FACTORS CONTROLLING THE REASSEMBLY

OF THE MICROVILLOUS BORDER OF THE

SMALL INTESTINE OF THE SALAMANDER

LEWIS G. TILNEY and ROBERT R. CARDELL, JR.

From the Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and the Department of Anatomy, University of Virginia, Charlottesville, Virginia 22901

ABSTRACT

Hydrostatic pressure, when applied to segments of the small intestine of the salamander, causes a tremendous reduction in number of microvilli and a loss of the terminal web. The intestinal epithelium strips off from its deeper layers at the level of the basement membrane. When the pressure is released and this epithelial sheet is allowed to recover, the microvilli and its terminal web reappear. Stages in the reformation of microvilli are described. In the earliest stages, foci of dense material seem to associate with the cytoplasmic surface of the apical plasma membrane. From this material, filaments appear and their regrowth is correlated with the extension of the microvilli. We suggest that the dense material nucleates the assembly of the filaments which, in turn, appear instrumental in the redevelopment of microvilli. This concept is supported by the existing literature. Further, since neither the microvilli nor the terminal web reappear on any surface but the apical surface, even though the apical and basal surfaces are bathed with the same medium, we suggest that information in the membrane itself or directly associated with the membrane dictates the distribution of the dense material which leads to the formation of the microvilli and ultimately to the polarity of the cell.

In this report we attempt, in a preliminary way, to provide information on some of the factors which appear to be active in guiding the assembly of the microvillous border of the small intestine. These factors ultimately may be responsible for determining the polarity of the cell. This investigation was stimulated by observations made on the gastrulae of the sea urchin *Arbacia punctulata*. It was noted that the few microvilli present on the apical surface of ectodermal cells appeared to break down during the application of hydrostatic pressure (Tilney and Gibbins, 1969). Likewise, the region immediately surrounding the basal body of the cilium, a region which is believed to be homologous with the terminal web characteristic of intestinal epithelial cells and of proximal convoluted tubule

cells of the kidney, appeared to solate during compression. Thus, we felt that hydrostatic pressure might be used as an analytical tool to break down the microvilli and the terminal web regions of a highly differentiated cell. If this could be achieved, then a study of the recovery of the cells following their induced alterations should provide information on structural components of the cell which are important in establishing and maintaining polarity. More specifically, we were anxious to determine what controls the length and diameter of the microvilli and why microvilli are limited to the apical surface of the cell.

We chose as experimental material the salamander intestine because the absorptive cells of this tissue have a highly differentiated apical surface

consisting of organized arrays of microvilli and a thick terminal web. In addition, amphibians are particularly satisfactory for experimentation; not only are they cold-blooded, enabling experiments to be carried out at room temperature, but also an adequate amphibian Ringer's solution is available for such experiments.

MATERIALS AND METHODS

Animals

Triturus (*Notophthalmus, Diemyctylus*) *viridescens* were obtained from ponds near Charlottesville, Va. and maintained in an aquarium at room temperature. They were fed three times per week with ground meat.

Preparation of the Intestine

The salamanders were anaesthetized with MS 222 (Meta-aminobenzoic acid ethyl ether). The proximal half of the intestine was removed and placed in a dish filled with aerated cold-blooded Ringer's solution. The intestine was cut transversely into short ring-like segments and transferred to the pressure apparatus.

Pressure Apparatus

The apparatus used in these experiments has been described by Marsland (1950). The pressure bomb was modified slightly from the original design (see Tilney and Gibbins, 1969).

Experimental Procedure

In each experiment most of the segments were placed into the pressure bomb. The remainder were kept in Ringer's solution and served as the controls. In order to maintain the appropriate oxygen tension, the Ringer's solution was aerated for 15 min prior to compression. Compression was carried out for 20 min at 6500 psi. Some of the segments were fixed as soon as technically possible after compression, whereas others were placed in fresh Ringer's solution under constant aeration until fixation 15 min, 30 min, and 90 min later.

Fixation for Electron Microscopy

Adequate fixation was achieved by the addition of glutaraldehyde to the Ringer's solution. We pretreated the glutaraldehyde (50%, Biological grade, Fisher Scientific Co., Pittsburgh, Pa.) with $BaCO_3$, centrifuged to clear, then neutralized the glutaraldehyde with NaOH. The segments at the desired time were immersed in Ringer's solution containing 2% pretreated glutaraldehyde. Fixation was carried out for 1½ hr at room temperature. The segments were

washed in Ringer's solution and postfixed in 1% OsO_4 in 0.1 M phosphate buffer at pH 7.0. The tissue was dehydrated rapidly and embedded in Epon 812 or Araldite. Ultrathin sections were cut with a diamond knife on a Porter Blum MT II ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Philips 200 electron microscope.

1 μ sections were cut and examined either by phase-contrast microscopy or by light microscopy after staining (Richardson et al., 1960).

RESULTS

The Fine Structure of the Incubated Segments of the Intestinal Epithelium

We could find no morphological difference in segments of small intestine fixed immediately after removal from the salamander and segments which had been incubated for 1½ hr in aerated amphibian Ringer's solution. The segments reported here are adjacent to segments, from the same animal, which were experimentally treated with hydrostatic pressure.

The fine structure of the intestinal absorptive cell has been the subject of numerous recent papers (Cardell et al., 1967; McNabb and Sandborn, 1964; Mukherjee and Williams, 1967) and, even though most of these studies have been directed to mammals, the information is applicable to the amphibian intestinal epithelial cell. Nevertheless, we would like to emphasize certain features of this cell because they are essential in appreciating the changes which occur during and following pressure administrations. The apical surface of the intestinal epithelial cell shows the most dramatic response to pressure treatments; therefore, it is appropriate that we give detailed consideration to this region. The surface of the intestinal epithelial cell bears the characteristic microvilli, which are 1.6–2.0 μ in length and 0.08–0.1 μ in diameter (Fig. 1). In cross-section they generally appear hexagonally packed and are separated from one another by a minimum distance of 0.01 μ. In oblique sections the regularity of their packing can be appreciated by the formed moiré patterns.

Closer examination of a microvillus reveals a core of filaments, each approximately 50 A in diameter. These filaments insert into a dense material at the tip of the microvillus and they extend basally the length of the microvillus, finally terminating in the terminal web. Their base in the terminal web does not appear specialized. Because of the parallelism and similar length of the micro-
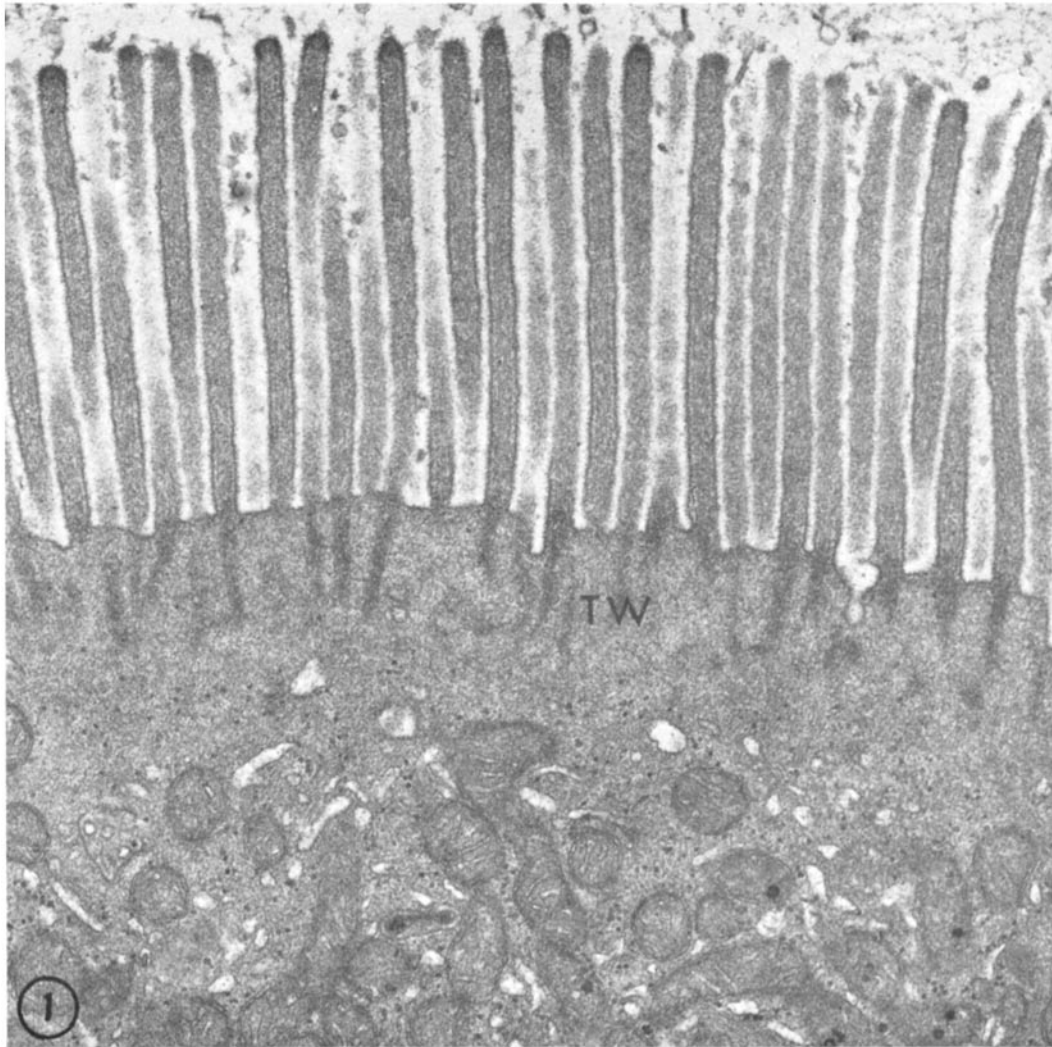
FIGURE 1. Electron micrograph of the apical end of an epithelial cell from an intestinal segment incubated for 1½ hr at room temperature in aerated cold-blooded Ringer's solution. Numerous microvilli project and each shows a compact core of filaments. The filaments extend from the dense tips of the microvilli into the terminal web (*TW*). The terminal web separates formed elements of the cytoplasm, e.g. mitochondria, endoplasmic reticulum, and ribosomes, from the free surface. ×39,000.

villi, transverse sections through the microvilli near their tips will cut some through the dense material, others just distal or apical to this point. In sections cut through the proximal portion of the dense material, we have counted as few as eight filaments. Adjacent microvilli have 9, 10, 11, 13, and 15 filaments. Near the base of the microvilli, identified as such in transverse section by the frequent appearance of two or more filament bundles enclosed within a single limiting membrane, we characteristically count 18–20 filaments in each bundle (Fig. 2). In general, the filaments are tightly packed, frequently appearing hexagonally packed; the cores of filaments are separated from the limiting membrane by 200–300 A, as has been described by McNabb and Sandborn (1964) and Mukherjee and Williams (1967).

The terminal web area varies in thickness from

0.3 to 0.6 μ, and is composed of fine fibrillar material. Present within it are apical vesicles (Cardell et al., 1967), glycogen granules, and an occasional microtubule. It is of importance to note that mitochondria, endoplasmic reticulum (ER), vesicles, and fat droplets are excluded from the terminal web area. Microtubules are frequently encountered parallel to the terminal web and just beneath it.

The lateral margins of the absorptive cells interdigitate with those of their neighbors. Near the apical surface are the characteristic junctional complexes (see Overton and Shoup, 1964). A series of desmosomes are usually located beneath this complex.

Underlying the basal surface of the mucosal cell is the amorphous extracellular basement membrane or basal lamina.

Fixation 4 Min following Decompression after 30 Min at 6500 psi

In most cases the intestinal epithelium, while remaining as a sheet, strips off from the underlying connective tissue and smooth muscle layers. This behavior of the mucosa appears to be related to, if not a result of, the pressure-induced solation of the basement membrane. This sheet of epithelium tends to curl and fold back on itself in such a way that it becomes difficult to establish whether a par-
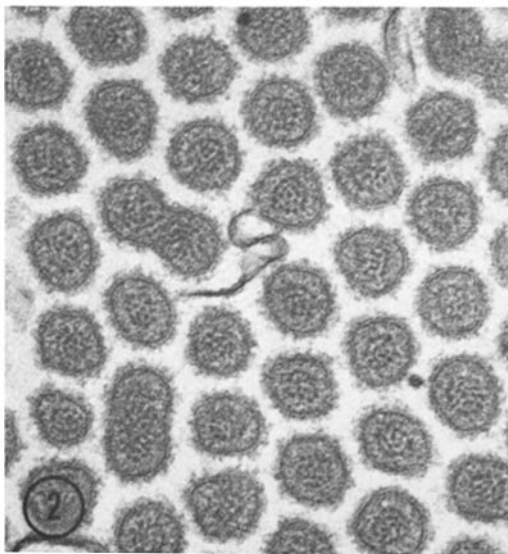

FIGURE 2   Transverse section of microvilli, illustrating the central core of filaments within each. ×105,000.

ticular region is located at the level of a crypt or near the tip of the villus.

The apical surface of the cell is prominently rounded; often this surface projects into the lumen as a bulbous lobe (Fig. 3). If one computes the increase in surface area, it is maximally about 3- or possibly 4-fold. This change in surface area is brought about by an actual increase in the apical surface since the lateral and basal surfaces are unchanged. Of greatest interest is the tremendous reduction in numbers of microvilli (Figs. 3 and 4); in fact, in some sections only one or two are present along the apical margin. Part of this decrease should be ascribed to the increase in surface, for now microvilli would be separated by greater distances. However, in most instances the separations of microvilli are greater than could be accounted for by the increase in surface, and thus the reduction in numbers of microvilli cannot be solely due to an increase in surface brought about by retraction of microvilli. Also, we find considerable debris in the lumen which appears to be due to a fragmentation of the microvilli. It appears, therefore, that the dramatic decrease in microvilli number is due to both retraction as well as fragmentation of the microvilli. We should emphasize, however, that there is considerable variation from cell to cell in the degree of apical rounding or protuberance formation and in the percentage loss of microvilli.

Coupled with the dramatic reduction in microvilli is a virtual elimination of the terminal web (Compare Fig. 1 with Fig. 4). Now organelles and inclusions such as mitochondria, ER, lipid droplets, and vacuoles can be found within 500 A of the free surface (Figs. 4 and 5). This indicates a 5- to 10-fold decrease in thickness of the terminal web. Vesicles have been found within 100 A of the free surface.

In a careful examination of the apical surface, we found many examples of what appeared to be early stages in the reformation of microvilli. Some of these are illustrated in Figs. 5, 6, and 7. The earliest stage in microvillus formation appears to be the accumulation of a dense material in spots on the cytoplasmic surface of the limiting membrane (Fig. 5 a). A similar material is characteristically present at the tips of untreated microvilli. In slightly longer microvilli, filaments can be seen extending from this dense material into the cortical region of the cell (Fig. 5 b and 5 c). Most frequently these short microvilli extend from the apical surface at an acute angle (Figs. 5 c and 6), an obser-
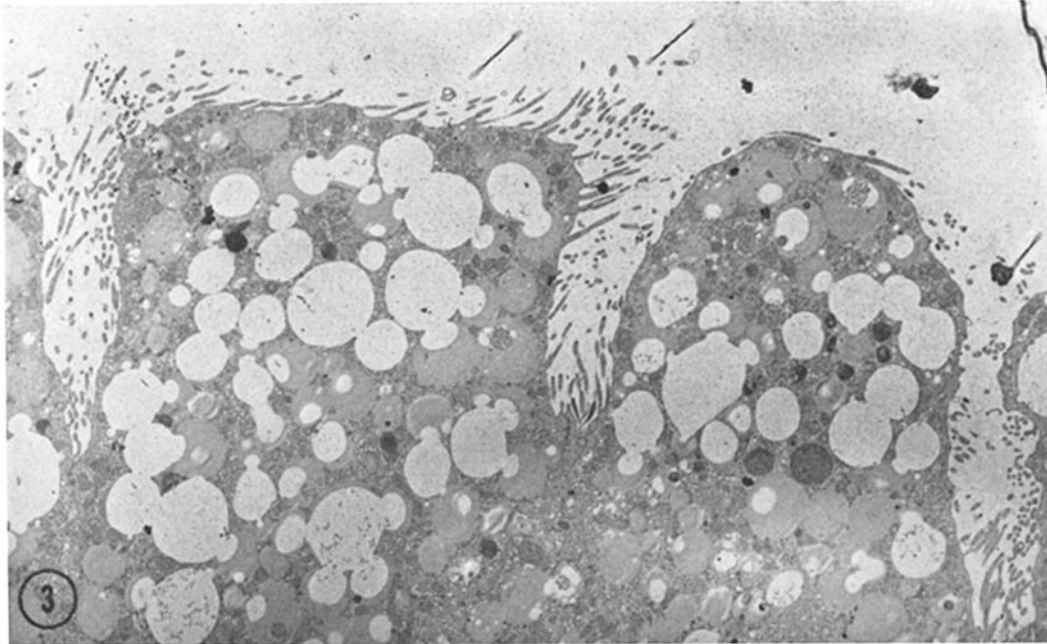
FIGURE 3 Low-magnification electron micrograph through the apical portion of two intestinal epithelial cells. The intestine was subjected to 6500 psi for 30 min, then fixed within 4 min after decompression. Of particular interest are the prominent bulging of the apical cell surface, the tremendous reduction in numbers of microvilli, and the loss of terminal web. Numerous lipid droplets are present in these cells. ×5000.

vation made earlier by Overton and Shoup (1964). We should emphasize, however, that every microvillus contains a central core of filaments which insert into dense material at the tips of the microvilli (Figs. 5 and 7 b). In transverse section the number of filaments in these short microvilli are comparable to that in untreated microvilli, a minimum of eight at their tips, with an average of 16. Forked microvilli are common (see Fig. 7). The cores of filaments from all branches merge together to form a common cluster of filaments in the trunk. What appears to be the initial stage in the evolution of a fork is depicted in Fig. 5 c.

Microtubules are extremely common just beneath the limiting membrane where they are oriented parallel to the apical surface of the cell (Figs. 4 and 5). In grazing sections through the free surface the microtubules appear to form almost a network. We have been unsuccessful in determining their origins or insertions.

Large, nonmembrane-limited, nearly spherical droplets up to 1.5 μ in diameter are common in these epithelial cells. They contain a fibrillar material which is similar in its morphological char-

acter to the fibrillar material which composes the terminal web of untreated cells. In most instances these droplets are present only in the apical part of the cell (Fig. 8, although depicting the apical part of an epithelial cell fixed 15 min after the release of pressure illustrates these droplets).

Fixation following 15 Min Recovery after Compression at 6500 psi

Two observations at this time period appear to be of special significance: (a) the apparent increase in numbers of microvilli, and (b) the partial reformation of the terminal web (Fig. 8). The microvilli show striking variations in length from 0.2 μ to 2.3 μ, even in the same section on the same cell. Often they extend from the free surface at an acute angle, as in the previous stage, but there is a greater tendency for them to lie normal to the apical cell surface. Branching is common.

The microvilli all contain filaments, in general, tightly packed. The bundle of filaments at the base of each microvillus is tightly packed.

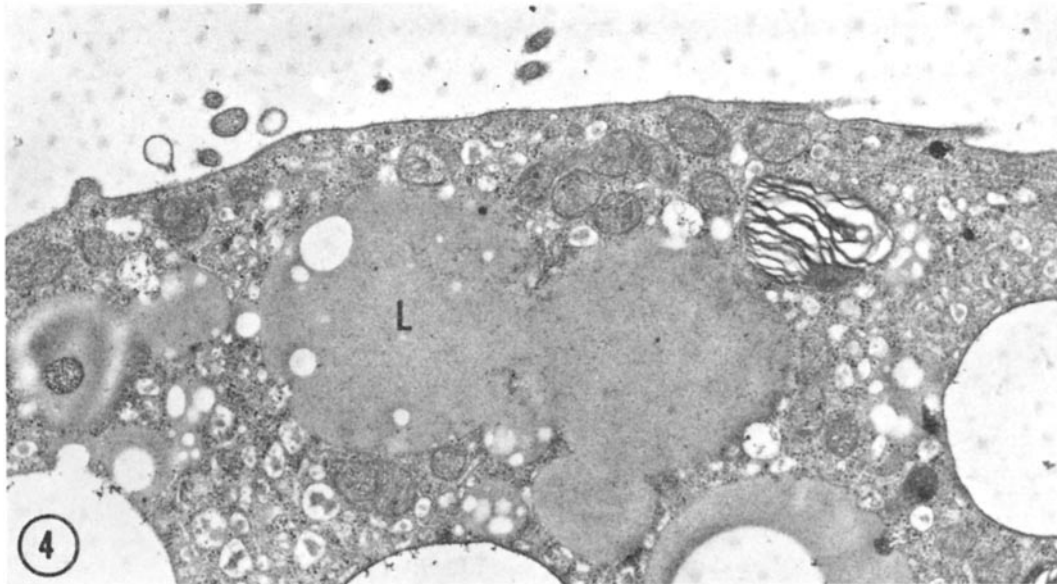The terminal web varies in thickness not only

FIGURE 4   Apical surface of an intestinal epithelial cell from an intestinal segment which had been subjected to pressure for 30 min at 6500 psi, then fixed 4 min after decompression. Note the virtual absence of microvilli and the apparent loss of the terminal web. Lipid droplets (L) are common. ×19,000.

from cell to cell but within portions of the same cell. In some cells it is 0.3 μ in thickness, a 3- to 5-fold increase from the preceding stage. It is composed of an interwoven mat of fine fibrils and appears to be formed by the dispersion of the fibrillar material present in the droplets (Fig. 8). These droplets are generally located just beneath the microvillous border and, since their margins are not membrane limited, they blend imperceptibly into the terminal web region. Of considerable interest is the fact that organelles and inclusions such as fat droplets, mitochondria, ribosomes, ER, and vacuoles are not "trapped" in the reforming terminal web. Rather, the fibrillar material making up this layer appears to be deposited apical to these organelles, thus displacing them basally.

Microtubules are most frequently oriented parallel to the apical surface, at the base of the developing terminal web.

The lateral and basal margins remain largely unchanged. No microvilli are seen extending from them, and terminal web material does not appear to be deposited near their limiting membrane.
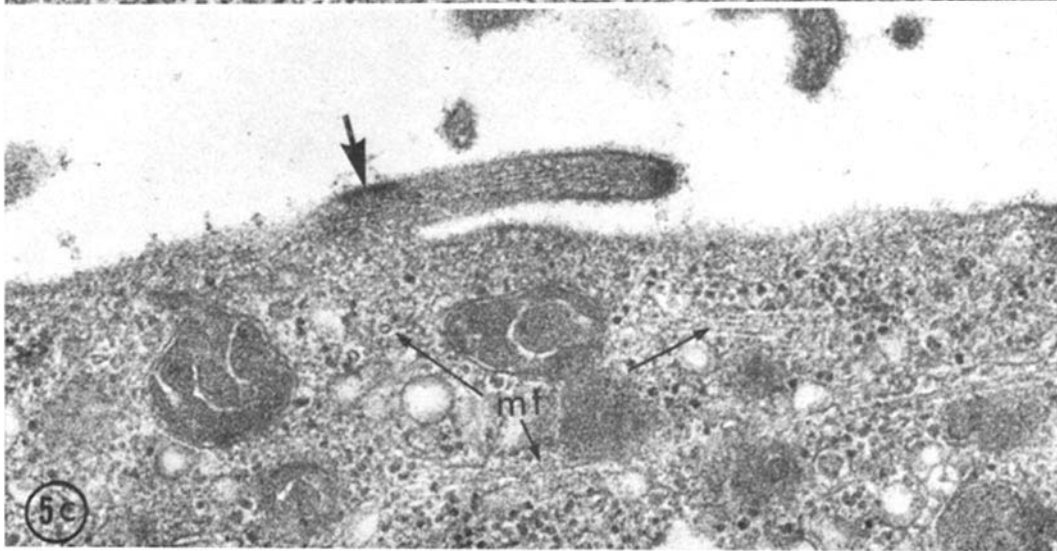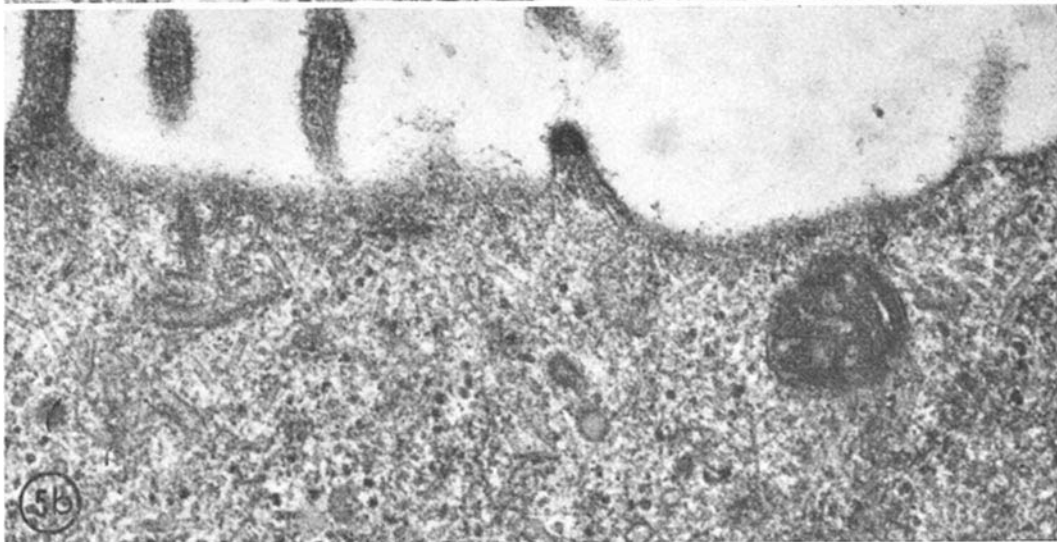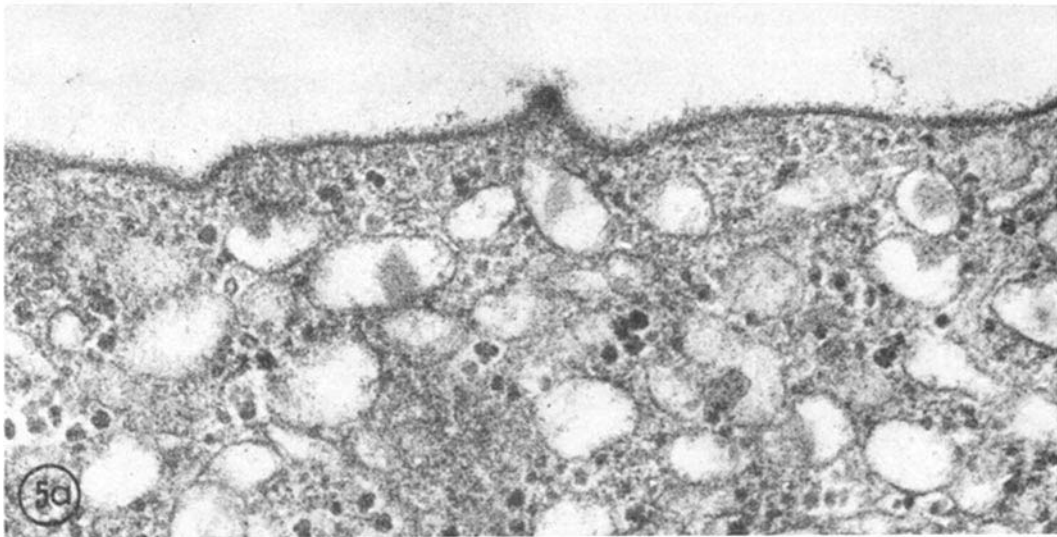
The apical cell surface appears less rounded than in the preceding stage. In cells where the terminal web is more developed, rounding is not prominent.

Fixation following 30 Min Recovery after Compression at 6500 psi

The microvilli remain variable in length; branching is common. Within each microvillus is a central core of filaments (Fig. 9) which penetrate the terminal web. This layer, although of varying thickness, approaches 0.35 μ at its thickest point. Neither microvilli nor terminal web material is found associated with the lateral or basal surfaces (Fig. 10). From these surfaces, however, we occasionally find projections similar to the microspikes of Taylor (1966). These are more varied in diameter than microvilli, lack the dense tips, and do not appear to contain the central core of filaments characteristic of microvilli. Similar processes are present in untreated cells. No evidence of a basement membrane is visible (Fig. 10).

Fixation following 1½ Hr Recovery after Compression at 6500 psi

The overall impression we get from examining sections at this stage is that most of the absorptive cells resemble untreated cells (compare Fig. 11 with Fig. 1). In each segment examined there are regions in which individual cells are clearly under-

going autolysis; this was concluded by the appearance of broken membranes, of swollen or pycnotic mitochondria, and of bloated microvilli.

The microvilli, although still frequently branched, appear now to be of relatively uniform length per cell and to extend normal to the apical cell surface (Fig. 11). Transverse sections through the microvilli reveal a compact core of hexagonally packed filaments whose numbers vary from 8 to 26, depending on the level at which they are sectioned.

The terminal web measures up to 0.5 μ in thickness. Basal to this mat like layer are microtubules, mitochondria, a few fat droplets, vesicles of ER, and ribosomes. These organelles or inclusions are not present in the terminal web proper. A number of droplets containing the fibrillar material remain. These, as before, are present near the apical cell surface.

The lateral and basal surfaces do not appear significantly different from those of the preceding stages; no evidence of microvilli or of a deposition of terminal web material could be found.

DISCUSSION

The Reformation of Microvilli

In all the microvilli examined in specimens prior to compression or during a stage in the reformation following decompression, there is *invariably a central core of filaments*. This appears to be true even of microvilli whose total length is 0.1 μ or less. Since the microvilli have an axial ratio of more than 30 and contain little else within them besides the central core of filaments, it seems reasonable to conclude that the filament bundle not only supports the microvillus but is necessary for its regrowth. It now becomes of interest to inquire into what factors orient as well as control the numbers of filaments in each microvillus, for these factors must ultimately pattern the apical surface of epithelial cells.

As illustrated in Fig. 12 and pointed out above,

one of the first steps in the reformation of microvilli appears to be an association between a dense mass of material and the cytoplasmic surface of the apical plasma membrane. This material appears identical to the dense material present at the tips of all microvilli. Shortly thereafter, filaments can be seen inserting on this dense material. As the microvilli increase in length, so do the filaments. A reasonable hypothesis for the patterning of the filaments in each microvillus would be that the dense material acts to nucleate the assembly of the filaments. If the size of this dense material is controlled, then not only the direction of growth would be determined, but also the number of filaments and ultimately the width of each microvillus would tend to be constant. An analogy here might be made with the growth of a cilium. In this case, however, the 9 + 2 pattern of microtubules is nucleated by the basal body. Nevertheless, the basal body gives direction to growth and as well determines the width of the cilium.

This hypothesis is consistent with what we have observed in regard to the formation of branched microvilli. What appears to be the earliest stage in the evolution of a branch is the appearance of the dense material at the limiting membrane of an already formed microvillus (see Fig. 5 c). Another stage includes the attachment of filaments to the dense material at the tip of each branch. Where the branches join, the filaments intermingle. Transverse sections through the crotch reveal twice the normal number of filaments.

We would predict from the above hypothesis that the filaments, once nucleated from the dense material, would all show unidirectional polarity. It is of interest, therefore, to relate these findings to recent studies of Ishikawa, Bischoff and Holtzer (1969). These investigators found that heavy meromyosin, when added to glycerinated segments of small intestine, attached to filaments in the microvilli and displayed the arrowhead configuration found when heavy meromyosin is added to actin. Of greatest interest is the fact that, as in the case

FIGURE 5 These micrographs illustrate what appears to be a sequence in the reformation of microvilli. Segments of the intestine were fixed 4 min after decompression. Pressure treatment was carried out for 30 min at 6500 psi. Microtubules (*mt*) are common in these cells. (*a*) Note the dense material associated with the limiting membrane. (*b*) Extending from the dense tip of this short microvillus are some short filaments. (*c*) The arrow indicates a bit of dense material at the base of a developing microvillus. This appears to be an early stage in the evolution of a fork. ×63,500.
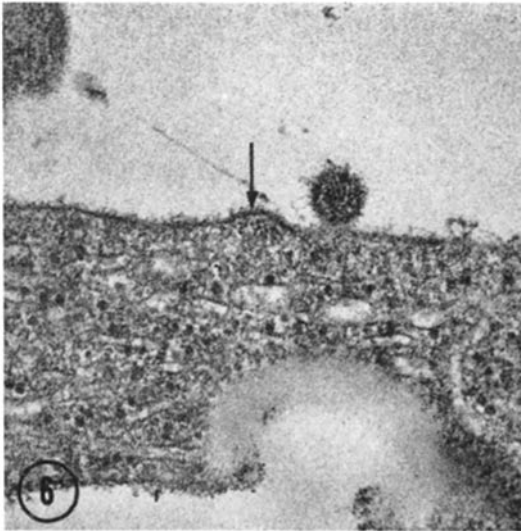
FIGURE 6 Portion of the apical end of an intestinal cell fixed 4 min after the release of pressure. The arrow indicates the base of a microvillus in transverse section. Note the bundle of filaments. Since most reforming microvilli exit from the surface at an acute angle, this plane of section is not surprising. ×65,500.

of actin, all the arrowheads point in the same direction on adjacent filaments. Thus the filaments are unidirectionally polarized.

Recently it has been reported that the microvilli along the terminal surface of the proximal convoluted tubules of mammalian kidneys undergo rapid contractions or pulsations (Thuneberg and Rost-gaard, 1969). It is reasonable to implicate the filaments in the microvilli as being responsible for this motion. If indeed these filaments turn out to be actin or actin-like as suggested by their binding with heavy meromyosin (Ishikawa et al., 1969), then we appear to have a system in the microvilli very similar to that in muscle where the dense material may correspond to the Z band material of skeletal muscle or the dense bodies of smooth muscle. It may be no coincidence, therefore, that the directions of the arrowheads relative to the dense material at the microvilli tips and the Z band material of muscle are identical.

The Redevelopment of the Terminal Web

As a general rule, associated with microvilli is a thickening of the subjacent ectoplasmic layer, usually referred to as a terminal web. Our observations are consistent with this homology, so that as
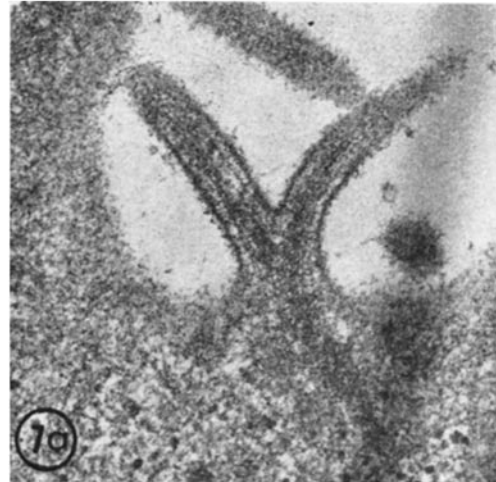
FIGURE 7 Two examples (a and b) of forked or branched microvilli from segments of the intestine fixed 4 min after the release of hydrostatic pressure. Each branch contains a filamentous core. ×57,000.

the microvilli reform so does the terminal web. Perhaps the terminal web provides a mat-like base against which the filaments are able to push, thereby enabling deformation of the surface of the cell. Yet, clearly the terminal web must also provide support for the apical surface of the cell. This
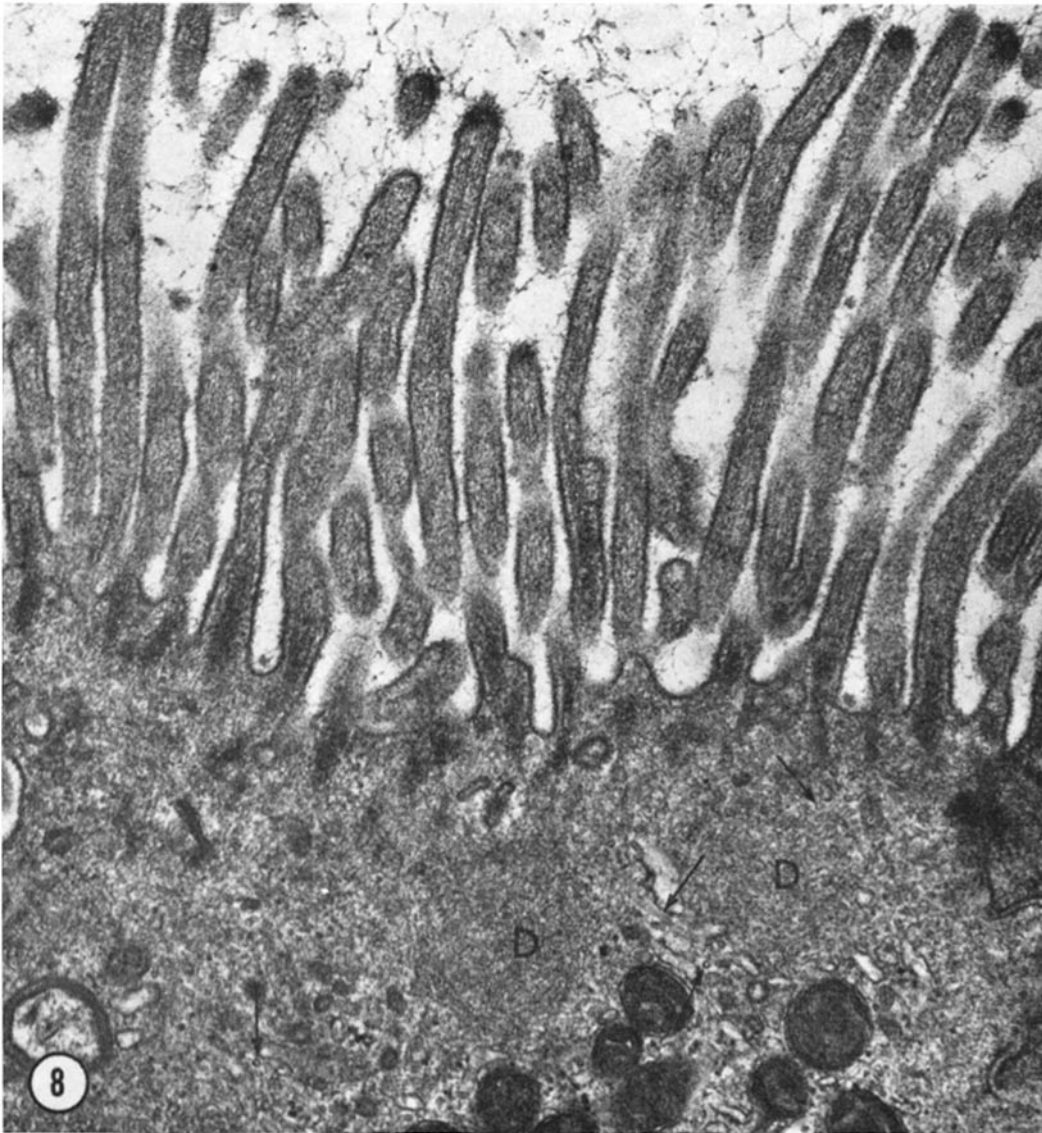
FIGURE 8 Apical end of an epithelial cell fixed 15 min after the release of hydrostatic pressure. Droplets containing a fibrillar material are present in apical end of this cell. Microtubules are common as well (see arrows). ×40,000.

is the only surface completely exposed to the environment, an environment which, after feeding, must be abrasive and hostile. The other surfaces of the cell are protected either by junctions with their neighbors or by extracellular coats such as the basement membrane beneath which are organized arrays of collagen and smooth muscle. We have found that the apical surfaces of the ectodermal

cells fixed immediately following compression show a rounded profile which in some cases bulges far out into the lumen. Yet, as recovery progresses, the apical surfaces become progressively more flattened. This flattening appears temporally related to the redevelopment of the terminal web, and it seems reasonable that they may be causally related. Of significance to the next chapter is the fact that
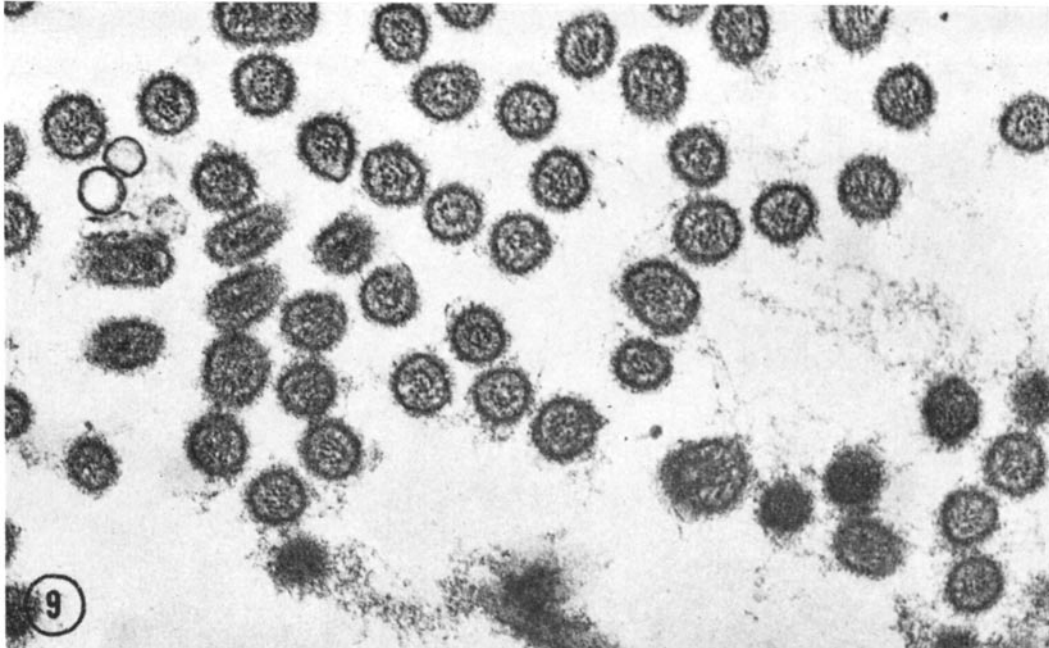
FIGURE 9   Transverse section through microvilli of a segment of intestine fixed 30 min after the release of pressure. A central core of filaments is present within each microvillus. ×72,000.
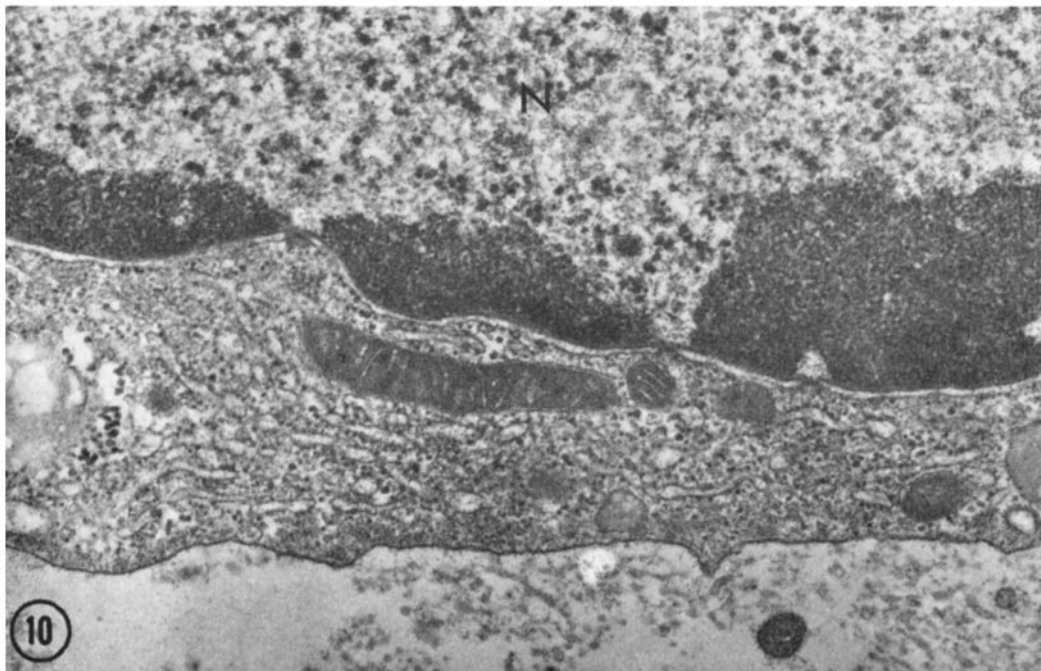

FIGURE 10   The basal portion of an intestinal epithelial cell fixed 30 min after the release of pressure. A portion of the nucleus (N) is visible. Note the absence of any basement membrane. ×33,000.
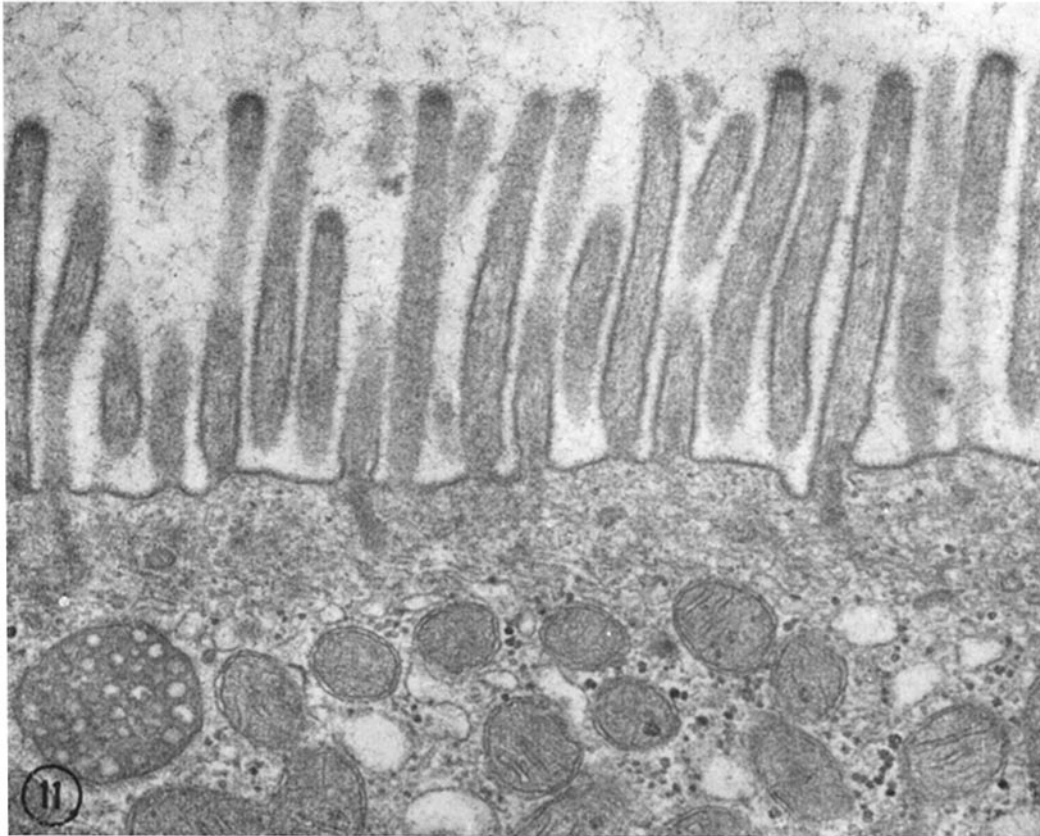
FIGURE 11   Apical region of a cell fixed 1½ hr after the release of hydrostatic pressure. Compare this figure with Fig. 1 and Fig. 4. Note the abundant microvilli and the prominent terminal web. ×45,000.

the terminal web always reforms just beneath the apical membrane, thus displacing organelles and inclusions basally. These particles are rarely trapped within the developing terminal web, as if there is a strong affinity of the apical cell membrane for the filamentous material which makes up the terminal web.

The Determination of Polarity

As set forth in the introduction, we would like to know what factors are capable of polarizing animal cells and where they are located. Before relating our observations to polarity, we would like to mention some observations and experiments on the distribution of pigment granules in the sea urchin (*Arbacia punctulata*) egg. Prior to fertilization the pigment granules can be found throughout the egg cytoplasm. Upon fertilization, however, these granules move to the cortex of the egg

(Harvey, 1956). Centrifugation, even at high speed (40,000 g), is relatively ineffective in displacing these granules. Simultaneous treatment of the eggs with hydrostatic pressure and centrifugation, however, concentrates the granules at the heavy pole (Zimmerman and Marsland, 1956). When this treatment is discontinued the granules migrate back to the cortex, eventually distributing themselves equally over the surface. These granules then show a distinct polarity, a polarity that seems to be induced by fertilization. There can be little doubt that the attraction of these granules to the surface of the egg must reside in the membrane or in a very thin layer beneath it.

The observations presented in this study extend the information obtained from *Arbacia*. We have demonstrated that in a unidirectional polarized cell, the intestinal epithelial cell, hydrostatic pressure leads to the destruction of most of the micro-
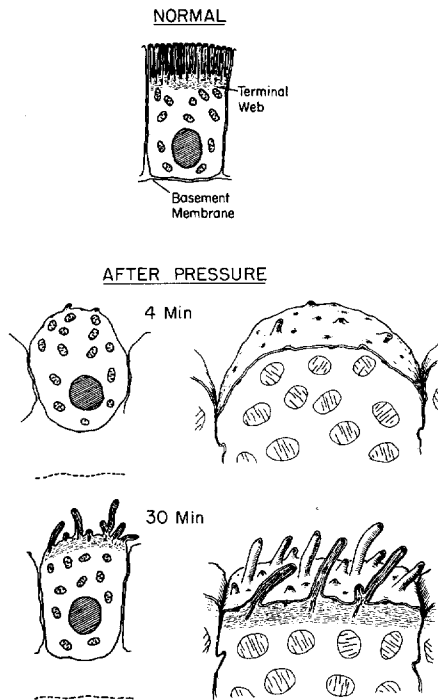
NORMAL

AFTER PRESSURE

4 Min

30 Min

FIGURE 12 This simplified drawing depicts the effects of hydrostatic pressure on the intestinal epithelium and two stages in the reformation of the microvilli and the terminal web following decompression.

villi, and the disappearance of the terminal web. Large cytoplasmic organelles and inclusions can now approach within 500 A of the free surface. Upon release of the pressure both the microvilli and the terminal web reform, yet of great interest is that during recovery these structures have *only formed on the apical cell surface, never on the lateral or basal surfaces.* This specificity for the apical surface does not appear to be the result of the environment surrounding the cell, for we demonstrated that the epithelium, when subjected to pressure and its release, disassociates from deeper layers at the level of the basement membrane. Thus, at least the basal surface, and, to a certain extent, the lateral surfaces as well, are subjected to the Ringer's solution which bathes the apical cell surface. If the reformation of the striated border is analyzed in greater detail as presented in the previous sections, it seems reasonable to predict that the filaments which appear important in the reformation of each microvillus assemble from discrete spots of dense material which become attached to the cytoplasmic side of the apical plasma

membrane. Further, the terminal web becomes redeposited directly beneath, as well as presumably within, the existing 500 A ectoplasmic zone, for organelles and inclusions such as segments of ER, mitochondria, and ribosomes are not enmeshed in the reforming terminal web, but are actually displaced basally. Logically then, we are led to the conclusion that the membrane proper should be held ultimately accountable for the apical pattern of the cell, for it seems to associate with the dense material which leads to the orderly assembly of filaments which, in turn, is related to microvillus formation. Thus the membrane limiting the apical end of the cell may account for the polarity of the cell.

It might be argued, nevertheless, that the recovery of the microvilli and the terminal web only on the apical surface is not surprising, for it is in this region of the cell that most of the filament precursors have concentrated upon their pressure-induced disassembly. Two points argue against this position. First of all, since hydrostatic pressure dissolves the plasmagel (see Marsland, 1956), one would expect that in 30 min, by Brownian motion or by cytoplasmic streaming, some of the precursors to the filaments would become redistributed to other parts of the cell. Of course, it is unlikely that the droplets themselves would be displaced markedly. Since these droplets are not membrane limited, it is reasonable to expect some of their contents to become distributed throughout the cell. Thus, occasionally one might expect to see a microvillus projecting into the extracellular space between cells or extending from the basal surface of a cell. We have not found one. Secondly, the fact that the terminal web never traps larger, formed elements within it, as it reforms, argues for a strong attraction to the limiting membrane.

From studies on the isolation of the striated border, it is now clear that at least 10 enzymes are associated with this border (Forstner et al., 1968; Miller and Crane, 1961). In fractionation studies of the isolated border, these enzymes appear to be associated with the membrane fraction, there being little if any activity present in the fibrous terminal web fraction (Forstner et al., 1968). It is also clear that there must be carrier systems for uphill transport of sugars and amino acids (see Kinter and Wilson, 1965; Goldner et al., 1969) as well as ions (Curran 1960, 1965) in this border, and it seems likely that they will be found situated in the membrane proper. Histological studies such as those of Hugon and Borgers (1966) demonstrate that one of the enzymes, alkaline phosphatase, appears to

be confined to the membrane limiting the microvilli, being absent on the lateral membranes. It seems reasonable to suspect that many of the other enzymes are also present exclusively on the apical membrane, and that there is limited or no activity on the membranes limiting the other margins of the cell. The position of these enzymes and "pumps" or other factors, therefore, may in turn attract the dense material to the membrane which ultimately leads to the formation of a microvillus. Thus we like to think of the plasma membrane as an imperfect mosaic in which the spatial location of specific enzymes and transport mechanisms could in turn lead to the association of discrete masses of dense material which ultimately leads to the morphological polarization of a cell, including not only the microvillous border but also its associated terminal web.

The idea that membranes on different surfaces of a single cell are different is not new, especially in reference to numerous efforts to locate active transport mechanisms. Certainly electron microscopy has not helped our understanding. Yet the idea that a limiting membrane on one surface of a cell is different from that on another cell surface and that this difference is capable of leading to the polarization of a cell has been largely neglected, even though studies on secretion make this concept imperative. For example, secretion of zymogen granules from the pancreas is accomplished, once the appropriate signal is given (by secreting in vivo or reserpine as well as a number of other agents in vitro), by the fusion between the membrane limiting the granules and the plasma membrane lining the lumen. The granules apparently do not fuse with each other or with the lateral or basal surfaces.

An extensive embryology literature exists which indicates that there resides in the "cortex" of developing oocytes a pattern which is expressed subquently in development. This "information" cannot be centrifuged out of the cortex. In most studies, however, the techniques employed, as well as the systems chosen for examination, are not sufficient to differentiate the cortex into the membrane proper and the cortical cytoplasm. Thus the imprecise term "cortex" has been used (see Arnold, 1968; Curtis, 1962; Elbers, 1969; Raven, 1961; and Verdank, 1968). A possible exception is the recent report of Elbers (1969). In this study the author presents evidence that cortical pattern, which is lithium sensitive, may in fact be located in the limiting membrane. In summary then, we would

like to suggest that structural and functional polarity may be ultimately provided by differences in the membranes of the cell.

BIBLIOGRAPHY

ARNOLD, J. M. 1968. The role of the egg cortex in cephalopod development. *Develop. Biol.* 18:180.

CARDELL, R. R. JR., S. BADENHAUSEN, and K. R. PORTER. 1967. Intestinal triglyceride absorption in the rat. An electron microscopical study. *J. Cell Biol.* 34:123.

CURRAN, P. F. 1960. Na, Cl and water transport by rat ileum *in vitro. J. Gen. Physiol.* 43:1137.

CURRAN, P. F. 1965. Ion transport in the intestine and its coupling to other transport processes. *Fed. Proc.* 24:993.

CURTIS, A. S. 1962. Morphogenetic interactions before gastrulation in the amphibian *Xenopus laevis*— the cortical field. *J. Embryol. Exp. Morphol.* 10:410.

ELBERS, P. F. 1969. The primary action of lithium chloride on morphogenesis in *Lymnaea stagnalis. J. Embryol. Exp. Morphol.* 22:449.

FORSTNER, G. G., S. M. SABESIN, and K. J. ISSELBACHER. 1968. Rat intestinal microvillus membranes, purification and biochemical characterization. *Biochem. J.* 106:381.

GOLDNER, A. M., S. G. SCHULTZ, and P. F. CURRAN. 1969. Sodium and sugar fluxes across the mucosal border of rabbit ileum. *J. Gen. Physiol.* 54:362.

HARVEY, F. B. 1956. The American *Arbacia* and Other Sea Urchins. Princeton University Press, Princeton, N. J.

HUGON, J., and M. BORGERS. 1966. A direct lead method for electron microscopic visualization of alkaline phosphatase activity. *J. Histochem. Cytochem.* 14:429.

ISHIKAWA, H., R. BISCHOFF, and H. HOLTZER. 1969. Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* 43:312.

KINTER, W. B., and T. H. WILSON. 1965. Autoradiographic study of sugar and amino acid absorption by inverted sacs of hamster intestine. *J. Cell Biol.* 25:19.

MARSLAND, D. A. 1950. The mechanisms of cell division; temperature-pressure experiments on the

cleaving eggs of *Arbacia punctulata*. *J. Cell Comp. Physiol.* **36**:205.

MARSLAND, D. A. 1956. Protoplasmic contractility in relation to gel structure: temperature-pressure experiments on cytokinesis and amoeboid movement. *Int. Rev. Cytol.* **5**:199.

McNABB, J. D., and E. SANDBORN. 1964. Filaments in the microvillous border of intestinal cells. *J. Cell Biol.* **22**:701.

MILLER, D., and R. K. CRANE. 1961. The digestive function of the epithelium of the small intestine. II. Localization of the disaccharide hydrolysis in the isolated brush border portion of intestinal epithelium cells. *Biochim. Biophys. Acta.* **52**:293.

MUKHERJEE, T. J., and A. W. WILLIAMS. 1967. A comparative study of the ultrastructure of microvilli in the epithelium of small and large intestine of mice. *J. Cell Biol.* **34**:447.

OVERTON, J., and J. SHOUP. 1964. Fine structure of cell surface specializations in the maturing duodenal mucosa of the chick. *J. Cell Biol.* **21**:75.

RAVEN, C. D. 1961. Oogenesis: The Storage of Developmental Information. Pergamon Press Inc., New York.

RICHARDSON, K. C., L. JARRETT, and E. H. FINKE. 1960. Embedding in epoxy resins for ultra-thin sectioning in electron microscopy. *Stain Technol.* **35**:313.

TAYLOR, A. C. 1966. Microtubules in the microspikes and cortical cytoplasm of isolated cells. *J. Cell Biol.* **28**:155.

THUNEBERG, L., and J. ROSTGAARD. 1969. Motility of microvilli. *Fed. Eur. Biochem. Soc. Madrid.* 270.

TILNEY, L. G., and J. R. GIBBINS. 1969. Microtubules in the formation and development of the primary mesenchyme of *Arbacia punctulata*. II. An experimental analysis of their role in the development and maintenance of cell shape. *J. Cell Biol.* **41**:227.

VERDANK, N. H. 1968. The effect of removal of the polar lobe in centrifuged eggs of *Dentalium*. *J. Embryol. Exp. Morphol.* **19**:33.

ZIMMERMAN, A. M., and D. MARSLAND. 1956. Induction of premature cleavage furrow in the eggs of *Arbacia punctulata*. *Biol. Bull.* **111**:317.