

A COMPARATIVE STUDY OF THE ULTRASTRUCTURE OF MICROVILLI IN THE EPITHELIUM OF SMALL AND LARGE INTESTINE OF MICE

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ABSTRACT

A comparative analysis of the fine structure of the microvilli on jejunal and colonic epithelial cells of the mouse intestine has been made. The microvilli in these two locations demonstrate a remarkably similar fine structure with respect to the thickness of the plasma membrane, the extent of the filament-free zone, and the characteristics of the microfilaments situated within the microvillous core. Some of the core microfilaments appear to continue across the plasma membrane limiting the tip of the microvillus. The main difference between the microvilli of small intestine and colon is in the extent and organization of the surface coat. In the small intestine, in addition to the commonly observed thin surface "fuzz," occasional areas of the jejunal villus show a more conspicuous surface coat covering the tips of the microvilli. Evidence has been put forward which indicates that the surface coat is an integral part of the epithelial cells. In contrast to the jejunal epithelium, the colonic epithelium is endowed with a thicker surface coat. Variations in the organization of the surface coat at different levels of the colonic crypts have also been noted. The functional significance of these variations in the surface coat is discussed.

INTRODUCTION

Since Zetterqvist (18) first described the ultrastructure of the columnar absorbing cells of the mouse jejunum, several reports (3, 7, 10-12, 14, 17) have led to a fuller understanding of the structural components of the microvilli of intestinal epithelial cells in a variety of animals. None of these reports, however, has attempted to differentiate structurally among the microvilli of epithelial cells, according to their locations in the intestine. It seems to us that this type of study may be important in understanding the role played by the microvilli of the epithelial cells in the different functions of the small intestine and colon.

With a view, therefore, to enlarging our knowledge of the anatomical differences, if any, between the microvilli of the epithelial cells lining different

regions of the mouse intestine, we have made a comparative analysis of the fine structure of microvilli of epithelial cells located on the villi or crypts in both the small intestine and colon.

MATERIALS AND METHODS

Small pieces of mucosa from the jejunum and colon of NZCW strain mice were immediately fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.3) (5, 15), and then postfixated in 2% osmium tetroxide in phosphate buffer of the same pH. The blocks were dehydrated in graded alcohols and embedded in Epon 812 (9). All procedures up to 95% alcohol were done at temperatures between 0° and 4°C. Sections of varying thicknesses were cut on a Porter-Blum Ultramicrotome with glass knives having 40° angles (obtained from an LKB Knifemaker). Thicker sec-

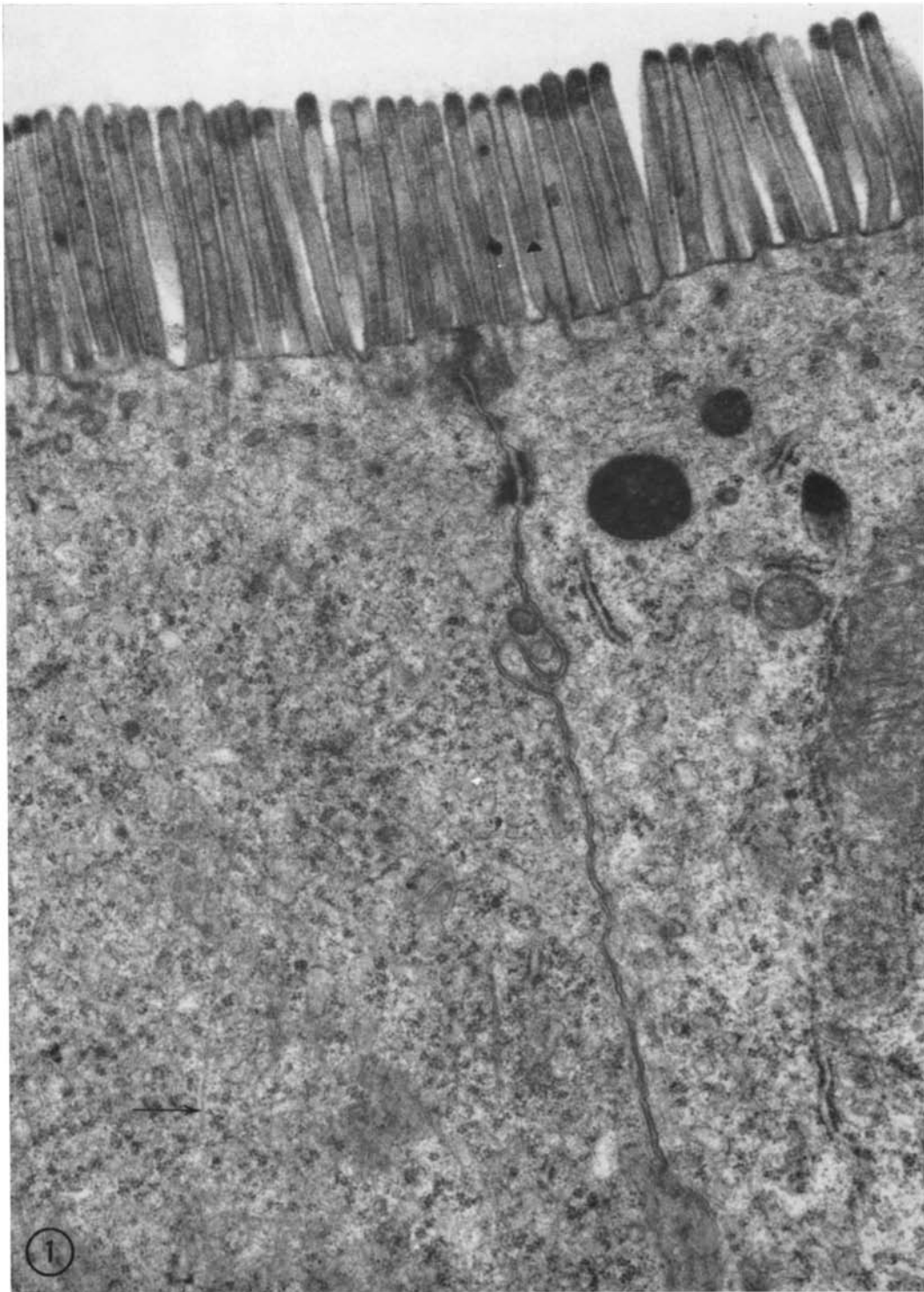


FIGURE 1 An electron micrograph of the apical region of two adjacent absorbing cells from mouse jejunum, showing the absence of a thick surface coat. The microvilli, however, are covered by a fuzz which can be detected more clearly at the tips. Arrow indicates a cytoplasmic microtubule (diameter ~ 200 A) proceeding towards the terminal web. Several cytoplasmic microtubules may be observed. $\times 27,000$.

tions were cut for light microscopy as a routine procedure. The sections for electron microscopy were stained with lead citrate (13) and some of them were double-stained with saturated aqueous uranyl acetate and lead citrate (6). The sections were examined in a Hitachi HU11A electron microscope operating at 75 kv, with double condenser lenses and a 50 μ objective aperture. Electron micrographs were taken at magnifications varying between 3,500 and 40,000.

OBSERVATIONS

Plasma Membrane

The triple-layered membrane limiting the small intestinal and colonic microvilli has an over-all thickness of 90–110 A; the outer layer is fairly constant at 25–30 A and the clear intermediate layer at 30 A, but the inner dense layer shows a thickness of greater variation, between 35 and 45 A (Figs. 5, 7, and 15).

Core

The structural detail of the core of the microvillus is essentially similar in small intestinal and

colonic epithelial cells. The core consists of two zones: a central zone, containing packed microfilaments, and a peripheral zone without such structures (Figs. 7 and 14). The dimensions of these zones vary considerably, depending on the diameter of the microvilli and the number of microfilaments within them. The filament-free zone is usually 200–300 A wide, which is very similar to the dimension observed by McNabb and Sandborn (10) in microvilli of the small intestinal epithelium in rat. However, the number of microfilaments in the microvillus varies from 10 to 50 and the center-to-center distance from 180 to 240 A. In strictly transverse sections the microfilaments appear to lie in a hexagonal array (Figs. 7, 13, and 14).

Some of the microfilaments in longitudinal sections appear as tubules for a short distance (Fig. 6), but in transverse sections these tubules can be detected with far more clarity (Figs. 7 and 14). The tubules seem to be composed of a wall about 25–30 A thick and their total diameter

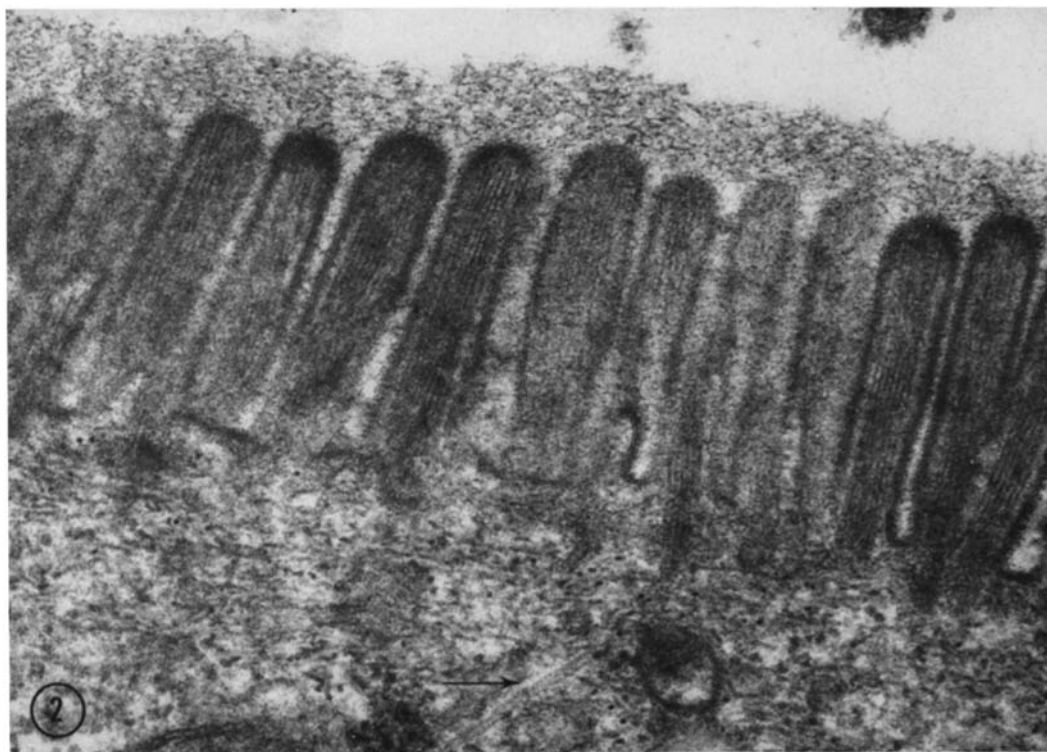


FIGURE 2 In some areas of the jejunal villus a thick (0.1 μ) surface coat may be observed in addition to the fuzz over the microvilli of the absorbing cells, as shown in this micrograph. Note a cytoplasmic microtubule (arrow) in the terminal web region. $\times 71,000$.

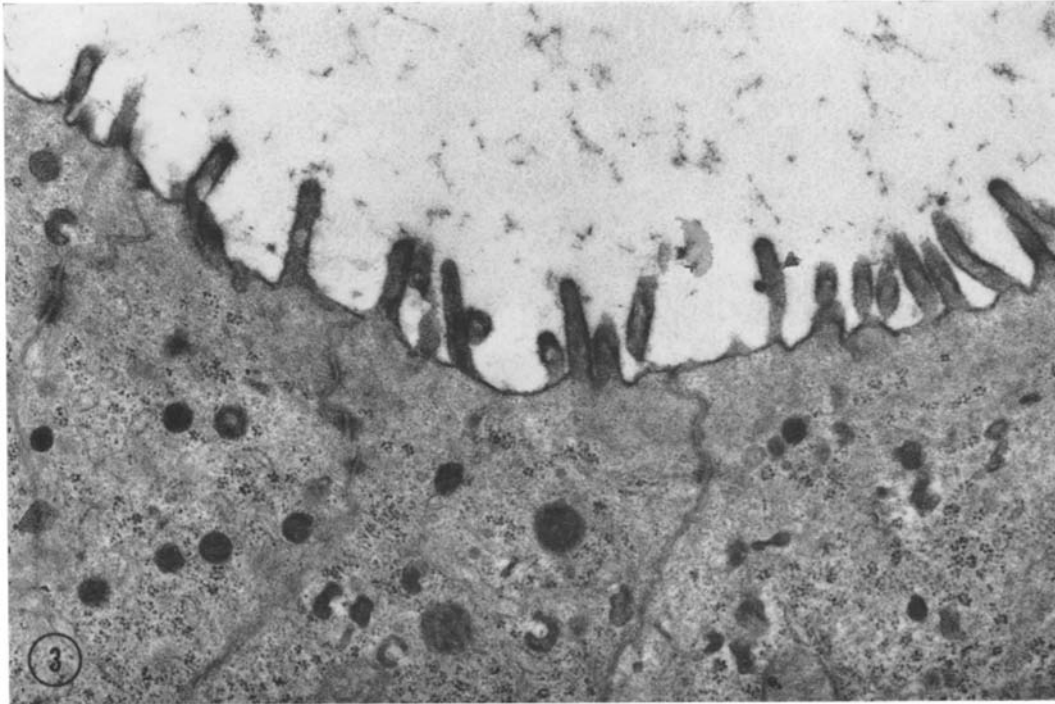


FIGURE 3 A section through the apical portion of several absorbing cells lining a small intestinal crypt, showing the absence of a thick surface coat. $\times 22,000$.

varies between 60 and 110 Å. Towards the terminal web region the microfilaments extend for a variable length and seem to disappear without any communication with the filaments of the terminal web.

In the tip of the microvillus occasional microfilaments seem to continue across the plasma membrane and then disappear in the network of surface coat material (Figs. 5 and 15).

Surface Coat

A surface coat has been observed on the microvilli of both small intestine and colon. The extent and the characteristics of the surface coat vary considerably according to the position of the microvilli in the villus or the crypt.

In the small intestinal epithelium the absorptive cells have a very thin layer of fine filaments which radiate from the plasma membrane limiting the microvillus both on its tip and lateral borders (Figs. 1 and 5). This layer closely follows the plasma membrane and seldom exceeds 500 Å in thickness. This characteristic of the surface coat, which is identical to the fuzz described by other

workers (17), is noticeable on the microvilli of most of the absorbing and goblet cells lining the villus or the crypt (Fig. 3).

In addition to the fuzz, a well-organized surface coat has been observed occasionally on the absorbing cells lining the tip or the lateral borders of the villus (Figs. 2 and 4). This surface coat is composed of fine filaments which arise from the plasma membrane and branch frequently to form a network disposed in the form of a layer over the microvillus. The width of this layer is very variable but rarely exceeds 0.1μ from the tip of the microvillus. The thickness of this coat varies considerably among adjacent cells, as may be observed in Fig. 4 in which only the cell in the center shows a well-organized surface coat. This cell is flanked on one side by a cell with a poorly developed surface coat and on the other side by a cell without any surface coat, except the fuzz covering the microvilli. Similar observations were made frequently, but in most instances a well-organized surface coat could be observed to spread over a few cells in a row and then either

to disappear abruptly at the cellular border or else to taper off over a few cells.

In the colon, the absorbing cells lining both the lumen of the gut (Fig. 8) and the crypts (Figs. 9–11) have a prominent surface coat which usually has a thickness of 0.1–0.25 μ but rarely may extend up to 0.5 μ from the tips of the microvilli. The characteristics of the surface coat vary with the location of the absorbing cell within the crypt.

In the cells lining the lumen of the gut (Fig. 8) and the higher regions of the crypts (Fig. 10) the filaments constituting the surface coat form a very compact meshwork, which is closely applied to the tips of the microvilli of the absorbing cells. In low power electron micrographs (Fig. 10) this compact surface coat appears as a continuous, dense layer close to the striated microvillous border of the absorbing cells, interrupted only at the levels of the goblet cells. The mucus within

the crypt is usually separated from the surface coat by a clear zone (Fig. 10).

Deeper within the crypt a surface coat of the same dimension as that at the higher regions may also be observed (Fig. 9). However, in some of the crypts, areas of surface coat material seem to occupy portions of the lumen and to have connections with the surface coat on the cells lining the crypt. Such an area may be observed in Fig. 9 in which a zone of surface coat material seems to form a bridge over a portion of the lumen. This bridge apparently is connected to the surface coat at the sides of the lumen and to the absorbing cell situated in between the two goblet cells. The goblet cells in Fig. 9 do not show any well-developed surface coat.

Nearer to the base of the crypt (Figs. 11 and 12) the nature of the surface coat varies slightly from that observed in the higher regions. The surface coat filaments in this region appear to be loosely packed and show less branching. These

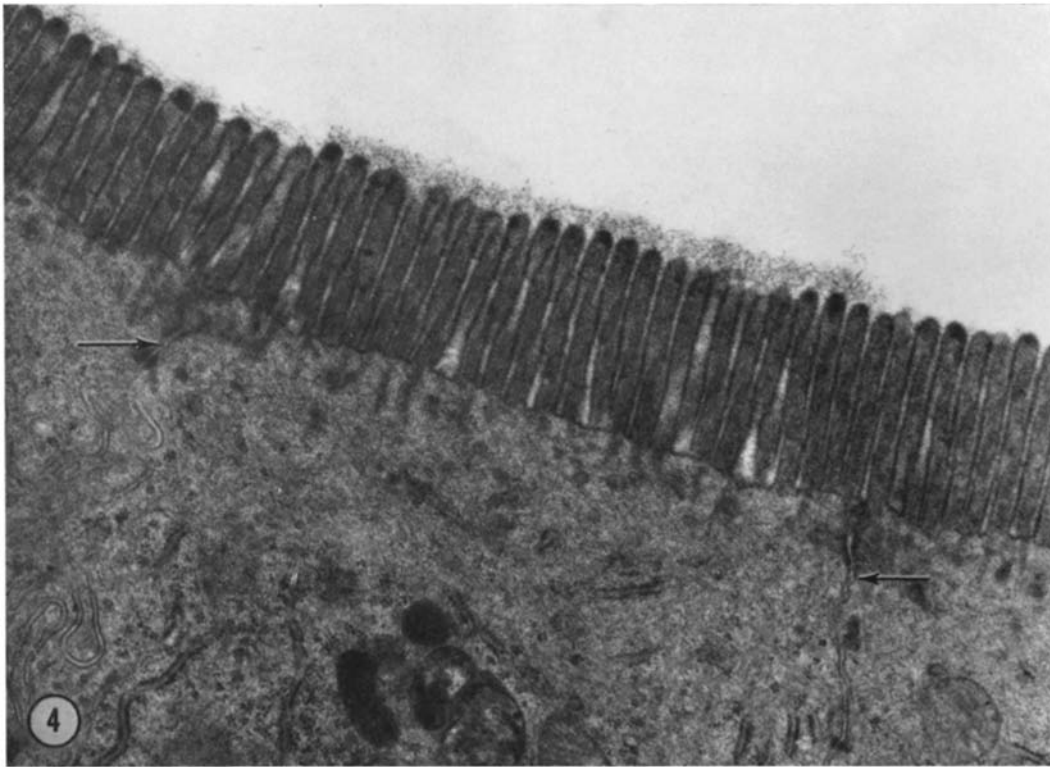


FIGURE 4 An electron micrograph showing the luminal borders of three absorbing cells lining the small intestinal villus. The cell in the center shows a characteristic thick surface coat limited at the cellular borders (marked by arrows). The cell to the left of this cell shows a poorly developed surface coat, and the cell to the right has apparently no surface coat. $\times 22,000$.

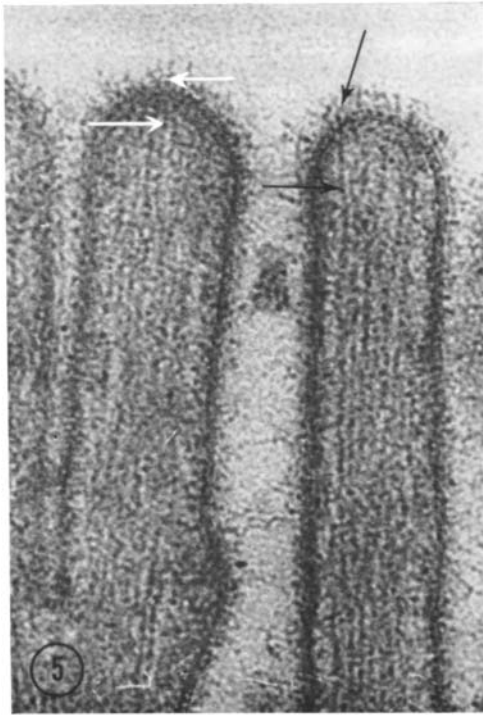


FIGURE 5 Longitudinal section of microvilli from jejunal villus, showing one microfilament running across the plasma membrane at the tip of the microvillus (black arrows). A microfilament situated in the core of another microvillus (white arrows) most probably continues across the plasma membrane but its course within the intermediate clear zone is not well defined. $\times 130,000$.

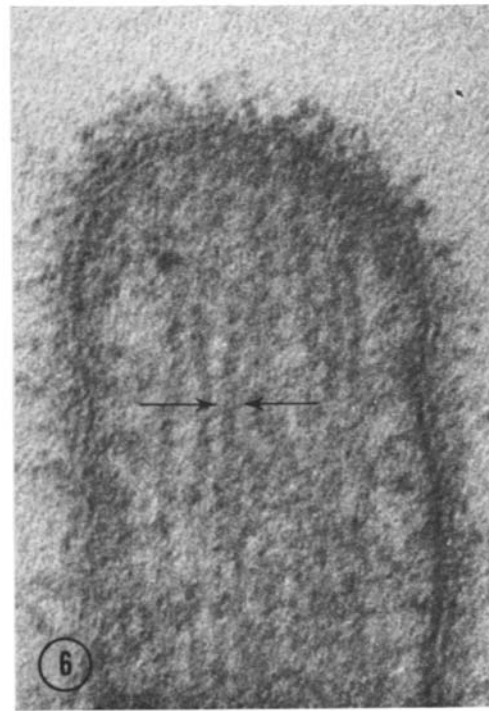


FIGURE 6 A high magnification micrograph of the tip of a microvillus from the jejunal epithelium cut longitudinally, showing the fuzz closely applied to the plasma membrane. Arrows indicate a longitudinally cut tubule seen only for a short distance in the core. $\times 270,000$.

filaments also seem to be more perpendicular to the plasma membrane and to extend usually for more than 0.25μ as measured from the tips of the microvilli. This difference in the characteristics of the surface coats in the upper and lower regions of the crypts may be compared in Figs. 15 and 12, respectively. Since the filaments of the surface coat are lightly packed at the lower level of the crypt, this coat appears in low-power electron micrographs as a lighter zone close to the tips of the microvilli (Fig. 11), instead of the dense layer observed in the higher regions of the crypt (Figs. 8-10). The goblet cells here occasionally show a surface coat but it is not so thick as that on the absorbing cells.

DISCUSSION

The thickness of the plasma membrane limiting the microvilli of both the small intestine and

colon is the same as that reported by Sjöstrand (16) in mouse intestinal epithelium, with the exception that the thickness of the inner dense layer is more variable; this may be due to the difference in the fixatives employed. It may be useful to mention here that, while trying to find a suitable fixative for this study, we have also observed that after fixation by Veronal-buffered osmium tetroxide (18) the thickness of the inner layer of the plasma membrane was not so variable.

In both the small intestine and colon the microfilaments of the microvillous core show a regular pattern of packing in the form of a hexagonal array. A similar arrangement of the microfilaments has also been reported by McNabb and Sandborn (10) in rat intestinal microvilli. But the center-to-center spacing of the microfilaments in the core of the microvillus obtained in this study on mice is greater than that reported by

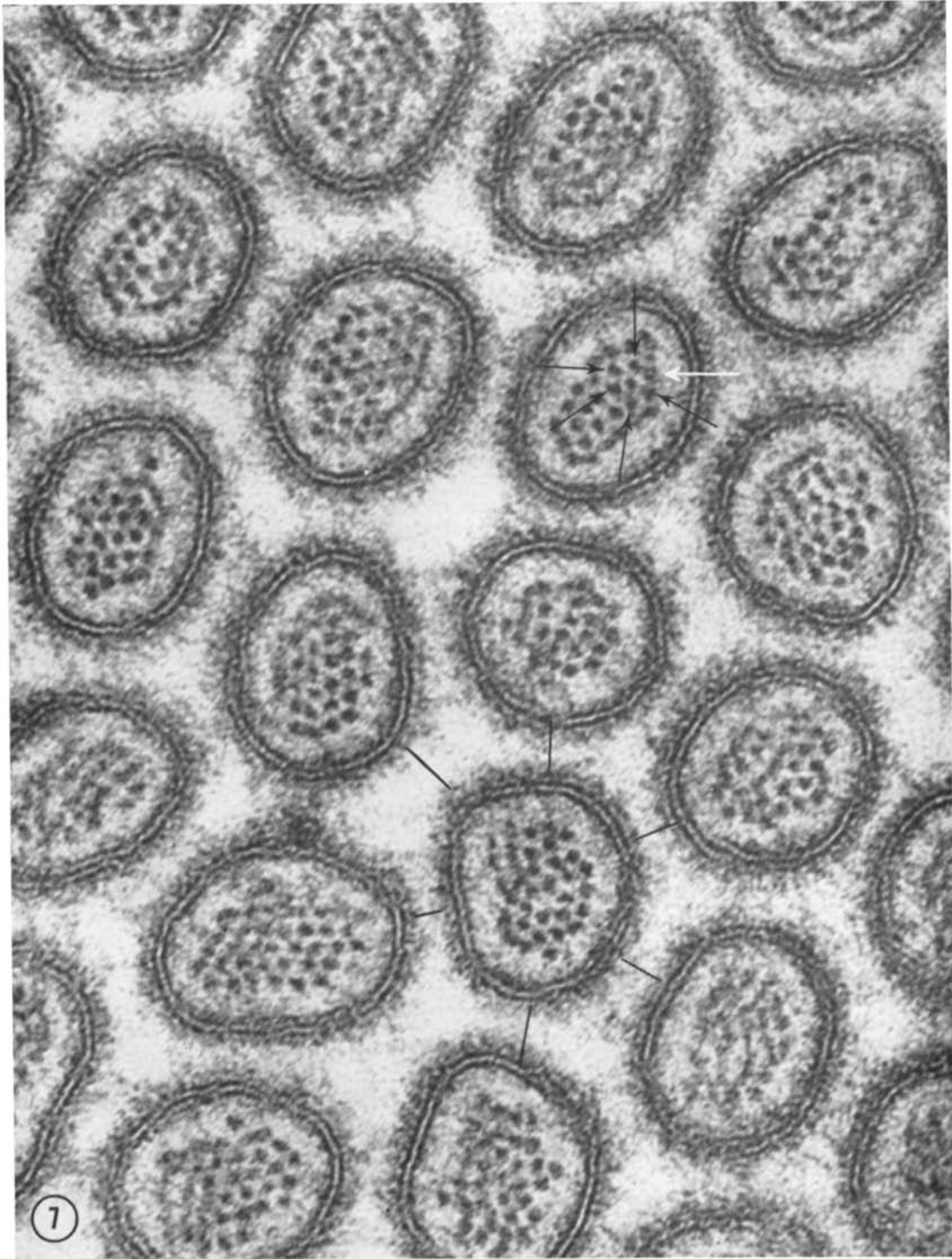


FIGURE 7 A transverse section through the striated microvillous border of the jejunal epithelium, showing the trilaminar plasma membrane, the filament-free zone without any apparent substructure, and the central core consisting of packed microfilaments. In the cores of some of the microvilli are seen the cross-sections of tubules (white arrow); the total diameter of each tubule ranges from 60 A to 110 A. Note the arrangement of the microvilli (lines below) and also of the microfilaments in the core (black arrows) which indicates packing in a hexagonal array. $\times 275,000$.

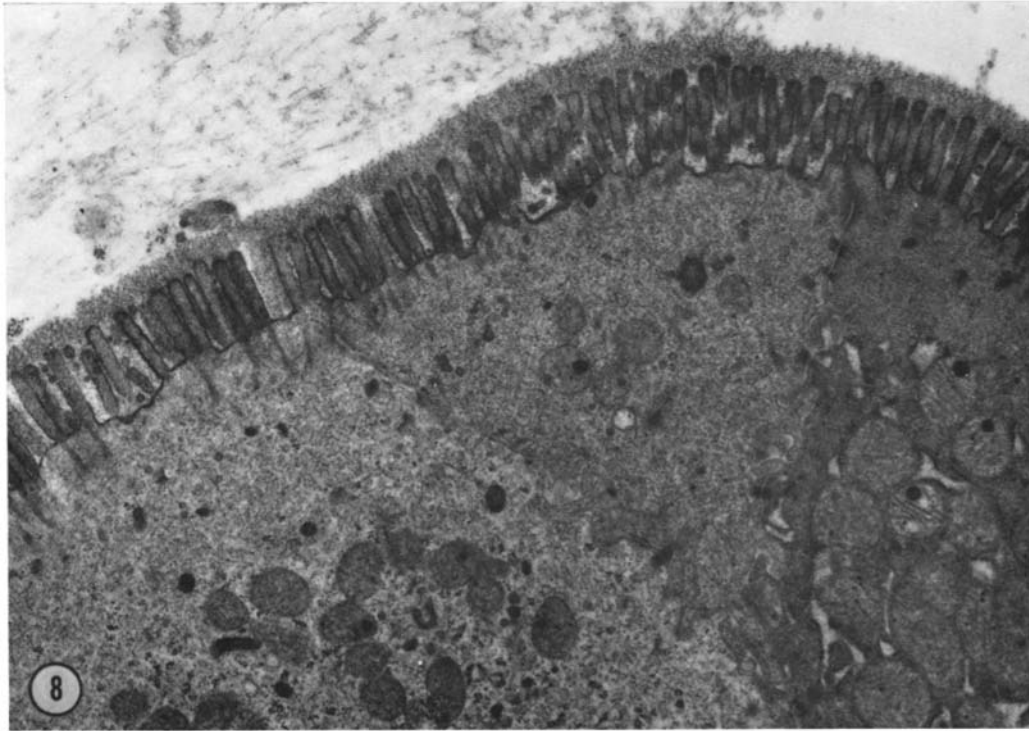


FIGURE 8 A portion of the true luminal border of the mouse colon, showing the thick surface coat (0.1-0.25 μ) covering the microvilli of the absorbing cells. $\times 16,000$.

the same authors (10) on rat intestinal microvilli. This disparity may be due to a species difference.

While comparing the hexagonal packing of the microfilaments with the packing of the microvilli observed in this study and also by Palay and Karlin (12) on rat intestinal epithelium, one cannot fail to notice the similarity between these two arrangements. It therefore seems reasonable to assume that this pattern has some obvious geometrical significance, e.g., the most efficient way of packing together identical elements.

Some of the microfilaments in the microvillous core appear as tubules with a total diameter of 60-110 A. Laguens and Briones (7) also made a similar observation on human intestinal epithelium, but the range of total diameter of the tubular structure was much greater than that obtained in the present study. These authors (7) also expressed the opinion that all the microfilaments were actually tubules. Our micrographs do not show conclusively that all the microfilaments are tubular structures. These

variations may be due to a species difference or to differences in the techniques employed.

At the tips of the microvilli of both small intestinal and colonic epithelial cells, some of the microfilaments of the microvillous core seem to run across the plasma membrane limiting the microvilli. The literature does not reveal any similar observation, although several workers have studied the characteristics of the microfilaments in microvilli of rat intestine (10) and human intestine (7). These authors (7 and 10) expressed the opinion that the microfilaments most probably end in the accumulation of dense material adjacent to the inner layer of the plasma membrane. This discrepancy may be due to a species difference, to the type of fixatives employed, or possibly to differences in section thickness. The thickness of the sections may be an important point for consideration since, from our own experience, the continuity of the microfilaments across the plasma membrane can only be observed in extremely thin sections which cut through the central longitudinal plane of the microvilli. In

regard to the feasibility of such an interpretation, the question of possible overlapping of fine filaments of the surface coat or of core microfilaments of the same or another microvillus must be taken into consideration. The fact that the sections on which these observations were initially made were colorless precludes the possibility of overlapping between the structures of the sectioned microvillus and the structures of another microvillus lying above it. Moreover, the fact that the triple-layered plasma membrane may be clearly observed over the tips of the microvilli (Figs. 5 and 15) discounts the possibility of overlapping by a superimposed microvillus. It is, therefore, presumed that the structures observed in Figs. 5 and 15 belong to one microvillus. In both of these electron micrographs the microfilaments which appear to run across the plasma membrane may be traced for quite a distance into the core of the microvillus. The

chance, therefore, that a fine filament of the surface coat is superimposed on a microfilament within the core, lying in the same plane, seems to be only a remote possibility. In addition, in Fig. 5 the microfilament (white arrows) belonging to the adjacent microvillus seems to be continuing through the plasma membrane, although its actual course within the clear intermediate layer is not clearly visible. In this instance, too, the chance that a fine filament of the fuzz lies in the same plane as the microfilament seems to be a very unlikely possibility. However, this observation needs further confirmation since it may have some interesting physiological significance.

One of the most important features of this study has been the observation of variations in the extent and disposition of the surface coat in the epithelium of both small intestine and colon.

In the small intestine the microvilli of the absorbing cells lining the jejunal villus and crypts

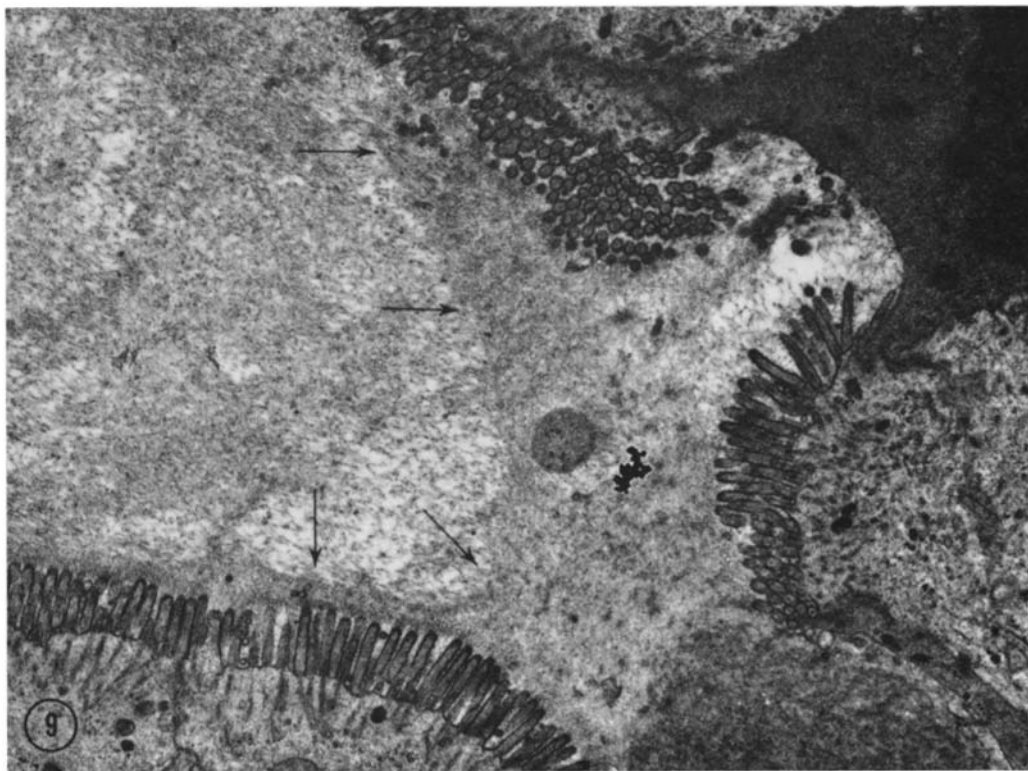


FIGURE 9 A section through the colonic crypt cut longitudinally at a level, which is higher than that in Fig. 11, showing the thick surface coat in close proximity to the microvilli. Arrows indicate a bridge of surface coat material, in the lumen, connected on either side to the surface coat over the cells lining the crypt. The bridge overlies two goblet cells and an absorbing cell, the surface coat of which is connected to this bridge. The goblet cells in this picture do not show any well-developed surface coat. $\times 13,000$.

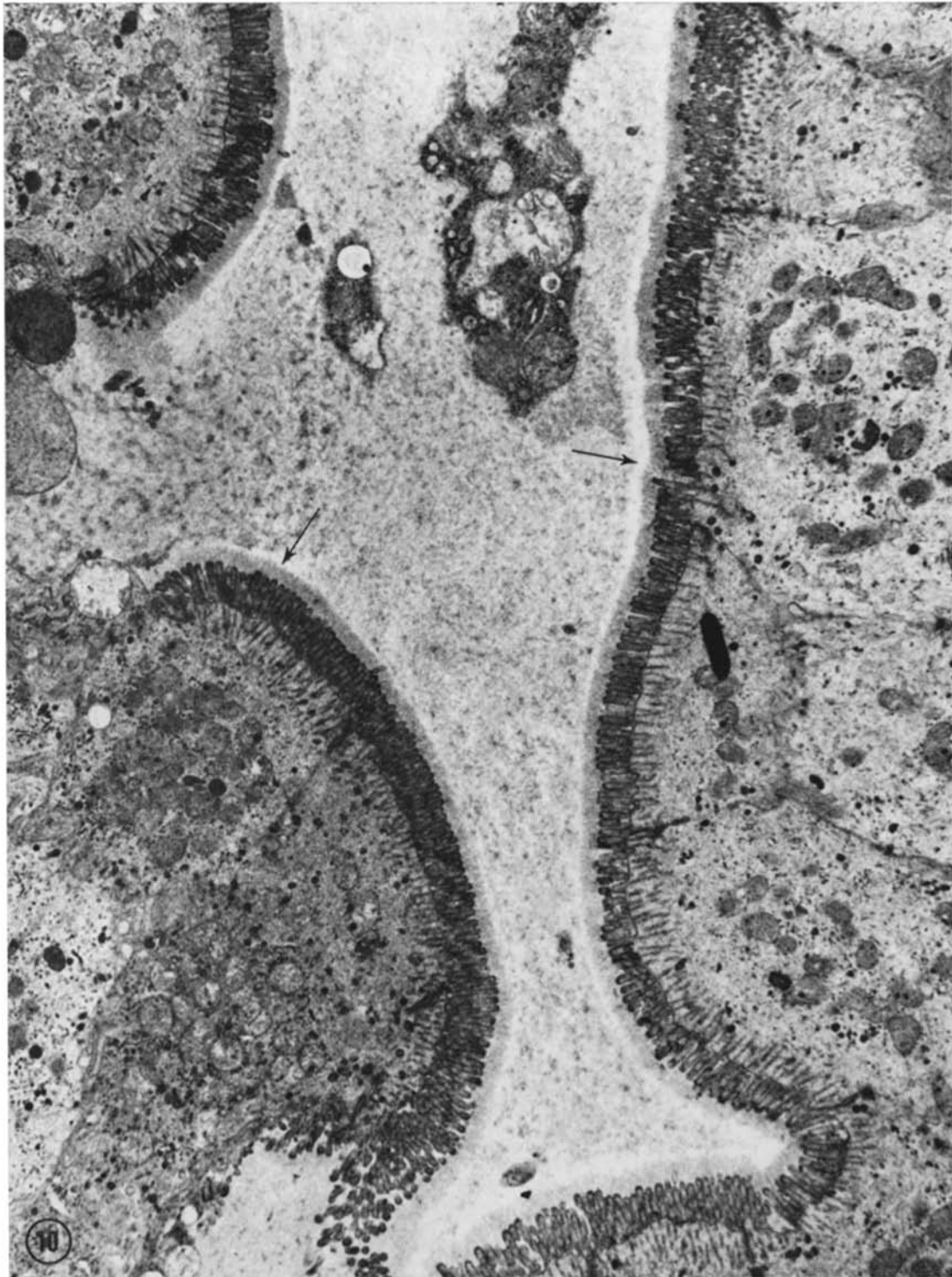


FIGURE 10 A relatively low-power electron micrograph of colonic crypt cut near to the luminal surface, showing the dense surface coat lining the lumen. Note the mucus in the lumen which is separated from the surface coat by a clear zone (arrows). The goblet cell lying on the left side of the lumen has no apparent surface coat. $\times 9,000$.

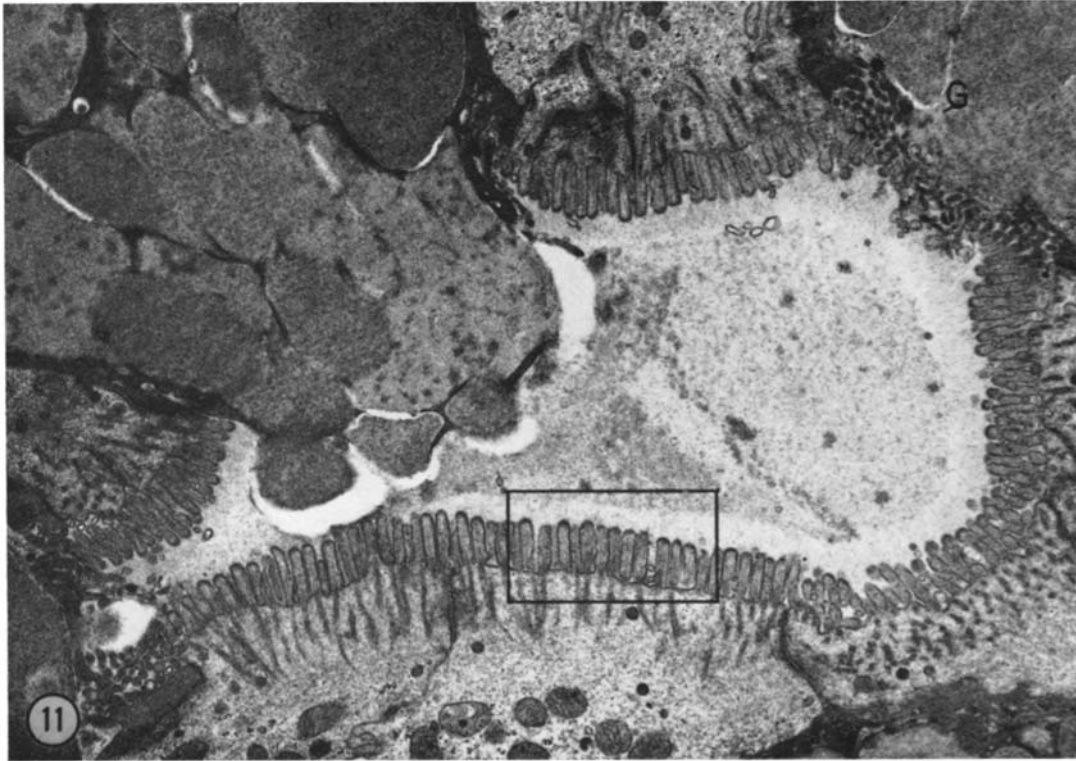


FIGURE 11 A portion of colonic crypt epithelium cut nearer to the base, showing a lighter zone closely applied to the microvilli. Within this zone fine filaments of the surface coat may be observed. The goblet cell (G) has a surface coat of lesser dimension than the adjacent absorbing cells. $\times 13,000$.

show most commonly a thin layer of surface coat which may be more appropriately designated as the fuzz of other workers (17). However, in addition to the fuzz, certain regions with a prominent surface coat were also observed but only on the absorbing cells lining the jejunal villus. The appearance of the surface coat in these regions is very similar to that observed in the colonic epithelial cells in the present study and also by Ito (3 and 4) in the intestinal epithelium of cat, bat, and human. The fact that localized areas of a well-developed surface coat are associated with a few absorbing cells in a row lining the jejunal villus only, and not with the cells lining the crypts, tempts us to assume that this form of surface specialization of the absorbing cells serves some specific function.

Another interesting feature of the surface coat in these regions is its limitation by the cellular boundary as illustrated in Fig. 4. This observation

strengthens the concept put forward by Ito (3) and Fawcett (2) that the surface coat is an integral part of the cell; it also reinforces our concept that the surface coat is a functional entity belonging to the cell and is not an extraneous coat.

In the mouse colon, in contrast to the small intestine, a thick surface coat is present over the microvilli of the absorbing cells lining the luminal borders and the crypts. However, the coat on the cells lining the higher regions of the colonic crypts differs slightly from that on the cells lining the lower regions of these crypts. The appearance of the surface coat in the higher regions of the colonic crypts and on the cells lining the luminal border is very similar to what has been observed by Ito (3 and 4) on the intestinal epithelium of other mammals, e.g. cat, bat, and human; but we have failed to obtain from the literature any observation similar to our finding on the structure of the surface coat at the basal regions of the crypts.

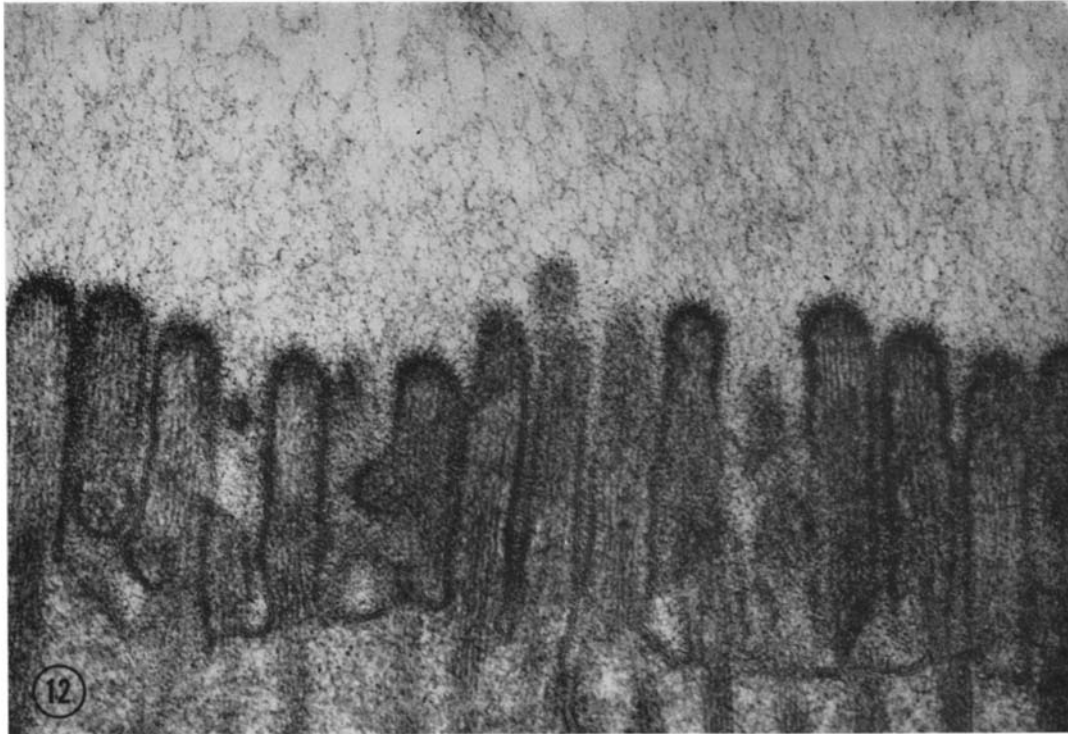


FIGURE 12 A high magnification micrograph of the rectangular area outlined in Fig. 11, showing the characteristics of the surface coat. The fine filaments of the surface coat show less branchings, and the individual filaments are oriented more perpendicular to the plasma membrane than they are in Fig. 15. $\times 85,000$.

Before the further significance of these variations can be discussed, the possibility that fixation artefacts cause this difference has to be considered. Ito (3) in his report on cat intestinal epithelium also stated that the appearance of the surface coat depended upon the fixatives and stains used. Although the same fixation was employed for all the experiments in our studies, sections taken from different portions of the same block showed this difference. Moreover, the cells did not show any damage which could be attributed to fixation. Therefore, it seems reasonable to assume that this difference is most probably due to a real dissimilarity in the organization of the surface coat filaments.

Cell proliferation in the intestinal epithelium has been reported (1, 8) to occur in the lower two-thirds of the crypt. Therefore, finding loosely packed, fine filaments in the surface coat near the bases of the colonic crypts probably indicates that

the surface coat is immature, whereas the more compact arrangement of filaments in the higher regions of the crypts indicates that the surface coat is more mature and is catering to the needs of a differentiating cell. That the structure of the surface coat in some portions of the jejunal villus is similar to the structure of the surface coat lining the higher regions of the crypts and the lumen of the colon supports the contention that a relationship exists between this form of "surface specialization" (2) and cellular differentiation. The absence of any typical surface coat on the cells lining the crypts of the small intestine poses an interesting problem, but it may be argued that the migration of epithelial cells may be faster in the the small intestine than in the colon. However, further work is necessary to confirm such an assumption.

The goblet cells in both the small intestine and

the colon do not show the sort of surface coat which has been observed over the absorbing cells, except for the fuzz on their microvilli. Only some of the goblet cells in the colonic crypts show an organized surface coat running continuously with the layer above the adjacent absorbing cells. In the colonic crypts, bridges of surface coat material have also been observed; this observation suggests that the surface coat over the goblet cells may have been contributed by the adjacent absorbing cells, since in these places very few filaments were actually seen to arise from the plasma membrane.

In conclusion, it is emphasized that the microvilli on both jejunal and colonic epithelial cells show a remarkable similarity in their inner structure but a considerable difference in their surface coats. Most of the detailed studies on the surface coats have involved intestinal epithelium

in the cat, bat, and human (3), although Ito (3) mentions that in mice, rats, and a few other species the surface coat exists as a thin layer. From our observations, it is apparent that the intestinal epithelium in the mouse exhibits interesting variations in the nature of the surface coat which have not been observed in the intestinal epithelium in cat, bat, and human (3). For this reason, intestinal epithelium in the mouse offers a particularly useful material for the study of the structure and function of the surface coat.

The authors wish to acknowledge Dr. F. O. Simpson for his valuable discussions and to Miss Margaret Stringer and Miss Janet Ledingham for technical assistance.

This study was supported by a grant from the Medical Research Council of New Zealand.

Received for publication 18 November 1966.

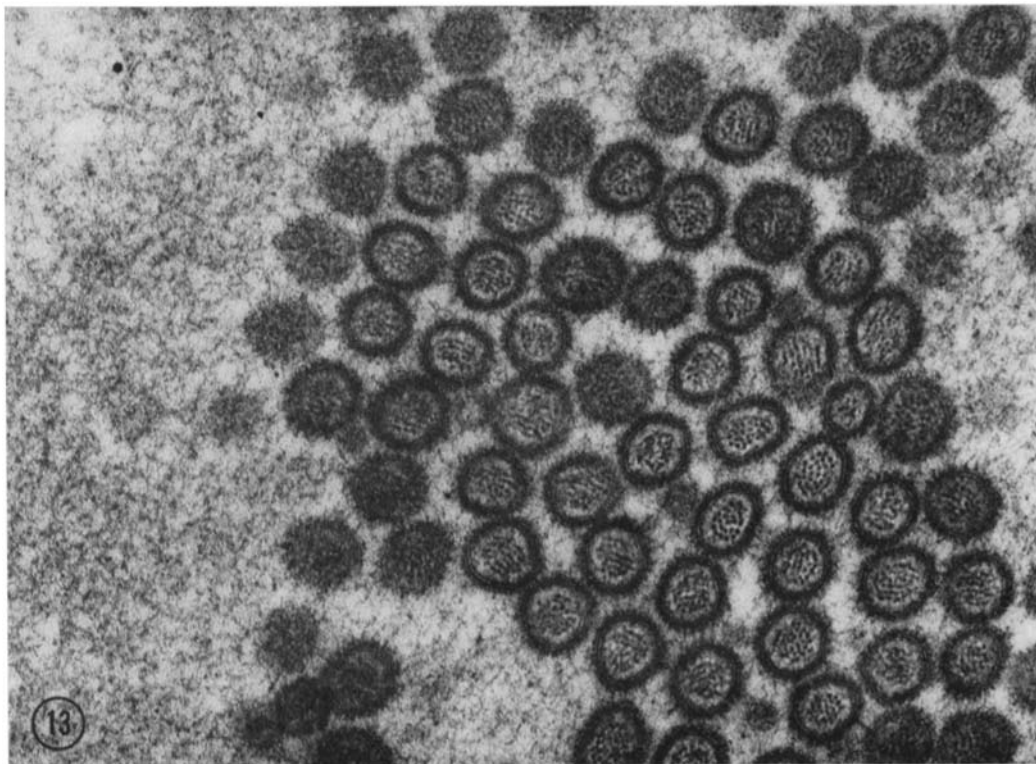


FIGURE 13 Transverse section through the striated microvillous border of the absorbing cells lining the true luminal border of the colon, showing the trilaminar plasma membrane, the filament-free zone, and the central core of packed microfilaments. Note the thick surface coat material on one side of the micrograph. $\times 68,000$.

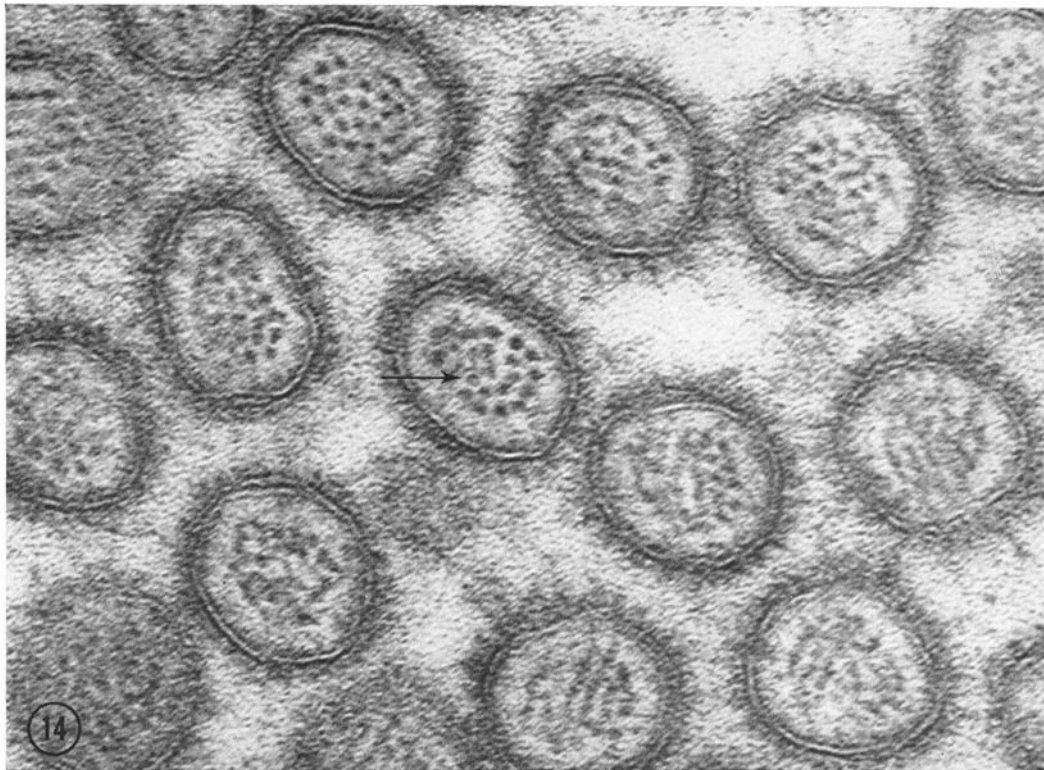


FIGURE 14 A high-magnification micrograph of a small area of the microvillous border in Fig. 13, showing that the packing of the microfilaments in the colonic epithelium is similar to that in the jejunal epithelium (Fig. 7). The arrow indicates a microfilament cut transversely which has the appearance of a tubule. Compare with Fig. 7. $\times 200,000$.

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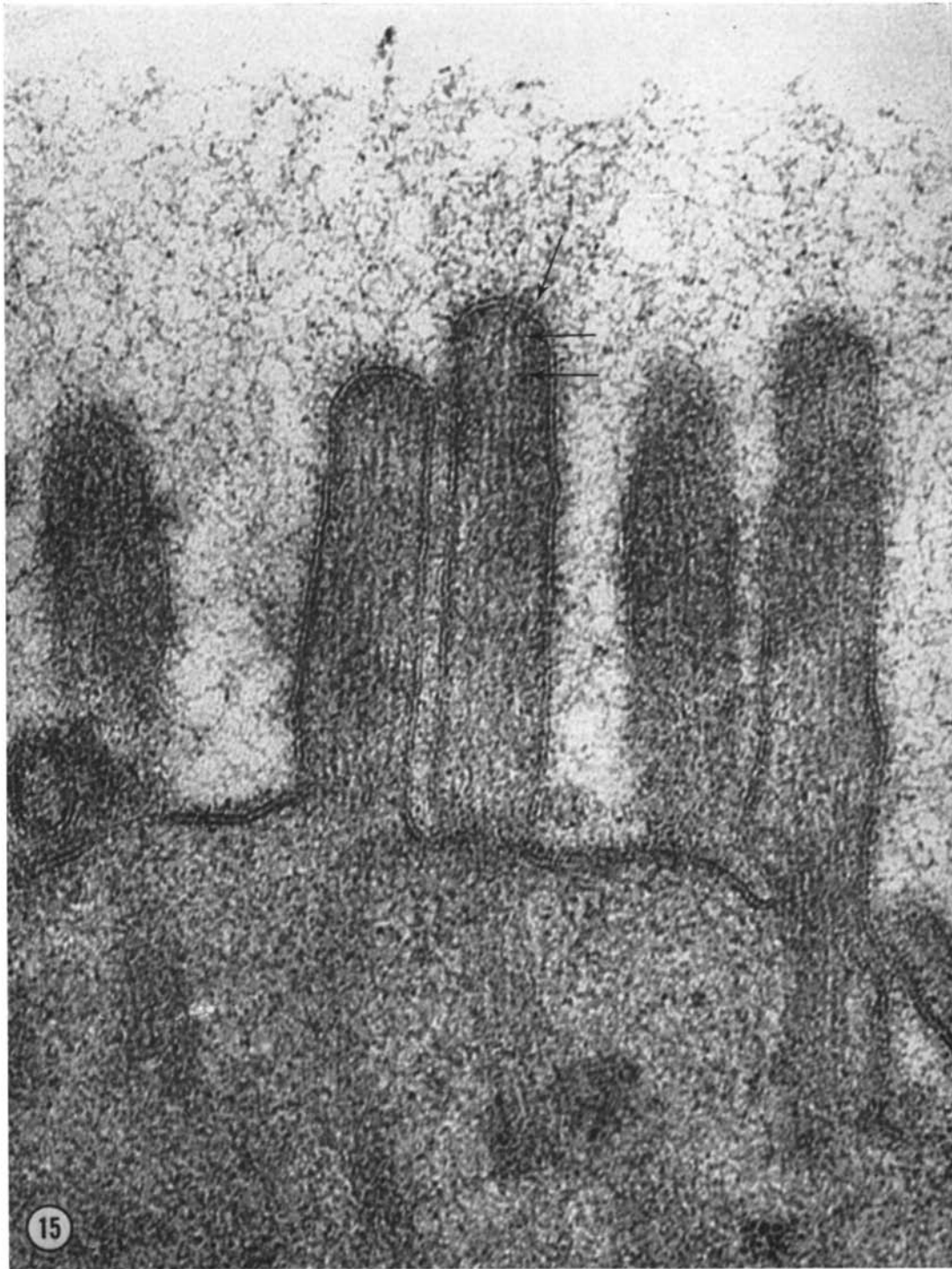


FIGURE 15 A high-magnification micrograph of a few microvilli (cut longitudinally) of absorbing cells lining the true luminal border of the mouse colon, showing the trilaminar nature of the plasma membrane and the characteristics of the surface coat. Note that the filaments of the surface coat in this micrograph are more compact than those of the surface coat seen in Fig. 12. Arrows indicate the continuity of a microfilament across the plasma membrane. $\times 160,000$.