DEMONSTRATION OF THE FUZZY SURFACE COAT OF RAT INTESTINAL MICROVILLI BY FREEZE-ETCHING

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Interest in understanding the structural and functional organization of the surface coat of cell membranes of animal, plant, and bacterial cells has been widespread. In particular the "fuzzy" surface coat of intestinal epithelial cells has received special attention, mainly for its strategic location at the absorptive surface of the cell and its unique structural organization as shown by the studies to date (e.g. 2–5, 7, 11). However, much of this knowledge has been obtained from thin sections of conventionally processed tissues, and considerable differences have been observed in the organization of the surface coat (3, 4, 9). From these investigations it seems uncertain whether such differences in the organization of the surface coat demonstrate in vivo differences or artifactual differences due to variations in the tissue processing methods. It was felt, therefore, that an attempt to demonstrate the surface coat by a technique alternative to conventional thin sections, such as freeze-etching, would be of value in resolving this uncertainty.

Previous freeze-etch studies have not yielded sufficient information to clarify details of the structural organization of the surface coat of epithelial cells or to enable meaningful comparison with our existing knowledge from thin-section techniques. Mukherjee and Staehelin (8) found that the surface coat of mouse intestine could not be readily identified in their freeze-etch preparations. Ito (4) reported that the surface coat of aldehyde-fixed cat intestinal mucosa was not visible if tissue specimens were glycerinated before freezing, but was visible if fixed specimens were frozen in distilled water. Ito's results showed the surface coat as consisting of a loose meshwork of filaments—a structure difficult to correlate with the surface coat structure seen in thin sections. Here we report that the surface coat of epithelial cells of rat rectum can be demonstrated clearly by standard freeze-etch procedures and that the appearance of the surface coat in replicas differs substantially from that found previously in both thin-section and freeze-etch studies.

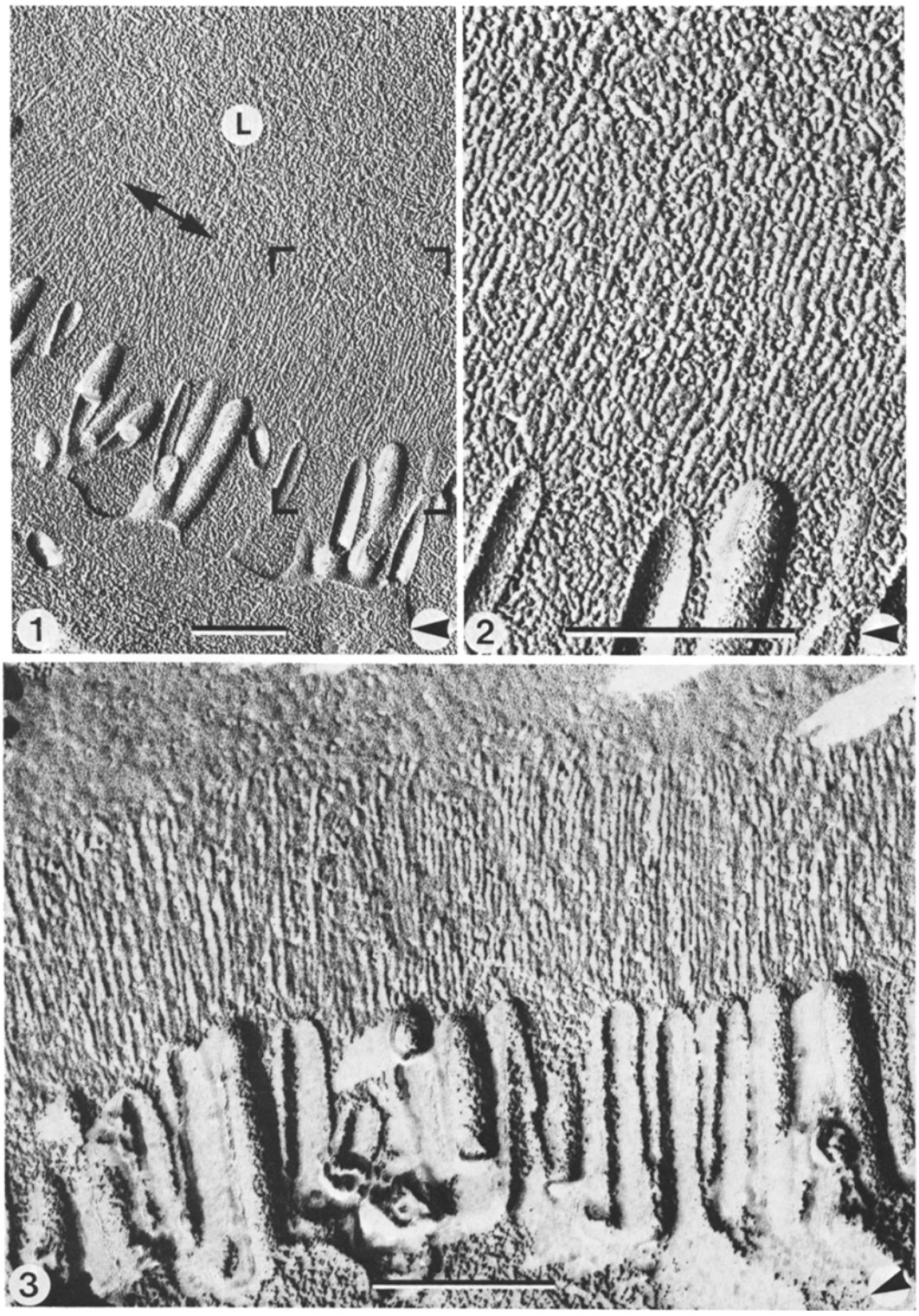
MATERIALS AND METHODS

For freeze-etching, small portions of mucosa from the rectum of D. A. Brown strain rats were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h at room temperature, rinsed in buffer, and transferred to 30% glycerol for 2 h. Pieces of mucosa of about 1 mm^3 were mounted on gold specimen disks, frozen in liquid Freon-22 at −150°C, and stored in liquid nitrogen. Specimens were fractured at −100°C and etched for 5 s at −100°C in a Balzers freeze-etch apparatus (Balzers AG, Balzers, Liechtenstein). The replicas were cleaned in sodium hypochlorite, mounted on Formvar-coated grids, and examined in a Hitachi HU-11E electron microscope.

For thin sectioning, pieces of glutaraldehyde-fixed mucosa were postfixed in 2% OsO_4 for 0.5 h, dehydrated in ethanol, and embedded in Epon 812. Sections were stained with uranyl acetate and lead citrate.

RESULTS

In replicas where the microvilli were fractured parallel to their long axis, a conspicuous surface coat was seen which formed a layer up to about 0.6 μm in thickness over the tips of the microvilli. Fig. 1 represents the appearance of the surface coat that was observed routinely in replicas. Preparations of the type illustrated in Fig. 3, in which surface coat details are displayed more clearly, were uncommon. (Such preparations probably resulted when ideal conditions of freezing, fracturing or etching, or a combination of all three, were achieved.) In Fig. 3 the extent of the surface coat is clearly defined, but generally the outer limits of the surface coat tended to merge with the granular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structure of the surrounding glycerol medium, making precise identification of the outermost margin of the surface coat more difficult (Figs. 1 and 2). However, the ordered structure within the surface coat region can be distinguished readily from the random, irregular structure of the glycerol medium.

The thickness of the surface coat in freeze-etch preparations was uniform over an individual cell, but showed a wide variation among different cells; stages of development that were seen ranged from no detectable surface coat on some cells through to the maximum thickness of surface coat of about 0.6 μm on other cells.

In replicas the surface coat was composed of filaments which extended, in parallel with each other, from the membrane surface of the tips of the microvilli into the lumen (Figs. 2 and 3). Individual filaments appeared to retain their identity from microvillus surface to termination in the lumen, and there was little evidence of anastomosis or branching (Fig. 3). The center-to-center spacing between adjacent filaments was approximately 195 Å in the replicas examined. In thin sections the surface coat consisted mainly of branching filaments which formed an open meshwork, although some individual filaments appeared straight for part of their length (Fig. 4).

DISCUSSION

This study has established that the surface coat of epithelial cells of rat rectum can be demonstrated successfully by standard freeze-etch techniques. The thickness of the surface coat in replicas was found to be cell-specific; surface coat thickness was uniform for a particular cell, but varied markedly among different cells. These observations are in agreement with previous thin-section studies of surface coat distribution (7, 10). However, freeze-etching reveals some essential differences in the organization of surface coat filaments. In contrast to the close-packed layer of branching surface coat filaments seen in most studies of conventionally processed tissues (2–4, 7), freeze-etching shows long straight filaments without any branching. The only parallel to this observation is found in the human colon, where the surface coat of the epithelial cells lining the surface epithelium showed similar long, nonbranching filaments (5). When samples of the tissues used for freeze-etching in the present study were examined in thin sections, the organization of the surface coat resembled in part the results of previous thin-section studies, i.e. a branching meshwork of filaments, while in some instances the filaments followed a straight course, similar to the appearance in replicas, before branching out as a meshwork. We suggest that this difference in the appearance of the surface coat filaments between thin sections and freeze-etch replicas could be attributed to the rigorous chemical treatments associated with the tissue processing methods for thin sectioning. It appears reasonable to assume that the filaments undergo some modification from a straight to a branched configuration during the post-glutaraldehyde stages of tissue preparation. The possibility that branching filaments may be obscured by the granularity of the glycerol medium is countered by preparations of the type shown in Fig. 3. Here the granularity of the glycerol is minimal, and branching filaments should be clearly visible; no branching filaments were evident in such replicas. If branching filaments were present, some of them might not be seen because of the shadowing, but certainly not all of them would be affected in this way. Ito (4) found that the surface coat of cat intestinal epithelium consisted of a very open meshwork of branching filaments in freeze-etch replicas, and that thin sections also showed a branching meshwork of filaments. However, these structures were not directly comparable since the filament diameter and filament-to-filament spacing were considerably greater in the freeze-etch replicas. Ito's freeze-

FIGURE 1 Freeze-etch replica showing a portion of the luminal surface of an epithelial cell of rat rectum. Surface coat filaments extend from the tips of the microvilli into the lumen (*L*). The double-headed arrow indicates approximately the distance into the lumen to which surface coat filaments reach. In Figs. 1–3 the arrowhead indicates the direction of platinum shadowing. Bar equals 0.5 μm. × 29,000.

FIGURE 2 Higher magnification of the area enclosed by the rectangle in Fig. 1, showing details of surface coat structure. × 70,000.

FIGURE 3 Another replica which illustrates the parallel filaments of the surface coat. × 56,000.

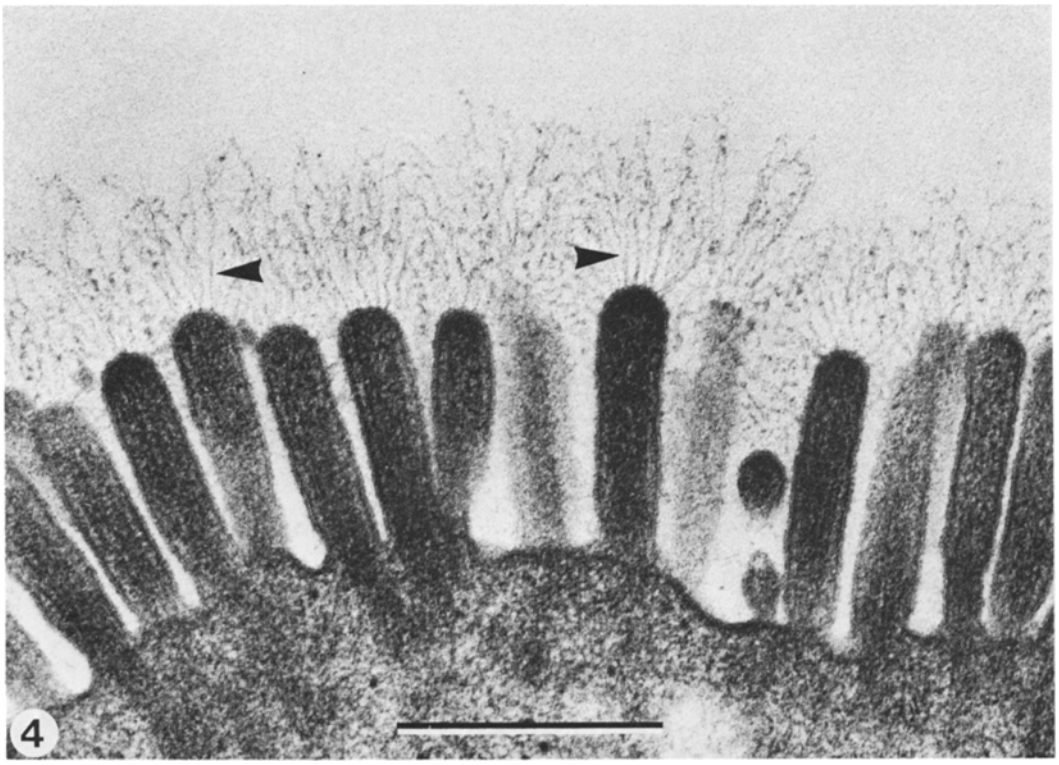

FIGURE 4 Thin section of an area comparable to that shown in Fig. 1. Note that the surface coat consists of both branched and unbranched (arrowheads) filaments. Bar equals 0.5 μm. × 70,000.

etch results may reflect the effect of freezing damage due to ice crystal formation since no cryoprotectant was used.

The difference in the morphology of surface coat filaments between conventionally processed and freeze-etched tissues raises some doubts as to the in vivo organization of the surface coat. Clarification of this aspect of surface coat structure is important since it has been suggested (3, 12) that one major function of the surface coat is to act as a barrier to the penetration of larger food particles closer to the microvillus plasma membrane. Thus, the surface coat would function as a filtration network, allowing only the smaller particles to approach the cell membrane. In view of the nonbranching nature of the filaments observed in freeze-etch replicas, it appears that the surface coat may have a more complex role in addition to the filtration function suggested by previous authors. The significant increase in the surface area available for direct contact with substances in the lumen, resulting from the projection into the lumen of long unbranched filaments, leads us to suggest a greater functional role for the surface coat filaments. Since the surface coat is composed of glycoproteins, it carries a significant amount of negative charge (1) which could act to adsorb positively charged ions in the lumen (5). Thus, long, negatively charged filaments projecting into the lumen would provide a considerable area for the adsorption of various positive ions. Evidence in support of this proposal is provided by recent studies on the attachment of ferritin particles to the surface coat filaments in human colon (6). Further studies are in progress to determine whether the structural organization of the surface coat of epithelial cells of rat rectum described here can be applied in general to the surface coat of intestinal epithelial cells of other species.

SUMMARY

In freeze-etch replicas of epithelial cells of rat rectum, the fuzzy surface coat is composed of discrete filaments which are aligned in parallel with each other and with the long axes of the microvilli.

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