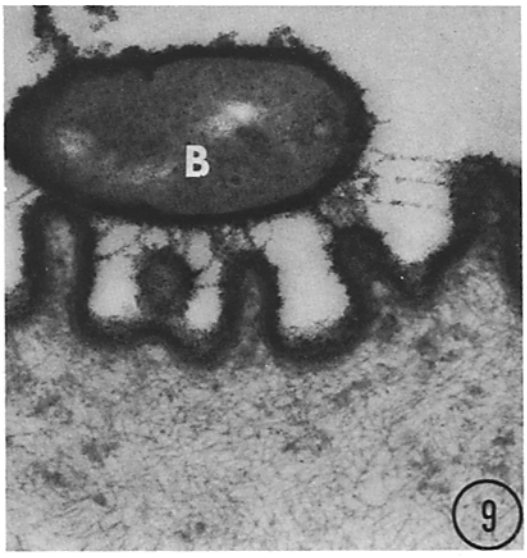
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Some, but not all, plicae possess a very fine filamentous surface coat (Fig. 8). Just subjacent to the plical and interplical plasmalemma there is often a thin (100–150 Å) electron-dense zone (Fig. 8). It may be that this electron-dense region serves to somehow reinforce the integrity of the plasmalemma against the environment. The intracellular plicae matrix consists of a disorderly array of fine filaments (40–60 Å) in a cytoplasmic matrix of variable electron density (Fig. 8). The rest of the cytoplasm of superficial lining cells is characterized by dense arrays of tonofilaments and few if any other cytoplasmic organelles or inclusions other than occasional mitochondria and cytoplasmic vesicles of variable sizes (Fig. 7). Staining with ruthenium red reveals that a polyanionic surface coat (glycocalyx) is present on microplicae (Fig. 9). This surface coat, which is probably acid mucopolysaccharide in nature, also uniformly covers the rest of the cell's exposed (free) surface.

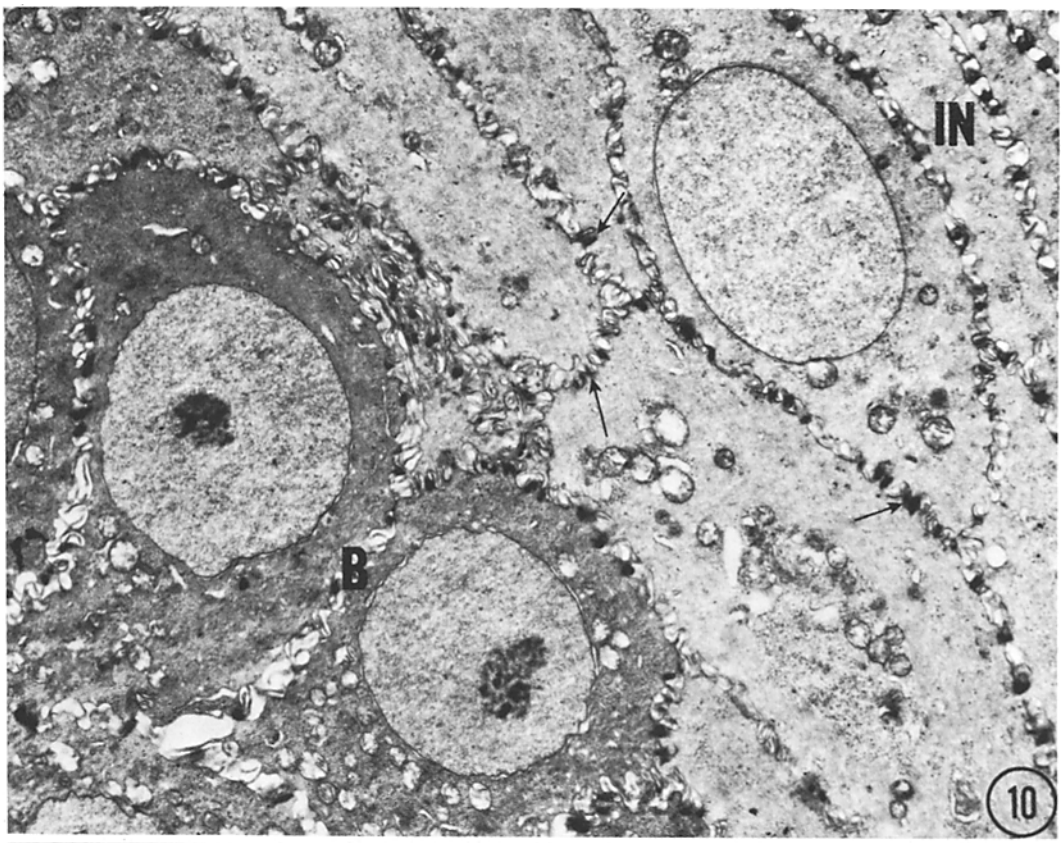
It is evident from thin-sectioned material that microplicae occur not only on the free surface of superficial stratified squamous cells but also on the basal aspect and sides of these cells as well (Fig. 7). Also evident is the fact that microplicae characterize all surface aspects of underlying cells down to and including the basal cell layer (Fig. 10). The basal cells, unlike overlying cells, are more rounded in surface outline and possess a full complement of typical cytoplasmic organelles. The microplicae on these cells interdigitate with and form desmosome-like adhesions with plicae of adjacent cells. The overlying flattened daughter cells still possess numerous cytoplasmic organelles and also exhibit numerous desmosome-like adhesions between closely interdigitating microplicae (Fig. 10). The desmosome-like adhesions between plicae are characterized by an electron-dense layer immediately subjacent to the plasmalemma, with tonofilaments converging upon these regions from the adjacent cytoplasm (Fig. 11). As the cells in the deep layers are displaced toward the luminal surface, they become increasingly flattened, lose most of their organelles, and exhibit a more dense array of tonofilaments. Microplicae on the basal aspect of the surface layer of cells still interdigitate to varying degrees with plicae on the dorsal aspect of underlying cells. These plicae interdigitations are, however, usually not so tight as in underlying layers, and desmosome adhesions are found only occasionally (Fig. 7).

## DISCUSSION

Although microplicae are an outstanding feature of cell surfaces, until recently they have been largely overlooked in the literature. One of the reasons for this oversight is that in the ultrathin sections studied by transmission electron microscopy, microplicae are easily mistaken for short, irregularly shaped microvilli. Although microplicae may, in fact, represent a modified expression of microvillus-like extensions, unlike most microvilli, microplicae do not have a cytoplasmic core characterized by straight microfilaments running parallel to their vertical axis. Also, microplicae do not usually exhibit the dramatic filamentous surface coats (7) and so-called terminal webs often associated with microvilli comprising brush borders.

The question arises as to the possible functional significance of microplicae. From the present investigation, it would appear that microplicae present on the free surfaces of superficial stratified squamous cells represent the same plasmalemma folds which once provided for intercellular interdigitation and adhesion between adjacent cells. In the underlying cell layers, such interdigitations and adhesions are probably necessary to maintain intercellular organization and integrity between adjacent cells. It has previously been shown that a variety of other cell types also possess interdigitating microplicae for apparently similar reasons (10). As the squamous cells are displaced toward the surface, microplicae remain but the tightness of plicae interdigitations as well as the number of desmosomes between plicae are significantly reduced. This, of course, permits the superficial layers to be more easily sloughed off and replaced by underlying cells. In view of these observations, one might conclude that microplicae present on the free surface of lining cells may merely represent nonfunctional remnants of plasmalemmal folds which once provided for intercellular interdigitation and adhesion. Although this may very well be the case, it is also possible that microplicae may continue to serve a useful purpose as a free surface microprojection.

As mentioned in the introductory paragraph, one of the characteristics that many microplicae-covered surfaces have in common is that they are regions which are normally subjected to considerable abrasive abuse. That is, these surfaces are



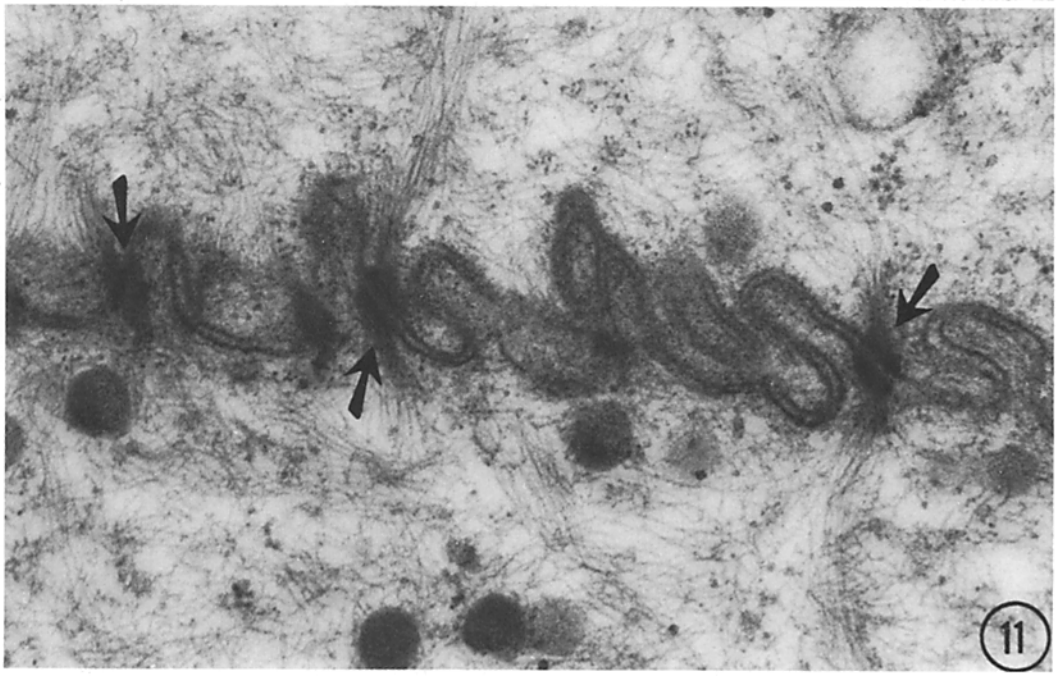




FIGURE 10 Basal (*B*) and intermediate (*IN*) layers of stratified squamous cells lining the esophagus (cat). At this level in the epithelium microplicae interdigitate and form desmosome-like junctions (some of which are indicated by arrows) with microplicae of adjacent cells. × 5,200.

FIGURE 11 Higher magnification view of desmosome-like junctions (arrows) between microplicae present on cells in the intermediate layer of the esophagus (cat). × 48,000

normally rubbing against either other surfaces or foreign objects. One might expect, therefore, that in order for the delicate plasmalemmas of lining cells to endure such abuse, they may be provided with a means by which to reduce friction. As noted in the present investigation, there is often an electron-dense zone subjacent to the lining cell's plasmalemma which may serve to reinforce the integrity of this plasmalemma. In addition, we would like to suggest that microplicae may also provide protection by (*a*) reducing the surface area of contact and thereby frictional resistance between opposing surfaces and by (*b*) holding by means of a polyanionic acid mucopolysaccharide surface coat and interplical grooves (microvalleys) a layer of cushioning and lubricating mucin. Another interesting point suggesting that microplicae may be a specific response to environmental abrasive abuse is indicated by the presence of similar macroscopic plicae patterns on the palmar surface of the hands and plantar surface of the feet (i.e. finger and foot prints). Here, again, are two regions of the body subjected to considerable abrasive abuse where nature has repeated itself at a macroscopic level in the production of plicae. It should be noted, however, that since microplicae also occur on some surfaces which are believed to be not subjected to surface abuse (5, 6), the above theory can only be considered speculative.

As a result of this investigation, it would appear that microplicae may be a ubiquitous feature of partially keratinized or nonkeratinized stratified squamous epithelial cells. It should be kept in mind, however, that other types of cells may also possess microplicae. We found, for example, that the so-called nonstratified cuboidal "dark" or "intercalated" cells lining kidney collecting tubules often exhibit microplicae (5). Even cultured cells exhibit plicae-like microprojections which are more commonly referred to as "ruffles" (13). Price (13) noted that ruffles usually arise along the leading edge of cells, migrate over the cell's central region, and are apparently involved in the endocytotic uptake of particulate material. There is, to our knowlege, no evidence at present that the microplicae studied in the present investigation are also involved in endocytosis.

From the observations made during this and a previous investigation (3), several relationships between the environment and the surface microextensions of cells have become apparent. When the outermost stratified squamous cells are exposed to air and thereby lose their wet mucin coat, these cells become fully keratinized and are without surface microprojections. One might consider the transitions from the inner wet to the outer dry orifices of the alimentary tract (mouth and anus) and the transition from the inner wet conjunctiva to the outer eyelid as examples of this. It is not unlikely that the lack of microprojections on these dry surfaces is due to their destruction by surface tension (resulting from drying) once exposed to air (12). When stratified squamous cells are protected by a liquid layer but are still subjected to considerable abrasive abuse, they apparently exhibit the microplicated free surfaces described in the present investigation and usually a significantly lesser degree of keratinization. Finally, when these same cells are in more protected regions of the body, such as the nasopharynx or anterior nasal cavity, microplicae appear to be replaced by longer finger-like microvillous projections and the cells exhibit even fewer signs of keratinization. In view of these observations, it would be most interesting to study the morphological alterations of cells' free surfaces in a given region when exposed to a variety of environmental situations.

In regard to the cornea of the eye, it should perhaps be noted that our contention that microplicae characterize these cells directly contradicts the conclusions drawn from another scanning electron microscope investigation of the cornea (11). In this earlier study, the author presented micrographs depicting corneal microplicae but contended that these plicae were merely microvilli lying down on the plasmalemma. We believe, of course, that this is a misinterpretation and demonstrates a lack of confidence in the critical point drying procedure which we have previously found capable of preserving the finest of microvillous extensions (12) and even the configurations of isolated inner mitochondrial membranes (4). We do, however, agree with the author's contention that corneal microextensions might aid in holding a protective tear film over the delicate corneal surface.

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