THE ENTERIC SURFACE COAT ON CAT INTESTINAL MICROVILLI

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ABSTRACT

The enteric microvilli of the cat, bat, and man are coated with a conspicuous layer composed of fine filaments radiating from the outer dense leaflet of the plasma membrane. This surface coat is prominent on the absorptive cells but is not so thick on the goblet and undifferentiated crypt cells. In other species the surface coat is poorly developed or inconsistent, but all intestinal microvilli have traces of such a coating over the tips and sides of the microvilli. Tissues prepared by the ordinary sectioning techniques for electron microscopy usually reveal this component when stained with uranyl acetate followed by lead staining. The surface coat is intensely periodic acid-Schiff (PAS) positive and reacts with Alcian blue or Hale's colloidal iron stain for acid mucopolysaccharide. It is also stained by toluidine blue at low pH. Repeated washings or incubation with various chemical agents have failed to remove or markedly alter the appearance of the coating, but extruded cells undergoing autolysis lose their surface coats. The stability, consistent presence, and intimate association of the mucopolysaccharide coat suggest that it may be an integral part of the plasmalemma rather than an "extraneous coat."

A prominent external coating of fine filamentous material is consistently observed on the surface of the cat intestinal striated border. Other species sampled in this survey included the human, rat, mouse, bat, shrew, dog, guinea pig, hamster, rabbit, beaver, gerbil, chicken, frog, and salamander. A thick coat such as that described here for the cat appears to be present in the bat and human intestine but a thinner layer of similar material is present on microvilli of all species examined.

It is hoped that the present study will stimulate further investigations aimed at determining the composition, biosynthesis, and functional significance of the enteric surface coat. Some of the information gained from such studies may further our understanding of the carbohydrate-rich surface layers that are present on many and possibly most cells.

The first fine structural observation of an external coating on the plasma membrane was that of Yamada (53), who published electron micrographs showing delicate filaments, the *antennulae microvillares*, radiating from the microvilli of mouse gall bladder epithelium. More recently, Peachey and Rasmussen (35) described a similar coat on toad bladder epithelial cells and interpreted the filamentous substance as a product secreted by certain cells and adsorbed onto the plasmalemma of the entire epithelium. Choi (11), studying the same tissue, further identified this coating, by histochemical methods, as a substance rich in mucopolysaccharides. A number of observations by different investigators have reported the presence of surface coats on various vertebrate and invertebrate epithelial cells (7, 15, 40, 51). Bennett (3, 4) has suggested that a polysaccharide-rich component is of widespread and possibly of universal occurrence on all cell surfaces. He proposed the general term *glycocalyx* for all such cell coats on microorganisms as well as plant and animal cells.

The prominent enteric surface coat was first observed on the bat intestinal microvilli (19), but extensive studies were not undertaken with this tissue because the animal did not lend itself to the contemplated radioautographic investigations. The cat intestinal mucosa was used for most of our morphological studies and for radioautographic investigations (20) on the biosynthesis of the surface coat that will be reported in a subsequent publication.

MATERIALS AND METHODS

The initial observations of the surface coat were made on intestinal mucosa fixed with s-collidine-buffered osmium tetroxide (5), but it was noted that phosphate-buffered osmium tetroxide (28) appeared to preserve the surface coat better. Most of the material used in this study was therefore fixed with phosphate-buffered osmium tetroxide. Glutaraldehyde (17, 44) followed by osmium tetroxide also proved to be a satisfactory fixative for the surface mucopolysaccharide, but it was not extensively utilized because the mucus of the goblet cells was not consistently preserved. Histochemical tests for the phosphatase reactions were carried out in tissue fixed in glutaraldehyde (17) or cold 10 per cent formalin. Although tissue preservation by formalin fixation followed by postfixation with osmium tetroxide was generally poor, the surface coat could, nevertheless, be demonstrated by this method. Potassium permanganate fixation (22) also preserved it to some extent but rather irregularly.

Tissues were embedded in Epon (23), methacrylate, or Araldite. Sections showing silver to gold interference colors were used for electron microscopy, and thicker sections from the same blocks were stained for study by light microscopy. The thin sections for electron microscopy were stained with saturated aqueous uranyl acetate followed by lead citrate (38, 50). Double staining markedly increased the density of the surface coat but lead staining alone was usually satisfactory.

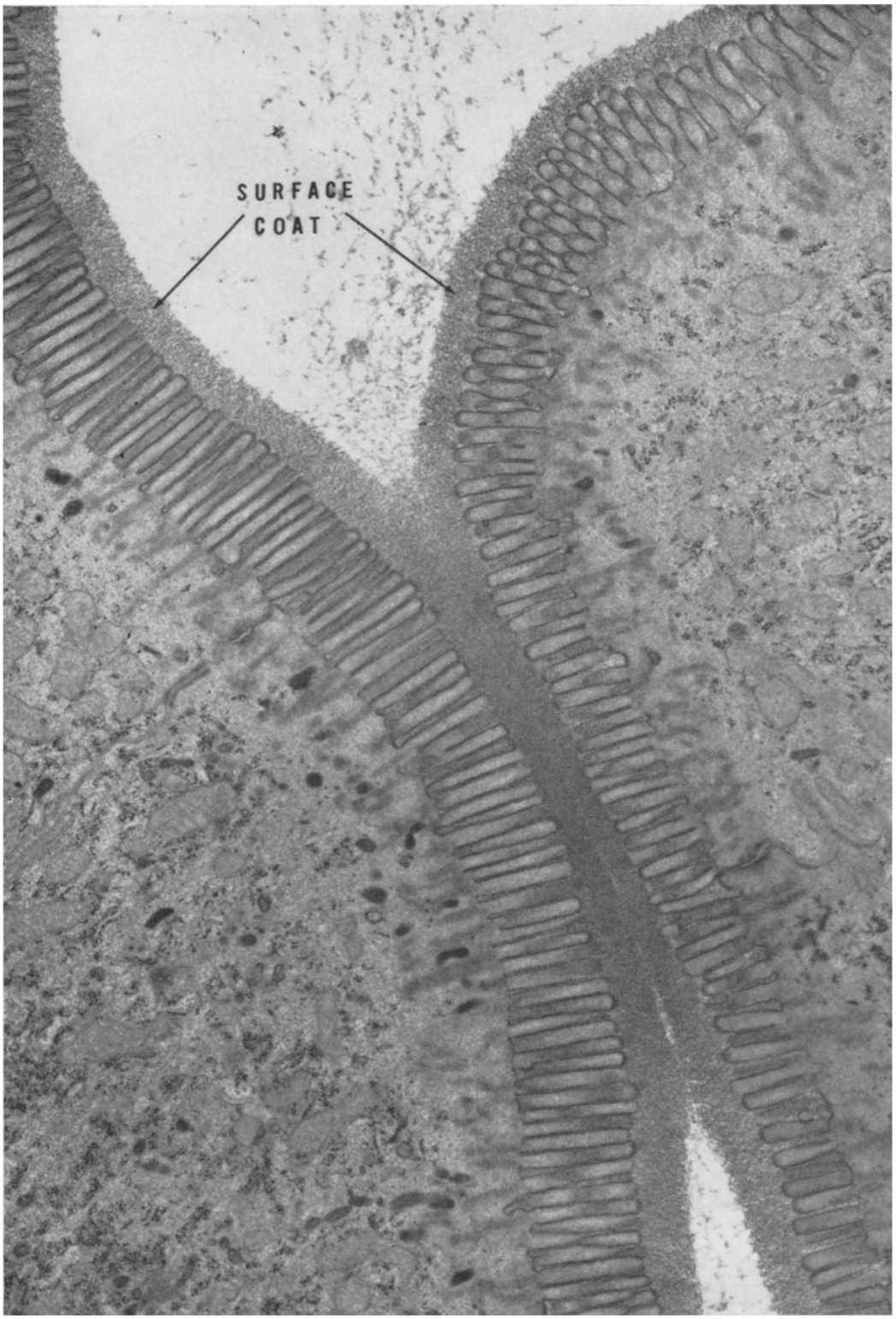
Histochemical staining tests on methacrylate or Epon sections for light microscopy included: the periodic acid–Schiff (PAS) reaction; 1 per cent Alcian blue in 3 per cent acetic acid (29) or in solutions buffered between pH 0.6 and 3.5; 1 per cent toluidine blue buffered between pH 0.5 and 9.0; Hale's colloidal iron reaction as modified by Mowry (29) for acidic carbohydrates. The specificity of Alcian blue staining was further tested by methylation of the tissue sections for 2 to 4 hours at 60°C in methanol containing HCl. Some sections were also saponified in 1 per cent KOH in 80 per cent ethanol after methylation. Electron microscope observations of localized reaction products were made on methacrylate sections reacted with Hale's colloidal iron early in this study and later with colloidal thorium at pH 2.1 according to the method of Revel (39). Sites of alkaline phosphatase activity at pH 9.4 and acid phosphatase activity at pH 5.0 were detected by incubating glutaraldehyde-fixed tissues in a Gomori medium as modified by Padykula and Herman (32), treated with buffered osmium tetroxide, and embedded in Epon.

OBSERVATIONS

Fine Structure of the Surface Coat

Electron micrographs of the intestinal absorptive cells of the adult cat show that the tips of microvilli are surmounted by a uniform surface coating of filamentous material which is rarely less than 0.1 μ and sometimes more than 0.5 μ thick (Figs. 1 to 9). This layer is present on the luminal borders of the epithelial cells throughout the entire length of the small and large intestine. A surface coating is also present on the goblet cell microvilli (Fig. 6), but it is often less prominent and at the margin of the goblet cell it may show an abrupt change in thickness compared to the adjacent absorptive cells. In late fetal and newborn kitten intestine, a filamentous coating is not a constant feature and when present it is not so thick as on the adult intestine. However, some goblet cells of newborn kitten intestines have a relatively prominent surface coating that extends

FIGURE 1 An electron micrograph of the apposed striated borders of cat intestina absorptive cells. The microvilli are topped with a conspicuous surface coating about 0.4 μ thick. This layer is the band that shows positive staining with PAS, toluidine blue, Alcian blue, and colloidal iron shown in Figs. 10 and 11 to 14. In regions where the coats are tightly juxtaposed, the filamentous meshwork appears to be compacted. The two layers may retain a thin line demarcating their margins but sometimes appear to be fused (Fig. 2). The floccular material in the lumen may be detached elements of the fuzzy surface coat or traces of secreted goblet cell mucus. × 16,000.


SURFACE
COAT

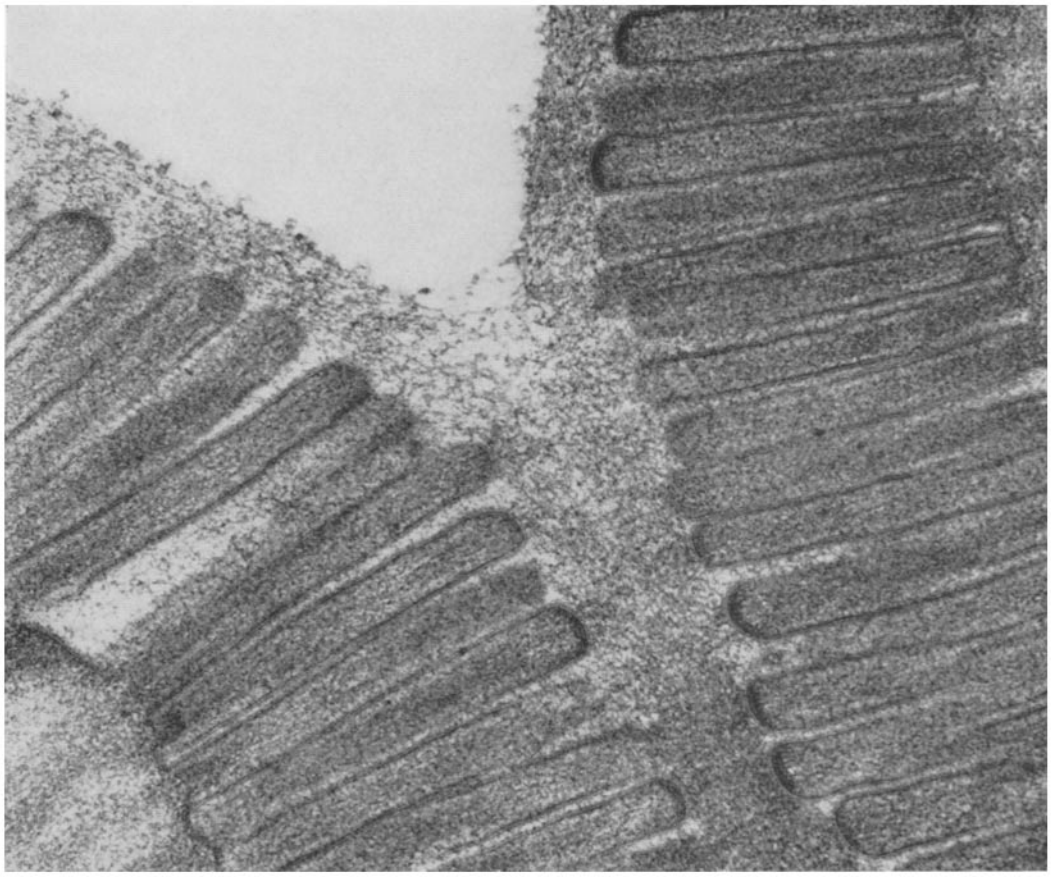


FIGURE 2 The apical surfaces of two cells from the cat ileum showing the apparent coalescence of the apical surface coats. The tips of microvilli are always separated by the mucopolysaccharide coating which is densest in regions of closest apposition. × 61,000.

farther into the lumen than the sparse coating on the adjacent absorptive cells. This difference serves to emphasize that the surface coat is not a general extraneous coating of the epithelium as a whole but is specifically related to the individual cells.

On the villus the surface coat is equally well developed on cells at the base and near the tip. Some cells at the villus tip are observed to be in the process of exfoliation. These cells and others that have been shed from the epithelium show a marked reduction in the amount of surface coat material. Fig. 9 shows the apical part of a cell that is still a part of the epithelium but is undergoing autolytic changes characteristic of extruding cells. Note that its surface coat is considerably thinner than that on the adjacent normal cell. The same micrograph shows a cell in the lumen that has been completely detached from the villus. This cell has undergone extensive changes. The microvilli are vesiculated and in some areas have disappeared. The surface coat is represented only by remnants of filamentous material.

The fine structure of the surface coat seems to vary with the preparative procedures and staining method used. In osmium tetroxide–fixed tissue sections examined without further staining, the surface coat is of very low density and may easily be overlooked. Lead staining at high pH brings it out more clearly, but double staining with uranyl acetate and lead is most effective. The total thickness of the surface coat layer as well as its density varies with the fixation and embedding medium. A thicker layer is preserved with glutaraldehyde fixation followed by postfixation with osmium tetroxide than with osmium tetroxide alone. When

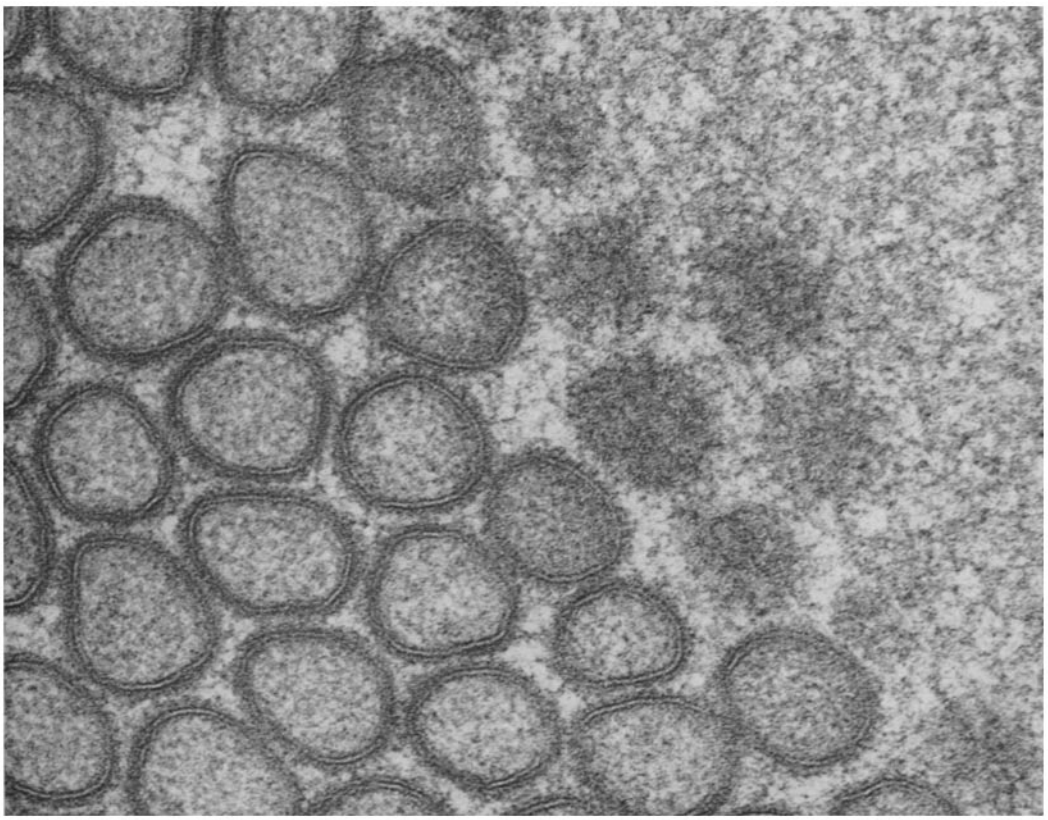


FIGURE 3 A transverse section through the striated border near the tips of cat enteric microvilli. The left part of the illustration shows near-normal cross-sections of microvilli. Radiating from the outer leaflet of the plasma membrane are fine filaments forming a moderately dense outer coating about 100 A thick. The remaining interstice is occupied by a loosely packed array of surface coat material. The right margin of the illustration shows an oblique section through the surface coat. Between this region and the transverse profiles (left) of the microvilli are dense circular areas which represent tangenital sections through the tips of microvilli. × 120,000.

different buffers were compared, it was found that osmium tetroxide buffered with phosphate preserved more of the surface coat than when Veronalacetate or s-collidine were used. Potassium permanganate preserved the trilaminar plasma membrane well but was the least favorable for the demonstration of the surface coat because it preserved less of the filamentous material than either of the other fixatives (Fig. 4).

In thin sections the layer may appear as a mat of branching fine filaments extending radially from the outer leaflet of the trilaminar plasma membrane (Fig. 5). The filamentous structure of the coating is not always apparent when it is densely packed and the sections are of sufficient thickness to cause superimposition.

Although the coating is more prominent on the microvillus tips, it is also present on the lateral surfaces as a sparse layer of shorter filaments. In favorable longitudinal or transverse sections of the striated border the surface coat is found to occupy much of the space in the interstices between microvilli (Fig. 3). However, areas in the striated border have been encountered where the microvilli are widely separated. The lateral surfaces of these microvilli have a coating about 300 to 600 A thick.

There is no significant fine structural difference in the coating on various parts of the microvillus but there is evidence of local differences in its stability. In preliminary electron microscope observations on cat brush border fractions isolated

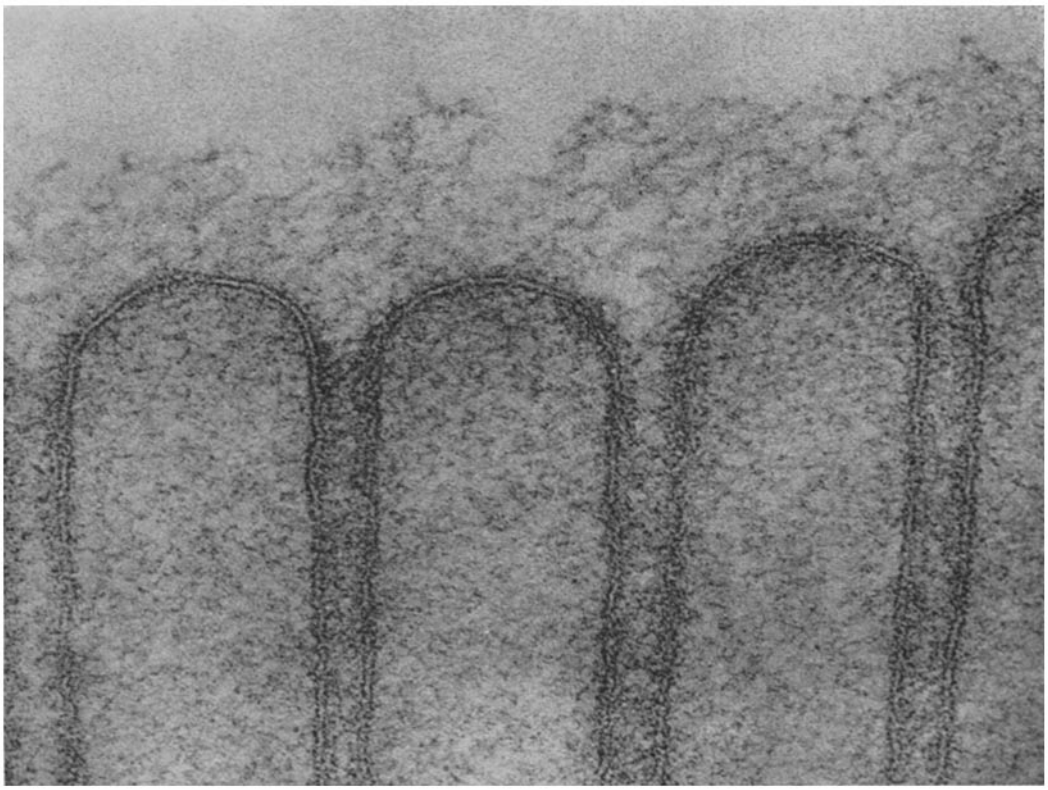


FIGURE 4 A micrograph of the apical parts of cat intestinal microvilli and their surface coat after potassium permanganate fixation. The trilaminar plasma membrane is clearly preserved but only a modest amount of the filamentous surface appendages is present. × 240,000.

by the method of Miller and Crane (27), strands of surface coat material were found at the apex of microvilli but only traces were found on the lateral surface. Although this suggests that the filaments at the tips are more resistant, it is conceivable that the lateral surface is more subject to mechanical abrasion by adjacent microvilli during homogenization and isolation.

The surface coat appears distinctly different in its fine structure from the free mucus found in the lumen. Although both components may appear filamentous, the orientation of the filaments in the two and their staining affinities are not the same. The surface coat is a thick layer of vertically oriented filaments attached at one end to the microvillus and is more or less perpendicular to the plasma membrane. Free mucus in the lumen secreted by goblet cells, or possibly by other mucous cells of the digestive tract, has a different appearance. It is rarely in direct continuity with the surface coat and is usually separated from the latter by a clear space of varying width. Although the mucus may appear to be composed of fine filaments, they have no prevailing or consistent orientation, in contrast to those of the surface coat. Moreover, lumenal mucus is often absent in micrographs whereas the surface coat is a constant structure.

Stability of the Surface Coat

In order to learn something of the composition and possible functional role of the surface coat, a number of chemical agents were employed to assess its solubility properties. The results with this approach have been largely negative but are nevertheless interesting in that they reveal that the surface coat has a surprising degree of stability. Neither washing the cat intestinal lumen with physiological saline for several hours nor *in vitro* incubation of inverted intestinal sacs and minced

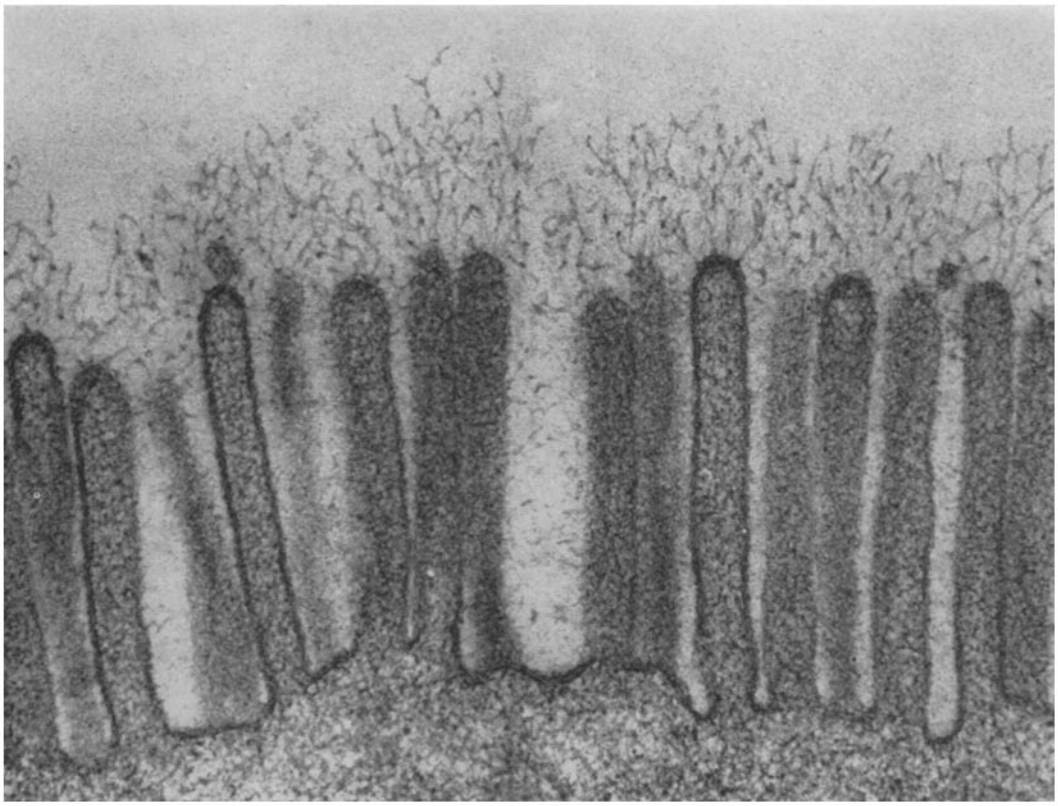


FIGURE 5 Intestinal microvilli from the bat intestine illustrating the branching, filamentous appearance of the apical surface coat after fixation with s-collidine-buffered osmium tetroxide and methacrylate embedding. × 85,000.

mucosal fragments dislodged the surface coat from cells as long as the integrity of the cells was maintained. Several methods known to be effective in removing the extraneous coats of marine eggs (9) were tried. Exposure of the cat intestinal mucosa to calcium-free saline or to solutions of Versene (EDTA) 0.005 M and 0.01 M was quite ineffective. Finally, attempts were made to remove the cat enteric surface coat with potent mucolytic substances and with enzymes that are known to be mucolytic or proteolytic. The agents employed included *N*-acetyl cysteine 0.1 M in Ringer's solution, chymotrypsin (Cytolav, Armour Pharmaceutical Co., Kankakee, Illinois) 140 Armour units/ml in acetate buffer at pH 5.6, hyaluronidase 30 and 78 units/ml in phosphate buffer at pH 6.05, diastase 2 mg/ml in Ringer's solution, neuraminidase 100 μg/ml in Ringer's solution, lysozyme 100 μg/ml in Ringer's solution, trypsin 100 μg/ml in Ringer's solution, papain 0.5 per cent in Ringer's solution, and *Streptomyces griseus* protease (Pronase, Kaken Chemical Co., Tokyo, obtained from Calbiochem Co., Los Angeles, California) 100 units/ml in Ringer's solution. Although in each case both *in vivo* application into isolated loops of intestine and *in vitro* incubation of minced intestinal mucosa were tried, none was effective in removing or markedly altering the filamentous coat. A critical evaluation of possible small changes in the surface coat after the varied treatments was not attempted. However, strangely enough, mucosal cells treated with lysozyme solutions seem to have a thicker surface coat than the controls.

To investigate the possibility that there might be visible changes in the prominence of the coating under different physiological conditions, bat intestines were examined during fasting or hibernation and after refeeding. No striking or consistent variation in the appearance of the coat was noted.

The surface coat seems to be an effective physi-

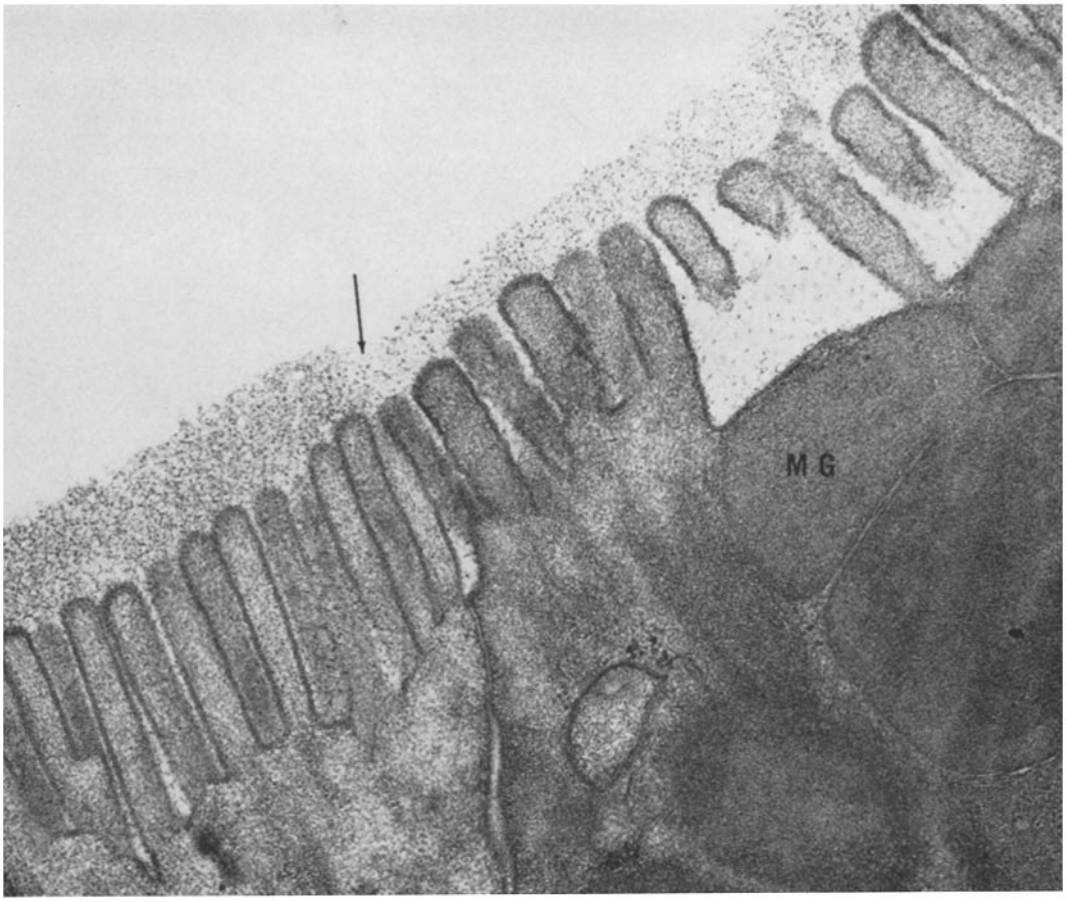


FIGURE 6 A section of the brush borders of a goblet cell and an intestinal absorptive cell in the cat ileum. The goblet cell has a thinner and less dense layer of surface coating. Note the abrupt change in the appearance of the coat at the junction between the cells (arrow). The cytoplasm of the mucous-secreting cell has greater density than the absorptive cell. *MG*, mucous granule. × 43,000.

cal barrier to relatively large particles such as indigenous bacteria, desquamated cells or solid food particles. Such formed objects do not penetrate the surface coat but remain on its outer surface. When surfaces of neighboring villi are tightly apposed, the coats may be somewhat compressed but a clear line of demarcation usually remains between the two layers (Fig. 1). The surface coat appears to be compressible to about one-half its original thickness and it resists disruption or displacement by mechanical forces that are usually encountered in the intestinal lumen.

To observe the effect of small particles on the enteric coat, colloidal thorium dioxide or carbon was placed in the lumen of an isolated loop of cat intestine. Dense particles of thorium dioxide were found in the filamentous surface layer of the striated border on some cells and less frequently in the corresponding coating between microvilli. Thus the coating acts as a mechanical barrier to large particles, but colloidal materials, large molecules and nutrients in solution are probably not restricted by the surface coat. Although it seems possible that some substances are accumulated or bound by the surface coat, no indication of such a process has been observed.

Light Microscope Observations

Having established the presence and dimensions of the surface coat by electron microscopy, it was realized that the thickness of it was such that it should be visible by light microscopy if it could

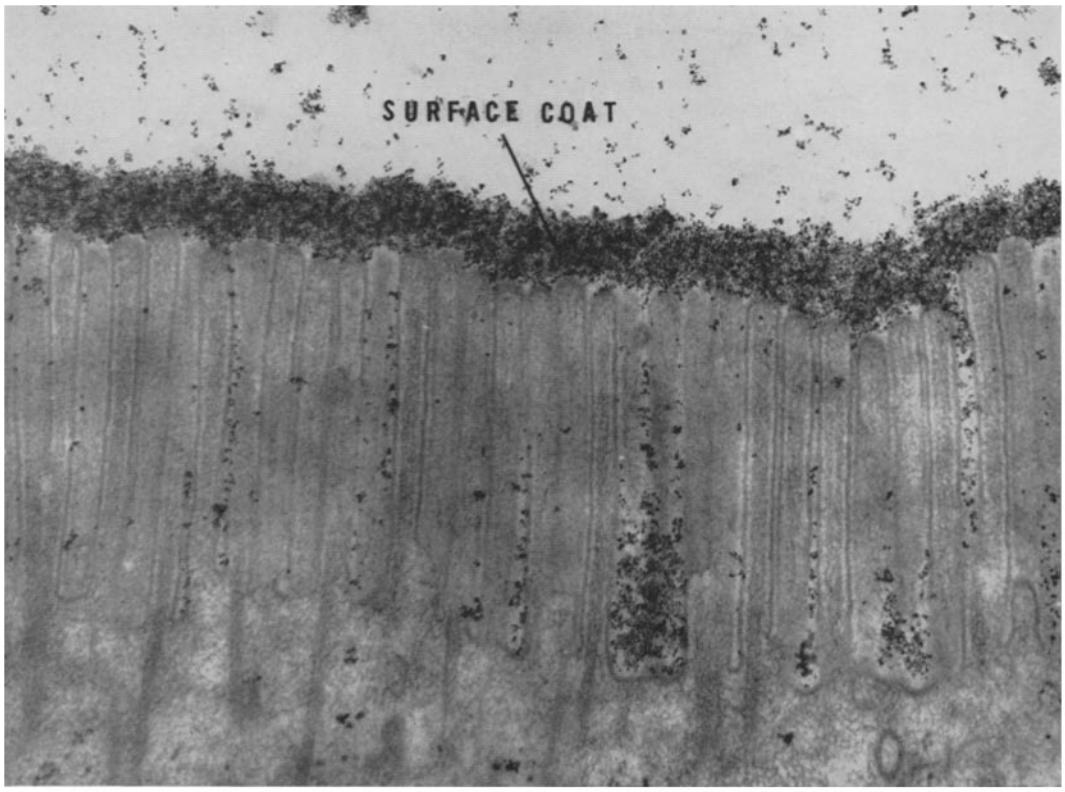


FIGURE 7 The striated border of the cat jejenum reacted with colloidal thorium dioxide to demonstrate acid mucopolysaccharide by the method of Revel (39). The surface coat surmounting the microvilli is heavily stained. Accumulations of thorium dioxide particles are also present in the interstices between microvilli. The scattered luminal reaction may represent small amounts of free mucus. × 45,000.

be stained. Application of the periodic acid–Schiff (PAS) method revealed a very intense reaction in the surface coat, while the underlying striated border formed by the closely packed microvilli was less intensely positive (Fig. 10). Without comparison of the relative thicknesses of the PAS-staining zones and the layers visible in electron micrographs, the intense staining reaction might erroneously be attributed to the striated border and the less heavily stained zone to the underlying terminal web. The differential staining of the surface coat and the striated border proper is most apparent in tissues fixed for electron microscopy and sectioned at a thickness of between ¼, and 2 μ. Even though paraffin sections, after conventional fixation, would be better suited for histochemical tests, the excessive thickness of routine paraffin sections does not permit a clear distinction of the surface coat which is usually not more than ½ μ thick. The acrylic embeddings were better suited for this purpose than either Epon or Araldite because of the greater staining intensity of the tissues that can be achieved, and it was not necessary to remove the plastic for the performance of the Alcian blue or Hale's colloidal iron stain.

Alcian blue buffered at pH 2.6 lightly stains the secreted mucus while the surface coat and goblet cells both show a strong reaction (Fig. 14). Above pH 3.0, Alcian blue staining is less specific. The intensity of Alcian blue staining is reduced when the pH is lowered but a reaction is still detectable at pH 0.6. The surface coat reacts quite well with Hale's colloidal iron stain while the goblet cell mucous granules stain only faintly and the mucus in the lumen is intermediate in its reaction (Fig. 13). These differences may not be a true indication of differential colloidal iron staining by may be due to slight differences in the penetration of the dye into the tissue section. The colloidal iron re-

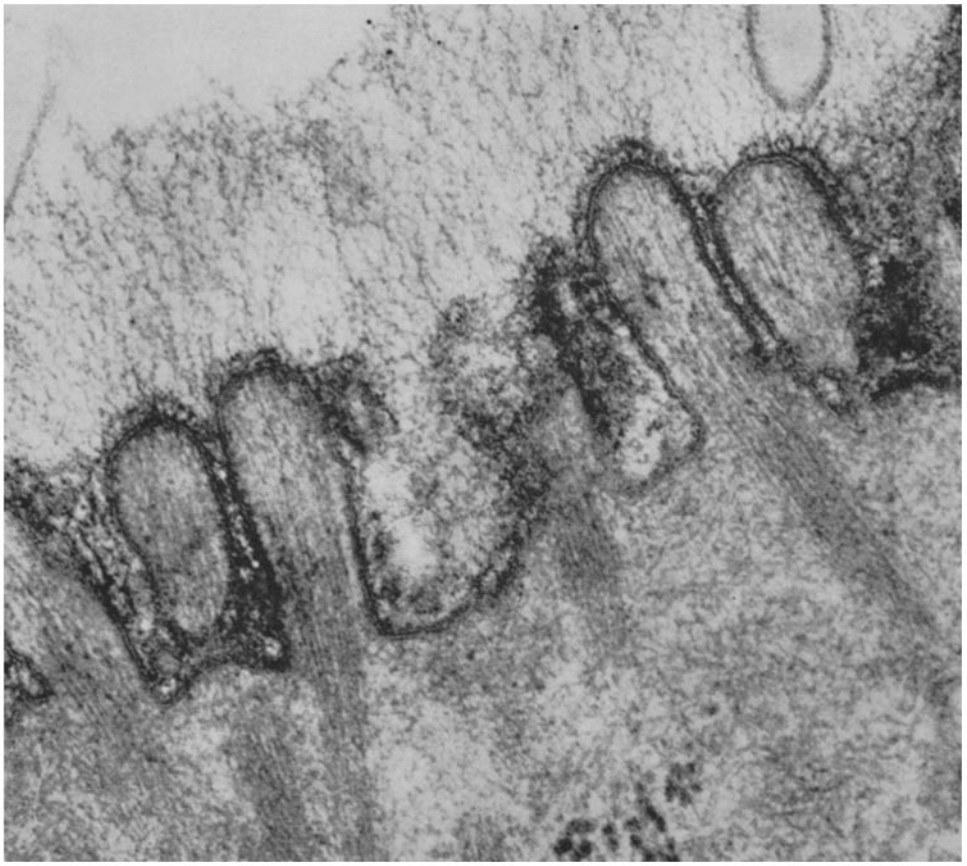


FIGURE 8 An electron micrograph of cat intestinal brush border reacted to show alkaline phosphatase activity. Most of the dense reaction product is limited to the surface coating immediately adjacent to the outer leaflet of the unit membrane. The filamentous material forming the remainder of the surface coat does not show enzymatic activity. Note that glutaraldehyde fixation preserves the filamentous appearance of the surface coat. × 90,000.

action is limited to the outer cut surface of the tissue section and does not seem to completely permeate the whole thickness of the section.

Toluidine blue stains the surface coat over the wide pH range of 0.1 to about 8.0. Below pH 1.0 the staining is restricted to a faint reaction in the surface coat and goblet cells, while no other tissue component is colored. There is little nuclear or cytoplasmic staining with 1 per cent toludine blue when it is buffered below pH 2.5, but goblet cell mucous granules and the surface coat are moderately stained (Fig. 12). Although accurate visual color discrimination is difficult in a structure as thin as the filamentous coating, this coating appears blue when stained at low pH and is violet to purple at neutral or slightly alkaline pH (Fig. 11). To retain these color differences as well as the selective staining at low pH, the slides must be rinsed, before examination, in a buffer solution of the same acidity as the staining solution. When tissues are stained in toluidine blue in alkaline solutions above pH 8.0, the surface coat cannot be distinguished from the striated border.

Tissue sections which were treated with a methanol-HCl solution for 2 to 4 hours at 60°C after osmium tetroxide fixation and methacrylate embedding showed a marked reduction in Alcian blue staining of the surface coat and goblet cells. Saponification of the methylated sections restored much of the original staining characteristics. Acetylation of sections in a 25:15 mixture of pyridine:acetic anhydride at 60°C caused only a

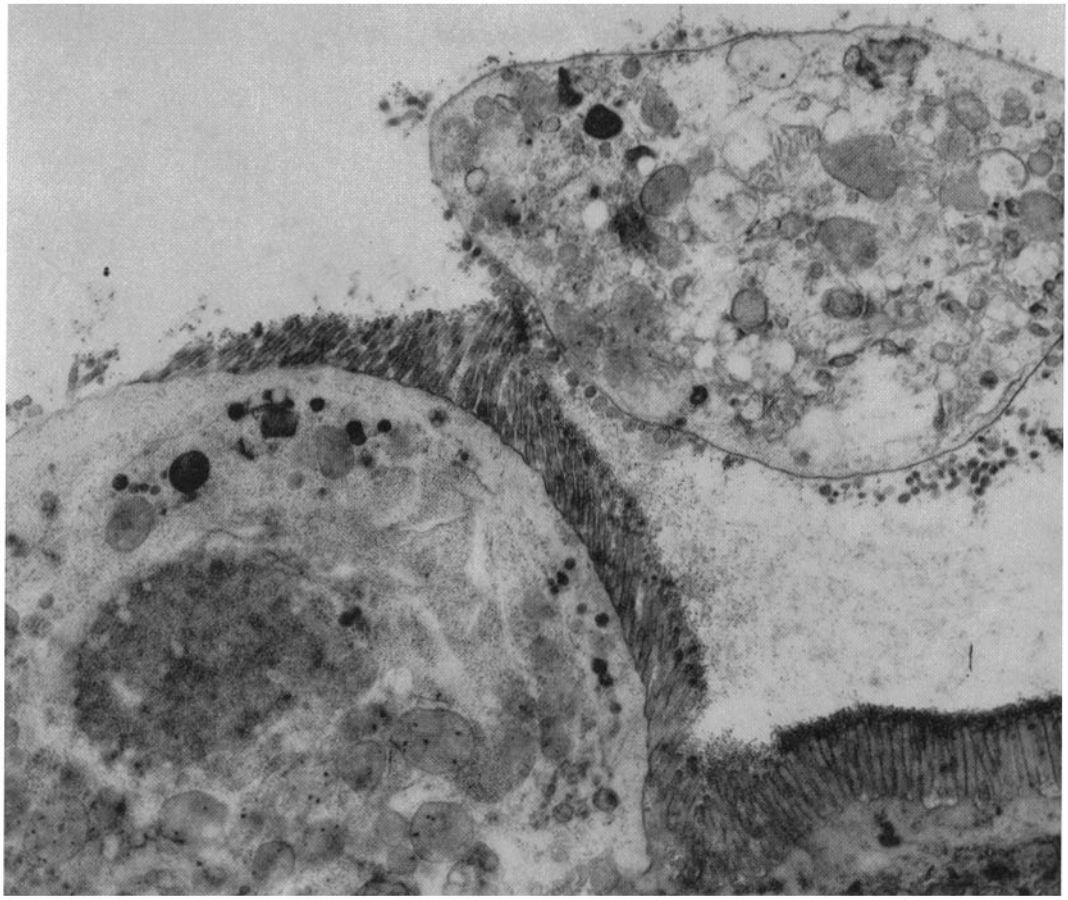


FIGURE 9 An electron micrograph of bat intestinal epithelial cells illustrating the disruption and loss of the surface coat during cell exfoliation. Part of a normal cell with its striated border and dense surface coat is seen at the lower right. The cell at the lower left margin is in the process of being extruded. Note that its surface coat and some of the microvilli are partially disrupted. At the upper right is a cell which appears to be completely detached from the epithelium. Only vesicular remnants of the microvilli and almost no surface coat material are present. × 12,000.

slight reduction in the Alcian blue reaction of the surface coat but almost completely blocked goblet cell staining. Under these conditions of methylation and acetylation the PAS reaction was not altered.

Histochemical Observations at the Electron Microscope Level

Examined with the electron microscope, re actions for acid phosphatase activity in the surface coat of the cat intestinal absorptive cells were negative. The alkaline phosphatase reaction, on the other hand, was quite strong and appeared to be localized in or on the trilaminar plasma membrane and in the immediately adjacent region of the surface coat (Fig. 8). The greatest part of the thickness of the surface coat, however, does not give a positive reaction for this enzyme.

Staining for acid mucopolysaccharides by Hale's colloidal iron technique was readily visible in the electron microscope before and after treatment with potassium ferricyanide. However, there is a moderate amount of non-specific colloidal iron deposition randomly distributed over the tissue and the empty lumen. A more discrete reaction on the surface coat was obtained with the colloidal thorium stain for acid mucopolysaccharides (39). With this technique most of the thorium dioxide

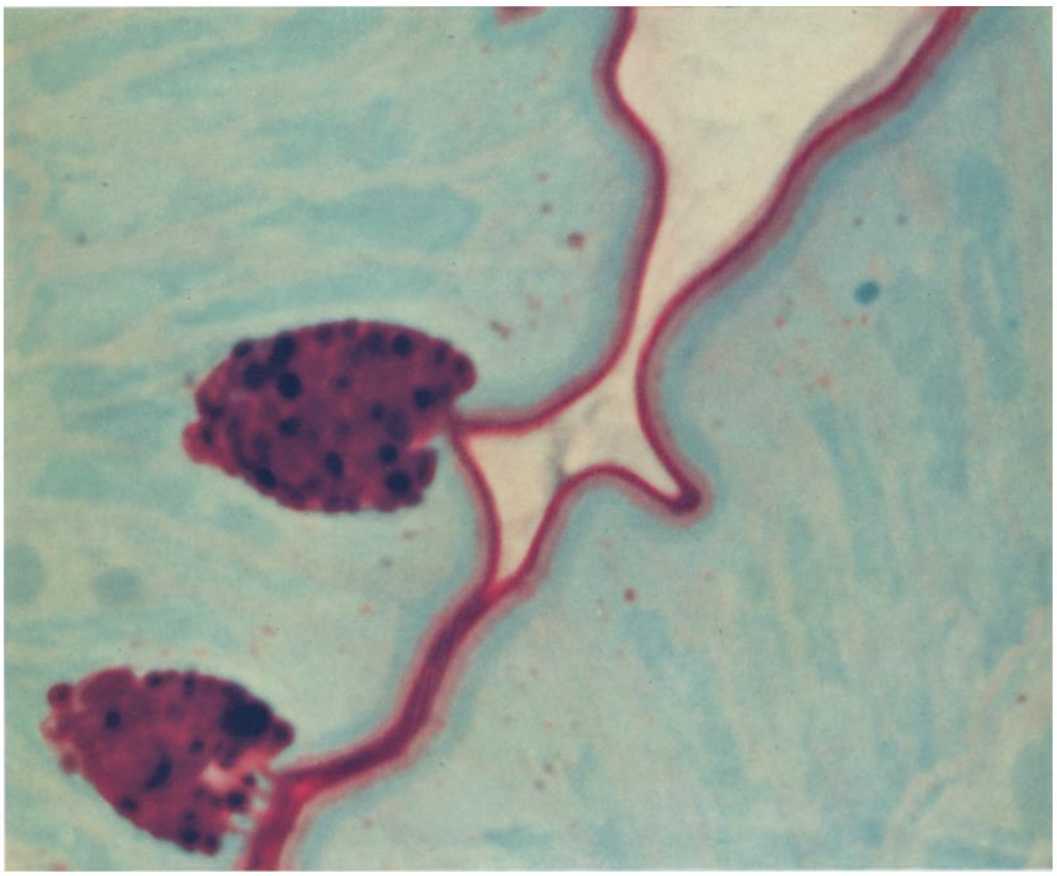
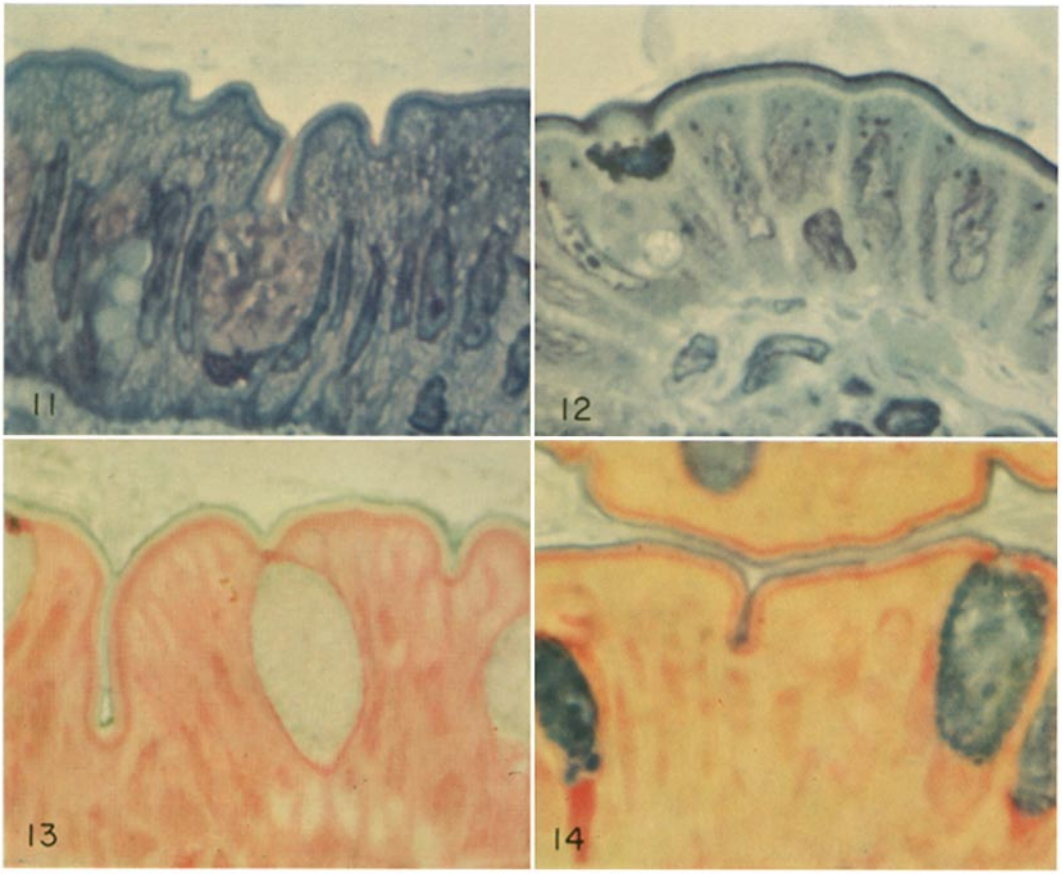


FIGURE 10 A color photomicrograph of cat enteric mucosa fixed with osmium tetroxide, embedded in methacrylate, sectioned at 1 μ, stained with the periodic acid–Schiff reaction (PAS) and counterstained with acid fast green. The intensely PAS-positive surface coats of the absorptive cells are the red bands extending from the upper right to the lower left. A clear luminal space separates the coats except at the bottom of the micrograph where the cells have their surface coats tightly apposed. The striated border immediately underlying the dense coating is lightly stained by the PAS reaction and delimited from the apical cytoplasm by the fast green-stained terminal web. The goblet cell mucous granules and the small granules in the absorptive cell cytoplasm are also PAS positive. The nuclei and cytoplasm of the epithelial cells are lightly counterstained with acid fast green. × 3000.

deposits were localized on the surface coat, while lesser amounts were found between microvilli, and only a few particles were present in the lumen or over the cytoplasm.

DISCUSSION

Although the number of species examined is limited, the presence of a thick enteric surface coat does not seem to be correlated with the dietary habits or with the taxonomic position of the animal. For example, among the *Carnivora*, the dog intestine has a relatively inconspicuous surface coat when compared to the cat intestine. The shrew enteric mucosa possesses microvilli with almost no detectable filamentous surface coat, in contrast to the prominent layer found in the bat. The rat and mouse consistently have only a very thin enteric coat, but in the hamster the coat seems to be variable. Bat intestines examined during different nutritional states did not show any notable difference in the prominence of the surface coat, but the possibility of differences in the rate of biosynthesis or the composition of the coat, remains to be evaluated. It is tempting to attribute some functional role to a structure so prominent and so



FIGURES 11 to 14 Color photomicrographs of cat intestinal mucosa fixed with buffered osmium tetroxide, embedded in methacrylate, sectioned at ½ or 1 μ, and stained as indicated.

FIGURE 11 A ½ μ section stained with 1 per cent toluidine blue at pH 7.9 and rinsed with buffer at the same pH. Note the purple-violet color of the goblet cell mucous granules and the surface coat. The striated border is less intensely stained and is distinctly delimited by the deeply stained, blue terminal web. × 2000.

FIGURE 12 A 1 μ section stained with 1 per cent toluidine blue at pH 2.8 and washed at the same pH. The surface coat is stained deep blue as are the goblet cell mucous granules. The striated border is less densely colored and the terminal web is almost unstained. Below pH 1.0 there is almost no staining of any cellular components except for the surface coat and the goblet cells. × 2000.

FIGURE 13 A section stained with Hale's colloidal iron reaction for acid mucopolysaccharides. An intense reaction is present on much of the surface coat while the goblet cells are less intensely stained. The counterstain (1 per cent acid fuchsin in 30 per cent acetic acid) stains the terminal web and other cellular components. × 2000.

FIGURE 14 An Alcian blue–stained section illustrating the intense reaction of the surface coat and the goblet cell mucous granules. Note that the free luminal mucus is faintly stained. Counterstained as in Fig. 13. × 2000.

strategically located on the absorptive surface as the enteric surface coat. However, the species variations show that this layer is not indispensable. A more essential component of the surface coat may be that part of the filamentous layer immediately adjacent to the plasma membrane. This portion corresponds to the sparse surface coat found on other species and is the probable site of phosphatase activity.

Published electron micrographs illustrating a surface coating on the free surface of cells are numerous: the surfaces of the cells of the gastric mucosa (14, 18, 21), intestine (7, 13, 36, 40, 46, 51), as well as of the toad bladder (2*a*, 11, 35), gall bladder (52), and other free surfaces. The endothelial cells lining the vascular system have also been noted to be coated with a thin layer of filamentous material (24). Examples of localized external coatings on plasma membranes are the coated pits and vesicles on the surface of liver cells and mosquito oocytes illustrated by Roth and Porter (42, 43). Similar structures have been implicated in the uptake of ferritin in the toad spinal ganglion cells by Rosenbluth and Wissig (41). Bessis and Breton-Gorius (6) and Fawcett (15) have described a mechanism for uptake of ferritin by erythroblasts involving the attachment of ferritin particles to small plaques of cell membranes having a filamentous coat followed by micropinocytosis of these local areas of specialization.

The most extensively studied external surface coat is the hair layer or mucoid slime coat of *Amoeba proteus* and *Chaos chaos* (7, 25, 26, 34). This surface coat also stains with the periodic acid–Schiff reaction (1, 33) and its fine structure (8, 34) has some of the characteristics of the enteric surface coat described here. Unlike the intestinal furry coat, the ameba hair layer has been shown to adsorb large amounts of electron-opaque particles such as thorium dioxide and ferritin. These particles are then ingested by pinocytosis (8, 25). Another feature of the ameba surface coat which is apparently lacking in the cells of the intestine is the ability of the ameba to shed or cast off its hair layer under certain experimental conditions (7, 30). During this process a new surface coat is formed. The studies of Marshall and his coworkers suggest that the external polysaccharide coat is an integral part of the cell membrane and seems to function as a structure on which materials are adsorbed prior to pinocytosis.

An analysis of the isolated cell membranes of *Amoeba proteus* with their mucoid coat has been made by O'Neill (31). This fraction was found to contain about 35 per cent lipid, 26 per cent protein, and 15 per cent total sugars. An analysis of the sugar fraction revealed the presence of mannose and galactose and only a small amount of glucose. The isolated plasmalemmae retained many of their fine structural characteristics and were found to resist dissolution by treatment with a number of reagents including trypsin, lysozyme, and hyaluronidase. Although the solubility characteristics of the surface coat on the ameba are not identical with or directly comparable to those of the enteric surface coats in the present study, the general similarity suggests that the surface coats may share related roles that are specific for different cells.

The histochemical findings reported here indicate that the enteric surface coat possesses some of the characteristics considered by Spicer (45) to be typical of sulfated, but weakly acidic, acid mucopolysaccharides. These include the positive PAS reaction, and the positive staining with Alcian blue and with toludine blue at low pH. Methylation of cat intestinal mucosa resulted in a reduction of surface coat staining with Alcian blue which was restored after saponification. This suggests that the groups responsible for the acidic nature of the polysaccharide are carboxyl rather than sulfate groups. On the other hand, studies to be reported in detail elsewhere demonstrate incorporation and apparent turnover of radioactive sulfur as well as glucose, galactose, and acetate into this layer. Further staining tests are necessary to determine more fully the histochemical characteristics of the enteric surface coat.

A positive reaction for alkaline phosphatase has been demonstrated in the intestinal striated border of many species by light microscopy, and this enzyme was the first to be demonstrated at the electron microscope level (10, 16, 37). In this early histochemical study, the dense reaction product was found localized on and between the microvilli. The alkaline phosphatase reaction thus seemed to be localized in the regions now found to be occupied by the surface coat material. It therefore seemed to be of interest to examine the distribution of this enzyme in species with a prominent surface coat. The present observations show the enzyme distributions to be quite similar regardless of the degree of development of the surface coat. The superficial part of the cat enteric sur-

face coat has no activity. A similar finding has been reported by Bartoszewicz and Barrnett (2*a*) who demonstrated nucleosidase activity on the plasma membrane of the toad bladder cells but none in the filamentous surface coating. Preliminary observations on the localization of adenosine triphosphatase and thiamine triphosphatase activities in the cat intestine indicate that these activities are limited to the same area that is positive for alkaline phosphatase. These observations suggest either that the enzymatic activity resides in the membrane and diffuses into the adjacent parts of the surface coat or that the latter has a functional zonation that is not represented morphologically.

The brush border region of the intestinal absorptive cell is an obvious site for localization of the terminal digestive processes prior to absorption of nutrients. To what extent the late stages of breakdown of nutrients occur in the lumen, in the plasmalemma, or immediately beneath the cell surface remains to be determined. Ugolev and his coworkers (47–49) consider that the extracellular site immediately external to the plasmalemma is the most important one for the terminal digestion of nutrients. Crane and his coworkers (12) have isolated brush borders of intestinal absorptive cells and found that almost all of the carbohydrate digestive enzymes were present in this fraction and have designated the brush border as the "digestive surface." Since both of these concepts place the important site where we find the surface coat, it seems reasonable to speculate that this filamentous material may in some way be involved in these physiological processes, as suggested by Fawcett (15).

The enteric surface coat possesses all of the defining characteristics of the glycocalyx, a term applied by Bennett (3, 4) to all external polysaccharide coats of cells including plant and bacterial cell walls, insect cuticles, ameba hair layers, the zona pellucida of eggs, basement membranes or the basal laminae of epithelial cells, type-specific red cell antigens, and the filamentous surface coat of various epithelial cells. Although these coats differ widely in their composition and form, they have in common a content of sugars. Moreover, most of them may be removed from the underlying plasma membrane by various treatments without markedly altering cell viability. In this sense it is reasonable to consider the glycocalyx as an extracellular or extraneous coat. On the other hand, the enteric surface coat has so far resisted removal by lytic agents and seems to be so intimately associated with the plasma membrane that no measure short of dissolution of the membrane removes it. Some of the agents used did alter the surface coat, and specific agents may yet be found that will detach the filamentous coat. Nevertheless this coat is unusually resistant and bears a more intimate relation to the unit membrane than most of the other structures included under the general term glycocalyx.

On the basis of morphological criteria the surface coat of the intestine is quite different from the basal lamina (basement membrane). The polysaccharide supporting layer of the epithelia appears as a continuous sheet not respecting cell boundaries and often underlying various cell types. On the other hand, the surface coat on a goblet cell may be distinctly thicker or, in some instances, less well developed than that on adjacent absorptive cells. A cell undergoing extrusion may lose much of its filamentous coat while adjacent normal cells retain their coats intact. Thus the surface coat does respect cell boundaries, and its condition depends upon the physiological state of the underlying cell. The basal lamina, on the other hand, is not so closely associated with the basal plasmalemma. In cells such as those in the kidney the plasma membranes have numerous basal infoldings which are not accompanied by incursions of the basal lamina. In contrast, the apical surface coat always conforms closely to the contour of the cell membrane at the free surface and probably extends into all tubular or pinocytotic invaginations. Because of these obvious differences between the filamentous surface coat and the basal lamina, it is of questionable value to describe these and other surface layers by a common term. The differences between them may be of greater significance in understanding their function than the properties they have in common.

There is little doubt that the surface coats or glycocalyces are cell products, but whether they are always synthesized by the cell that is coated is not established. Studies in progress with radioactive sulfate, glucose, galactose, and acetate seem to indicate that the enteric surface coat is synthesized by the particular cell on which it is found, just as the coat on the ameba or other isolated cells must be attributed to an intrinsic source. The question of whether to consider the enteric surface coat as an integral part of the membrane

or an extraneous layer is largely semasiological. For obvious reasons it is not possible to provide a definitive answer to the question as to the actual boundary between the living cell and its nonliving external environment. The unit membrane as defined by Robertson is generally regarded as the outer limiting barrier between the cell and its environment. In electron micrographs the cell membrane is seen as two dense lines with an intervening less dense space and is thought to be composed of a bimolecular layer of lipid with associated protein and/or carbohydrate on its outer surfaces. Just how far these peripheral layers of the membrane extend may be argued. In the case of the enteric surface coat, its consistent presence and the relation of the filaments to the outer dense lamina of the membrane both strongly suggest that this layer is not an extraneous coat in the usual sense, but is an integral part of the external leaflet of the plasma membrane.

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