THE FINE-STRUCTURAL ORGANIZATION OF THE BRUSH BORDER OF INTESTINAL EPITHELIAL CELLS

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SUMMARY

The fine structure of the brush border of intestinal epithelial cells of the mouse has been studied with both normal sectioning and freeze-etching techniques.

Freeze-etching reveals the plasma membrane of the microvilli as consisting of a continuous layer, that is split during the cleaving process, in which numerous particles, 5–9 nm in diameter, are embedded, while other particle-like structures, with diameters of 7–10 nm, appear attached to the true outer membrane surface. The mucopolysaccharide surface coats of the microvilli show up more clearly in sectioned material than in freeze-etched specimens.

Inside each microvillus 2 different filament systems can be demonstrated: (1) bundles of fairly closely packed and straight core microfilaments, which lead into the tip of the microvillus, and (2) short cross-filaments. Under suitable conditions the core microfilaments display a sub-unit structure with a repeating distance of approximately 6 nm. The diameter of a microfilament can vary along its length from 6 to 11 nm. Two strands of globular particles wound helically around each other seem to make up each microfilament. These and other data support the idea that the core microfilaments are actin-like. No substructure has been found on the cross-filaments, which have an orientation approximately radial to the axis of the microvillus and seem to be attached at one end to the core microfilaments and at the other to the inner surface of the microvillous membrane.

The interwoven terminal web filaments also show no substructure. They form a continuous flexible platform-like structure into which the bundles of core microfilaments extend. Some terminal web filaments appear attached to the plasma membrane between the microvilli. It is suggested that the core microfilaments represent mechanical supporting elements and that the terminal web and cross-filaments are tensile elements of the brush border. In addition all 3 filament systems may also be involved in possible contractile movements of the microvilli.

INTRODUCTION

In recent years a great deal of interest has been focused on the elucidation of both the fine-structural (Granger & Baker, 1949; Zetterqvist, 1956; Palay & Karlin, 1959; Millington & Finean, 1962; Sjöstrand, 1963; McNabb & Sandborn, 1964; Ito, 1965;
Laguens & Briones, 1965; Mukherjee & Wynn Williams, 1966, 1967; Boyd & Parsons, 1969) and the functional (Miller & Crane, 1961; Holt & Miller, 1962; Eichholz & Crane, 1963, 1965; Dahlqvist & Nordström, 1966; Oda & Seki, 1966; Crane, 1966, 1968; Moog & Grey, 1967) organization of the brush border. All these investigations have suggested that the brush border of the intestinal epithelial cell is a highly specialized sub-cellular organelle. Until now, however, our knowledge of the brush border fine structure has depended solely on studies of chemically fixed, dehydrated, embedded and sectioned material. Although this is the most versatile technique for studying cellular fine structure at present, its limitations have become particularly apparent in investigations of the fine-structural organization of the microvillus of the brush border. This is manifested in the failure to agree whether the filaments of the core of the microvillus are actually filaments (Millington & Finean, 1962; McNabb & Sandborn, 1964; Boyd & Parsons, 1969) or tubules (Millington & Finean, 1962; Laguens & Briones, 1965) or possibly both (Mukherjee & Wynn Williams, 1967). Similarly, Millington & Finean’s (1962) observations, presumably of physiological significance, that pores are present in the microvillous plasma membrane and that lateral filaments stretch between the core microfilaments and the lateral microvillous plasma membrane, have remained unconfirmed and perhaps unappreciated. It is thus apparent that a good understanding of which structures actually constitute the microvillus and how they are disposed, is still to be attained, in spite of our using improved fixation agents (Sabatini, Bensch & Barnett, 1963) and embedding chemicals (Ryter & Kellenberger, 1958; Luft, 1961).

Our success in applying the freeze-etching technique (Moor & Mühlethaler, 1963) to the fine structure of intestinal tight junctions (Staehelin, Mukherjee & Wynn Williams, 1969a, b) suggested that the same method could be applied with equal advantage to investigate the morphology of the microvillus of the intestinal epithelium. To this end we have made a combined conventional sectioning and freeze-etching study of the brush border of epithelial cells of the mouse intestine in the same manner as was done previously by Mukherjee & Wynn Williams (1966, 1967) using conventional techniques alone.

**MATERIALS AND METHODS**

**Sectioning**

Small pieces of mucosa from the proximal portions of jejunum and colon of NZCW mice were fixed for 2 h in 2.5%, glutaraldehyde in phosphate buffer (pH 7.2–7.4) and then postfixed for 2 h in 2% osmium tetroxide in the same buffer at the same pH. The blocks were then dehydrated in a graded ethanol series and embedded in Epon 812 (Luft, 1961). Sections of varying thickness were cut with either a Porter-Blum ultramicrotome or an LKB Ultratome III ultramicrotome with glass knives having ~ 40° angles (obtained from LKB Knife-maker). Sections for electron microscopy were all mounted on 75/300 mesh copper grids without any supporting membrane and double stained by immersing first in saturated aqueous solution of uranyl acetate and then lead citrate.
Freeze-etching

To prevent the formation of ice crystals during the freezing process 20–30 % glycerol was added either to a Ringer solution or to 2 % glutaraldehyde buffered at pH 7.2 with phosphate buffer and injected into 2–3 cm long ligated loops of the proximal region of the small intestine and colon of anesthetized NZCW mice, and in some controls of albino rats from the Charles River Breeding Laboratory. In other experiments the epithelial tissues were prefixed with buffered glutaraldehyde for 15–60 min before glycerol was added. A few control specimens were also processed without any pretreatment. During the infusion of the glycerol the animals were kept under anaesthesia for 15–30 min, following which small pieces of epithelium were removed, cut into cubes of less than 1 mm³, and rapidly frozen on copper disks in Freon 12 held at –150 °C. The freeze-etching was performed according to Moor & Mühlethaler (1963) on a freeze-etch apparatus produced by Balzers. Etching of the specimens varied from 3 s to 2 min at –100 °C. The encircled arrow on each micrograph indicates the shadowing direction.

Observations

Conventionally processed tissue

Our present observation on the microvillous fine structure is essentially similar to what has already been described in detail by Mukherjee & Wynn Williams (1967). Therefore the results will be only briefly summarized here. The microvilli of the jejunal and colonic epithelial cells have a remarkably similar fine structure with respect to the thickness of the plasma membrane (9–11 nm), the extent of the so-called ‘filament-free zone’ (20–30 nm) which separates the core from the plasma membrane, and in the characteristics and disposition of the core microfilaments. It is mainly in the extent and organization of the surface coat that the microvilli of the small intestine and the colon differ. Similar variations in the characteristics of the surface coat are also observed among epithelial cells lining the different regions of the colonic crypts.

In addition, however, some novel morphological features have been observed: (1) In very thin longitudinal sections of microvilli the core or axial microfilaments are constructed of spherical subunits which, when clearly observed, reveal a centre-to-centre spacing of about 6 nm. The arrangement of these subunits in such sections (Fig. 2) suggests that each core microfilament could consist of 2 helically wound strands. This structure would explain the variation in the apparent thickness of individual core microfilaments from 6 to 11 nm (Fig. 2). (2) The region surrounding the closely packed axial filaments of the microvillus, which is commonly known as the ‘filament-free zone’ in thin sections, possesses filaments, about 6 nm in thickness, crossing this zone either transversely or obliquely (Figs. 3, 4). These are termed ‘cross-filaments’ in this study and are probably identical to those originally described by Millington & Finean (1962) as the lateral strands or fibrils. Cross-filaments are difficult to discern in thicker sections, possibly due to overlapping effects or because of the electron density of the surrounding matrix. They appear to arise from the vicinity of the core and may sometimes show a connexion with the core microfilaments on one side and extend up to the plasma membrane on the other (Figs. 3, 4). No substructure could be discerned in these cross-filaments.
Freeze-etched specimens

The fine structure of microvilli as revealed by freeze-etching of epithelial cells of both the jejunum and the colon is similar and therefore both types will be described together. Furthermore, as the appearance of tissues pretreated with either glycerol/Ringer or glutaraldehyde/glycerol/phosphate buffer solutions is essentially similar, except for a few instances, the ultrastructure will be described without any special reference to the mode of pretreatment. Only when describing the exceptions will a reference be made to the method of pretreatment.

Microvillous plasma membrane. The microvilli of freeze-etched epithelial cells of the intestine are most frequently cleaved in such a way as to reveal surfaces of their limiting plasma membranes (Figs. 5-7). A careful examination of these surfaces indicates that in general they probably represent split inner membrane faces of the type proposed by Branton (1966). Supporting this interpretation are the following observations. Suitably cross-fractured microvillous plasma membranes appear to consist of 2 (each approximately 4-5 nm wide) parallel ridges (Figs. 11-13), which probably result from the individual fracturing of each half of the membrane combined with some splitting along the central plane (Branton, 1966; Staehelin, 1968a). When individual microvilli are obliquely cleaved (Fig. 6) 2 different surface views of their surrounding membrane are seen. The convex surfaces (pa) facing towards the external space are characterized by a ridge (4-5 nm wide) of etch-resistant material at their bases. The concave surfaces (pb) on the other hand, which face toward the microvillous core, can be seen to be continuous with and identical to the inner surface of the ridge material facing the pa surfaces (Fig. 6). It is therefore suggested, in line with Branton’s (1966) theory, that the surfaces pa and pb are complementary split inner plasma membrane faces. In the micrographs Figs. 5, 7 these 2 membrane faces can be examined in greater detail. The convex pa faces seen, for example, on the right side of Fig. 5, carry large numbers of small particles, 5-9 nm in diameter, except in the tip region of the microvilli. There the pa faces appear to be nearly devoid of the type of membrane particles that is exposed by the cleaving process. Only a few particles are generally present on the split inner membrane faces pb (see, for example, the left side of Fig. 5). If pa and pb are truly complementary membrane faces of the type proposed by Branton then we should be able to see not only membrane particles but also a matching number of holes in the membrane surfaces; pb faces should exhibit large numbers of holes, pa faces relatively few. Despite the fact that small depressions are difficult to recognize in replicas it is clearly seen in Fig. 7 that the number of small holes in the pb surfaces greatly exceeds the number detectable on the pa faces.

Besides revealing particles on the split inner membrane faces, freeze-etching has also provided evidence for what appear to be particle-like structures protruding from the true outer plasma membrane surface of the microvilli. These particles are most readily visualized along the edges of the pb membrane faces, i.e. where only the external half of the membrane is left, in freshly cleaved and unetched specimens (Figs. 5, 7). Under these conditions the frozen material of the lumen surrounding the micro-
Brush border fine structure

villi has an amorphous appearance allowing the protruding particles, 7–10 nm in diameter, to be distinguished from background irregularities.

**Surface coat.** Although the high degree of hydration and the fine, loosely packed filamentous structure of the mucopolysaccharide surface coat seem to preclude an unambiguous demonstration of this structure by means of freeze-etching, we have been able to obtain some information confirming at least the presence of a thin layer of material covering the outer surface of the microvilli.

By deep-etching frozen intestinal tissues not pretreated with glycerol it is possible to expose an amorphous coat, 10–30 nm thick, surrounding the whole microvillus (Fig. 8), which could represent the freeze-etch equivalent of the 'fuzz' known from sectioned material (see Mukherjee & Wynn Williams, 1967; and Figs. 2–4). Similarly, occasional halo-like irregularities in the fracture plane around the tips of some microvilli (Fig. 9) may also be related to the presence of this layer. However, in none of our freeze-etch specimens have our results been as clear-cut as in suitably stained sections. Furthermore, freeze-etching has provided us with no information at all on the enteric surface coat similar to what is seen in Fig. 10.

**Components of the microvillous matrix.** The structure of the core is best demonstrable in longitudinal fractures of the microvilli. The core microfilaments, i.e. the axial filaments disposed in the core region of the microvillus, are readily observed in freeze-etch specimens (Figs. 11–13 and 20), and in general these appear similar to those seen after conventional methods of sectioning and staining (Figs. 2–4). The individual microfilaments measure 6–11 nm in thickness and show a centre-to-centre spacing of between 12 and 24 nm (Figs. 11–13 and 19). At higher magnifications (Figs. 11, 12) of longitudinally fractured microvilli they appear to consist of subunits, with a repeating distance of approximately 6 nm. At the tip of the microvillus the microfilaments can be followed close to but never clearly right up to the inner surface of the plasma membrane. Supporting evidence for the continuation of the core microfilaments across the plasma membrane in the tip of the microvilli, as suggested from thin sections (Mukherjee & Wynn Williams, 1967) has not been obtained with the freeze-etching technique. At the base of the microvillus the bundles of core microfilaments seem to extend for variable distances into the filamentous meshwork of the terminal web (Figs. 20, 21).

Besides the core microfilaments, a second set of filaments can be clearly demonstrated inside microvilli processed with the freeze-etching method. These filaments, which bridge the 20–30 nm wide zone between the closely packed microfilaments of the core and the surrounding plasma membrane, usually run approximately at right angles to the axial microfilaments (Figs. 12–15) and have therefore been termed 'cross-filaments' in accordance with the similarly distributed fibrillar elements seen in sections (Figs. 3, 4). It may, however, be significant that in some microvilli, groups of cross-filaments take up angles with the core microfilaments which differ considerably from the 'standard' 90° (Figs. 13, 14). In the core region the 5–9 nm thick cross-filaments seem to have some association with the core microfilaments (Figs. 13, 14) while peripherally they extend out to the microvillous membrane (Figs. 13–16). Occasionally the cross-filaments appear to be attached through particles to the
microvillus membrane (Fig. 16). In transverse fractures they show a roughly radial orientation (Fig. 16). No clear substructure is discernible on the cross-filaments, although the shadowing along their length may appear somewhat irregular. The great variation in the apparent number of cross-filaments per microvillus (compare Fig. 12 with Fig. 13) makes it virtually impossible to estimate the actual number of filaments involved.

**Terminal web.** The mass of interwoven filaments of the terminal web have similar diameters to the cross-filaments of the microvilli and are usually distributed parallel to the brush border surface. Frequently some of the filaments of the terminal web appear to be anchored to the apical plasma membrane at the base of and in between the microvilli (Figs. 17, 18, 20 and 21). The outline of these filaments can often be traced for a few hundred Ångstroms on the exposed, supposedly split inner membrane face (Fig. 17).

It is interesting to note that in crypt cells there are significantly greater numbers of terminal web filaments attached to the plasma membrane (Fig. 17) than in those of the higher regions of the crypt or the villus (Fig. 18). Again, in the crypt cells of particularly the colon more filaments are seen to be attached to the plasma membrane than in the jejunum. Thus it seems that more terminal web filaments are attached to the plasma membrane in regions of the apical border of the epithelial cells, where the microvilli are widely spaced.

Cytoplasmic microtubules having nearly the same dimensions (~ 20 nm) as those observed in sectioned material (Mukherjee & Wynn Williams, 1967) are also seen after freeze-etching (Fig. 20). They usually terminate just below the terminal web.

**DISCUSSION**

The fact that most of the structural elements of the microvillus described in this paper have been found both in sections of chemically fixed and embedded materials as well as in freeze-etched specimens suggests that they represent cellular structures which exist in vivo.

**Plasma membrane**

Various authors (Sjöstrand, 1963; Farquhar & Palade, 1963; Mukherjee & Wynn Williams, 1966, 1967; and Dermer, 1967) have demonstrated that in sectioned material the plasma membrane of the brush border has an asymmetric appearance, the cytoplasmic opaque layer being thicker (3.5-4.5 nm) and more intensively stained than the peripheral opaque layer (2.5-3.0 nm). Our present freeze-etch study, on the other hand, has revealed the same plasma membrane as consisting of a continuous layer, possibly a predominantly lipid bilayer that is split during the cleaving process, in which numerous particles (~9 nm in diameter) are embedded. In addition, other particle-like structures appear to be protruding from the true outer membrane surface. How these two electron-microscope images are related to each other is difficult to envisage at present. Nevertheless, the regularly occurring asymmetric cleaving of the plasma membrane in freeze-etch specimens, which leaves most of the internal mem-
brane particles adhering to the inner, cytoplasmic half of the split membrane, suggests a possible connexion with the asymmetric stain deposition in sections. Fii & Branton (1969) have drawn attention to the fact that metabolically active membranes carry more freeze-etch particles than those showing little activity, thus implying that the particles might represent membrane-bound globular proteins with enzymic activities. Similar ideas have also been expressed by other authors (Moor & Mühlethaler, 1963; Mühlethaler, Moor & Szarkowski, 1966; Weinstein & Bullivant, 1967; Branton & Park, 1967; and Staehelin, 1968a, b). According to biochemical investigations the microvillous plasma membrane is rich in various enzymes such as alkaline phosphatase (Clark, 1961; Eichholz & Crane, 1965; Moog & Grey, 1967), adenosine triphosphatase (Oda & Sato, 1964; Overton, 1965; Pearse & Riecken, 1967), leucine aminopeptidase (Pearse & Riecken, 1967), invertase, maltase (Crane, 1968) and phosphomonoesterase (Holt & Miller, 1962). While adenosine triphosphatase seems to be predominantly located on the cytoplasmic surface of the plasma membrane (Oda & Sato, 1964; Overton, 1965), invertase and leucine aminopeptidase appear to be attached to the outer surface (Oda & Seki, 1966). The latter authors have been able to correlate the latter 2 enzymes with 4–6 nm particles seen on the external surface of isolated and negatively stained microvillous membranes. Under these conditions it seems not unreasonable to suggest that the similarly located particles in freeze-etch specimens could also correspond to the same 2 enzymes. Further studies will have to be made to determine if the particles revealed on the split inner membrane faces of microvilli could be enzymes too.

Surface coat

The layer of mucopolysaccharides covering the outer surface of the microvillous plasma membrane is now accepted as an integral part of the plasma membrane (Ito, 1965; Mukherjee & Wynn Williams, 1966, 1967; Revel & Ito, 1967). Following chemical methods of fixation and section staining this mucopolysaccharide coat appears either as a generalized surface fuzz (Figs. 2–4) surrounding both the tip and the lateral borders of the microvilli, irrespective of its location in the crypt or on the villus, or as a thick meshwork of fine filaments disposed in the form of a dense layer interposed between the lumen and the tips of the microvilli (Fig. 10) (Mukherjee & Wynn Williams, 1967). This latter has been termed the 'enteric surface coat' (Ito, 1965).

Freeze-etching has not revealed either of these surface coats in a very satisfactory way. This can be explained by the fact that the mucopolysaccharides probably exist in an extremely hydrated state, which, when frozen, is difficult to distinguish from the surrounding aqueous solution containing glycerol, soluble sugars and proteins.

Core microfilaments

The observation of a repeating substructure in core microfilaments both in thin sections and in freeze-etch replicas seems to confirm similar observations of Overton, Eichholz & Crane (1965) on negatively stained core microfilaments of isolated brush border fractions. Thus, by use of 3 different electron-microscope techniques, it can be established that the core microfilaments probably consist of subunits with a centre-
to-centre spacing of approximately 6 nm. It is interesting to note that the subunit structure of the core microfilaments can be detected only in freeze-etch specimens, which have not been prefixed with glutaraldehyde. The disappearance of the subunit structure could be caused by the covering up of the subunits with small protein molecules from the ground plasma which are cross-linked to the microfilaments during fixation.

The next question that arises is how the subunits are arranged in the core microfilaments. From the evidence we have at present, it appears that a linear arrangement of the subunits is unlikely, since it would be difficult to explain why the thickness of the core microfilament varies between 6 and 11 nm (Mukherjee & Wynn Williams, 1967, and the present study) or between 6 and 15 nm in the specimens of Laguens & Briones (1965). A more probable arrangement would seem to be the disposal of the subunits in 2 helically wound strands. Such a structure has been proposed by Hanson & Lowy (1963) for actin filaments. For this reason we have noted with interest the resemblance of the negatively stained isolated actin filaments of Hanson & Lowy (1963) with some of the core microfilaments seen in our thin sections (Fig. 2). The similarity between these 2 filament systems seems to go a step further, in that both the centre-to-centre spacing of 5.5 nm of the actin filament subunits and the variation of the thickness of these filaments between 7.5 and 9.5 nm are comparable to our measurements on core microfilaments. Furthermore, when the heavy-meryosin-labelling technique for identifying actin filaments is applied to epithelial tissues of the intestine, strong labelling of the bundles of core microfilaments of the microvilli is seen, while the terminal web filaments remain unchanged (Ishikawa, Bischoff & Holtzer, 1969). The combination of these histochemical results with the morphological observations presented in this paper seems to provide strong evidence that the core microfilaments in the microvilli are indeed actin-like, if not actin filaments.

The grouping of the usually straight core microfilaments into fairly closely packed, regularly spaced and cross-linked bundles of filaments, their extension into the tips of the microvilli, and the general spatial relationship of the filament bundles in respect to the microvilli suggest that one of their primary functions would be to support and maintain the microvillous structure. Overton & Shoup (1964), while studying the fine structure of the microvilli in maturing duodenal mucosa of chick, noted that the filamentous structure of the microvillous core appeared at about the same time as the rudimentary protrusions of the cell surface were being transformed into developing microvilli. Similar evidence has been presented for developing intestinal cells of Xenopus by Bonneville & Weinstock (1970) and for re-forming of microvilli after pressure-induced disassembly (Tilney & Cardell, 1969). Thus it seems apparent that the core microfilaments are associated with the very early stages of development of the microvilli, suggesting that they are essential structural features for maintaining these specializations of the cell surfaces.

Cross-filaments

Both thin sectioning and freeze-etching have confirmed the presence of cross-filaments in microvilli. Millington & Finean (1962), during their investigation on the
effect of hypo- and hypertonic saline on the brush border of the epithelium of rat jejunum, first suggested the presence of lateral strands or fibrils (synonymous with the term ‘cross-filament’ used in this study), in the microvillous matrix. The pictures shown at that time were not extremely convincing, and since such cross-filaments were more readily observed in specimens immersed in hypertonic saline, the question of their actual existence was debated even by the authors themselves. With the recent improvements of fixation, embedding and sectioning techniques several reports on the structure of the microvilli have appeared in the literature (McNabb & Sandborn, 1964; Donnellan, 1965; Laguens & Briones, 1965; Mukherjee & Wynn Williams, 1966, 1967). None of these recent investigators has confirmed or even suggested the presence of cross-filaments. This is probably because the sections examined were thicker than ours, since from our own experience it can be stated that in thicker sections there is usually considerable overlapping of the cross-filaments within the section thickness, and this, together with the background electron density of the matrix substance makes the proper delineation of single cross-filaments difficult. However, the clear evidence of cross-filaments in freeze-etch specimens, where their demonstration does not depend on any heavy-metal staining, leaves little doubt that cross-filaments do exist. The interpretation of cross-filament structures in freeze-etch micrographs is facilitated by the fact that they can be demonstrated both in longitudinal as well as in cross-fractured microvilli, a finding which leaves little room for possible alternative explanations.

Although freeze-etch micrographs suggest that the cross-filaments may be on one side attached to the core microfilaments, there is considerable evidence suggesting that the cross-filaments are chemically and structurally different from the axial microfilaments. Neither sectioning nor freeze-etching has been able to reveal any clear substructure in cross-filaments similar to the subunit pattern in core microfilaments. Furthermore, Crane (1966) reports that, while isolating different fractions of brush border of hamster intestine, it was observed that besides a clearly identifiable brush border plasma membrane fraction and a fraction containing core microfilaments (also studied by Overton et al. 1965), there were 2 more fractions that could not be identified with any known structural element of the brush border. ‘When appropriately diluted these fractions form fibrils, possibly identical with those reported several years ago’ by Eichholz & Crane (1963). It was consequently concluded by Crane (1966) that, since the core microfilaments or the microvillous plasma membrane did not comprise the entirety of substance of the microvillus, it was thus possible that these unidentified fractions that formed fibrils represented a different physical state of the material located between the core and the microvillous membrane. To us this conclusion seems to fit very well with the location of the cross-filaments and further suggests that the cross-filaments and the core microfilaments could be chemically different.

The cross-filaments appear to be associated not only with the core microfilaments but also either directly or through particles with the plasma membrane.

While most cross-filaments seen in our specimens are usually positioned at right angles both to the core microfilaments and the plasma membrane, groups of others occasionally take up different angles (Fig. 13). The significance of these
observations remains uncertain but they do indicate that the cross-filaments could be
more than simple tensile elements anchoring the plasma membrane to the core micro-
filaments. Indeed, the different arrangements of the cross-filaments may reflect the
existence of movement or even contractility of the microvilli. Taking the actin-like
properties, the packing and the relationship of the core microfilaments to the cross-
filaments and the plasma membrane into account, it seems not unrealistic to suggest
that by interacting, these elements could bring about a change in the shape of the
microvillus. Information supporting the idea of contractile microvilli is still very
scarce (Granger & Baker, 1949; Rostgaard & Thuneberg, 1968; Boyd & Parsons,
1969), but our present observations do add credence to this line of thought. It is con-
ceivable that contractile motions could, among other things, dramatically increase the
rate of transport of absorbed substances from the narrow microvillous projections
into the main cell body.

Terminal web

The filaments of the terminal web are similar to the cross-filaments in many re-
spects. These include the similarity in their thickness and the absence of any detectable
substructure. Furthermore, since some of the terminal web filaments are attached to
the inner surface of the plasma membrane, it appears likely that these filaments may
have some anchoring function similar to that discussed with regard to the cross-
filaments. In situations like the crypt epithelial cells, where there is a significant lack
of microvillous projections, more filaments are seen attached to the inter-microvillous
plasma membrane than in higher regions of the villus. This could serve as additional
evidence to indicate the likelihood of an anchoring function for these filaments to
hold the plasma membrane in position. A similar anchoring of fibrils to the surface
of the plasma membrane has recently been described in lymphatic tissues by Leak &
Burke (1968).

There seems to be ample evidence from this study and also from that of Brunser &
Luft (1970) that there is no direct connexion between the terminal web filaments and
the rootlets of the core microfilaments. However, the mechanical stability of the whole
brush border region, as shown by the absence of serious disruption of its overall
structure in isolated samples (Eichholz & Crane, 1965), suggests that some sort of
functional association exists between these 2 filament systems. Thus in line with the
opinion expressed by Brunser & Luft (1970) we also feel that structurally the mass
of interwoven filaments of the terminal web would be suitable to serve as a flexible
platform for the bundles of core microfilaments that support the microvilli.

Conclusion

In the schematic illustration (Fig. 1) we have attempted to summarize the presented
results so as to give an idea of the organization of the various structures occupying the
apical region of intestinal epithelial cells.

After taking all the presented data into consideration it seems likely that both the
cross-filaments of the microvilli and the filaments of the terminal web could act as
tensile elements for imparting stability to the microvillous plasma membrane, while
the bundles of core microfilaments would have a supporting function to the microvillus. Besides this static, structural interpretation of the function of the various filamentous elements we would like to suggest that they may also be involved in some dynamic aspects of the functioning of the brush border as a whole. The similarity of the core microfilaments to actin and the disposition of the different types of filaments with respect to the microvillous plasma membrane indicate a possible involvement of the filaments in microvillous contraction, if preliminary reports on the active movement of microvilli can be confirmed.

Fig. 1. Schematic diagram showing the relationship between the various filament systems in the brush border region of intestinal epithelial cells. cf, cross-filaments; f, 'fuzz'; mf, core microfilaments; pl, plasma membrane; sc, enteric surface coat; tf, terminal web filaments.

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Fig. 2. Electron micrograph of a longitudinally sectioned microvillus. The trilamellar appearance can be traced almost completely around the microvillus, thus indicating that the section is very thin. The core microfilament (mf), which has been nearly tangentially sectioned towards the upper half of the microvillus, reveals a repeating subunit structure of spherical particles best seen at the points marked (p). The disposition of the particles within the microfilament (observed more clearly in the region marked by thick arrow) suggests that they could be arranged in the form of a double helix. The thickness of the core microfilament varies between ~6 nm (thin arrow) and ~11 nm (thick arrow). Glutaraldehyde/OsO₄, UA + Pb. x 325,000.

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Brush border fine structure
Fig. 3. Micrograph of a longitudinally cut microvillus showing the features of the cross-filaments (arrows). The cross-filament (cf) appears to arise from a core microfilament and to continue laterally across the plasma membrane of the microvillus. This feature is very rarely seen in thin sections only and its significance is not clear. \( \times 230000 \).

Fig. 4. Another longitudinally cut microvillus showing a few cross-filaments (arrows). The cross-filament marked by double arrow appears to arise from a core microfilament and terminate on the inner surface of the plasma membrane. Compare this feature with those observed in Fig. 14. Figs 3 and 4 were from specimens prepared in the same manner as Fig. 2. \( \times 230000 \).
Brush border fine structure

3

4

cf
Fig. 5. Freeze-etched microvilli of the jejunum of a freshly cleaved, unetched specimen demonstrating what appear to be split inner membrane faces of the microvillous plasma membrane. The convex \( pa \) membrane faces seen on the right half of the picture carry large numbers of particles, 5–9 nm in diameter, while the concave \( pb \) faces on the left bear only few (see also Fig. 7). Notice the absence of particles on the \( pa \) faces in the tip region of the microvilli and the particle-like elements around the edges of the \( pb \) faces (arrowheads), suggesting the presence of small particles protruding from the true outer membrane surface into the lumen. \( \times 66000 \).

Fig. 6. Obliquely cleaved microvilli revealing both convex \( pa \) membrane faces and concave \( pb \) faces. At the base of the \( pa \) faces a thin ridge (\( r \)) of membrane material can be seen, the inner surface of which is continuous with and identical to the \( pb \) faces. It is therefore suggested that \( pa \) and \( pb \) are split inner membrane faces. \( \times 79000 \).

Fig. 7. Higher magnification of similar microvillous plasma membrane faces as shown in Fig. 5. The \( pa \) membrane faces are characterized by numerous particles of various sizes and by a few small holes. A careful examination of the \( pb \) faces, on the other hand, reveals, in areas with a suitable shadowing angle, the presence of large numbers of small holes (see encircled areas) and of only few particles. Thus the surface geometry of the faces \( pa \) and \( pb \) seems to support the hypothesis that they may represent complementary split inner membrane faces. Along the edges of the \( pb \) faces particle-like structures (7–10 nm in diameter) can be observed protruding into the intermicrovillous space (arrowheads). \( \times 126000 \).
Brush border fine structure
Fig. 8. Micrograph of microvilli frozen without prior glycerol infiltration and etched for 90 s. The deep etching has revealed the outer surface of a 10–30 nm thick continuous surface coat (f) surrounding the microvilli, which is probably identical to the mucopolysaccharide coat or 'fuzz' coat seen in thin sections. The arrow indicates where a piece of the coat has been broken away, revealing the underlying outer membrane surface. × 52000.

Fig. 9. Freeze-etched microvilli of a freshly cleaved, unetched specimen. Around the tip of the central microvillus a halo-like layer can be seen due to a slight change in the fracturing angle in this region. This change may be related to the presence of a mucopolysaccharide 'fuzz' layer around the microvillus. × 158000.

Fig. 10. An electron micrograph showing the filamentous nature and the extent of the enteric surface coat as observed in thin sections. Note the typical limitation of the surface coat at the intercellular border (arrow). Glutaraldehyde/OsO₄, UA + Pb. × 27000.
Fig. 11. High magnification of a longitudinally fractured freeze-etched microvillus. The microfilaments exhibit a beaded substructure with a repeating distance of approximately 6 nm. \( \times 158,000 \).

Fig. 12. Similar microvillus to that demonstrated in Fig. 11, with some microfilaments revealing a beaded substructure. On the left a prominent cross-filament is seen (arrow). \( \times 158,000 \).

Fig. 13. Longitudinal fracture through 2 microvilli. Besides the core microfilaments, which lead up into the tip of the microvilli, a number of cross-filaments (thin arrows) can be detected. The thick arrows indicate cross-filaments which seem to be attached to a core microfilament on one side. \( \times 100,000 \).

Fig. 14. Longitudinal fracture through a microvillus. Numerous cross-filaments (arrows) seem to be connected to the inner membrane surface of the microvillus. \( \times 136,000 \).

Fig. 15. A further example of a cross-filament ending on the inner membrane surface of a microvillus (arrow). \( \times 84,000 \).
Fig. 16. Cross-fractured microvilli exhibiting cross-filaments, which have a roughly radial orientation and lead from the core region of the microvilli to the inner membrane surface. Some of the filaments seem to be associated with particles on the membrane surface (arrows). $\times 105,000$.

Fig. 17. Micrograph showing an epithelial cell of the basal region of a crypt. Because of the removal of some of the cell contents the apical plasma membrane is seen as from within the cell. Large numbers of terminal web filaments appear to be attached to the exposed, split inner membrane surface at the base of and in between the widely separated microvilli ($m$). The outline of some of the filaments (arrows) can still be detected after they have inserted on the membrane surface. $\times 70,000$.

Fig. 18. Similar view to that in Fig. 17 but showing the plasma membrane of an epithelial cell of the jejunal villus with the typical closely packed microvilli ($m$). Only few filaments can be seen attached to the membrane surface (arrows). $\times 80,000$.

Fig. 19. Micrograph of freeze-etch microvilli, which have been cleaved in their tip region. It is clearly seen that the membrane faces of the tips of the lower-lying microvilli carry only few particles (see also Fig. 5). Within the matrix of the cross-fractured microvilli in the upper half of the picture small arrays of closely packed round elements (arrow) with spacings of approximately 12–17 nm are apparent. The position and the spacing of these arrays suggest that they could represent cross-sectional views of the closely packed core microfilaments. $\times 80,000$. 
Fig. 20. General view of the apical region of a freeze-etched epithelial cell of the small intestine showing microvilli fractured at different levels and the terminal web. The core microfilaments (mf) and the terminal web filaments (tf) can be compared with a microtubule (mt). The arrows indicate 2 terminal web filaments leading to the inner surface of the intermicrovillous plasma membrane. × 68 000.

Fig. 21. Terminal web region of an epithelial cell of the jejunum. The bundles of core microfilaments (mf) lead from the base of the microvilli (m) into the mesh of interwoven terminal web filaments (tf), where they disappear. Some of the terminal web fibrils which appear to be interwoven with the core microfilaments are marked with arrows. × 82 000.