CONTRACTION OF ISOLATED BRUSH BORDERS FROM THE INTESTINAL EPITHELIUM

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ABSTRACT

Brush borders isolated from epithelial cells from the small intestine of neonatal rats are able to contract in the presence of ATP and Mg^{2+}; Ca^{2+} is not required. Contraction is characterized by a pinching-in of the plasma membrane in the region of the zonula adherens and a subsequent rounding of the brush borders. No movement or consistent shortening of the microvilli is observed. The contraction appears to involve the 5- to 7-nm diameter microfilaments in the terminal web which associate with the zonula adherens. These filaments bind heavy meromyosin as do the actin core filaments of the microvilli. A model for contraction is presented in which, in the intact cell, terminal web filaments and core filaments interact to produce shortening of the microvilli.

Several recent studies have suggested that the microvilli of intestinal epithelial cells possess contractile properties (13, 18, 31, 32, 43, 45). This idea has been based largely on the finding that the longitudinal filaments which compose the cores of the microvilli are able to bind heavy meromyosin (HMM) in a manner similar to the way skeletal muscle actin binds this same protein (13, 31, 32, 45). In addition, the protein isolated from microvilli filaments is, by several biochemical criteria, indistinguishable from muscle actin (45). Two reports have described rapid, coordinated movements of microvilli in intact cells (35, 43). More recently, Mooseker (18) has reported the movement of the core filaments into the terminal web when demembranated brush borders, isolated from intestinal epithelium, are incubated with Ca^{2+} and adenosine 5′-triphosphate (ATP). We report here a different form of contraction in isolated brush borders, with membranes partially intact, which is also dependent on divalent cations and ATP. We describe two different populations of actin filaments both of which may participate in the contraction within intact cells.

MATERIAL AND METHODS

Animals and Biochemicals

Female, random-bred Sprague-Dawley rats were purchased with 9-day old young from Charles River Laboratories, Wilmington, Mass. Rats were housed in the laboratory until used.

HMM, isolated by the technique of Lowey and Cohen (14), was kindly provided by Dr. Thomas Pollard (Department of Anatomy, Harvard Medical School, Boston, Mass.). Cytochalasin B was obtained from Imperial Chemical Industries, Ltd., Cheshire, England. The drug was stored as a 1 mg/ml stock solution in dimethyl sulfoxide at 4°C. The sodium salts of ATP, ADP, AMP, GTP, and the tris(hydroxymethyl)aminomethane salt of adenosine 5′-triphosphate (Tris-ATP) as well as ethylenediaminetetraacetic acid (EDTA) and ethylene glycol bis(aminoethylether)tetraacetic acid (EGTA) were purchased from Sigma Chemical Co., St. Louis, Mo.

Isolation of Brush Borders

Epithelial cells were harvested from the upper one-half of the small intestine of 14- to 20-day old Sprague-Dawley rats by the method described by Evans et al. (6). Typically, two-three animals were used for a single preparation. Isolated cells were then lysed by suspension in 5 mM EDTA buffer, pH 7.4, at 0°C followed by passage three-four times through a 23 gauge needle and syringe. The brush borders were collected from the crude homogenate by the method of Forstner et al. (8) by first filtration through a small pad of glass wool to remove large debris and then centrifugation of the borders at 450 *g* for 10 min at 4°C. In one preparation, EGTA was substituted for EDTA in the buffers used to lyse isolated cells and collect brush border fragments. Brush borders collected by either method were then washed three times by resuspension in small aliquots of Hanks' balanced salt solution (BSS) (Microbiological Associates, Bethesda, Md.) or 0.1 M potassium phosphate buffer, pH 7.3, followed by recentrifugation. Brush borders were used immediately after isolation.

Light Microscopy

For light microscopy, portions of pelleted brush borders were resuspended in appropriate buffers or incubation media as described in Observations. A small drop of each suspension was placed on a microscope slide and was mounted with a cover slip suspended from the surface of the slide by small pieces of a second cover slip to avoid crushing the sample. Slides and cover slips were first thoroughly rinsed with 5 mM EDTA buffer and distilled water before use. The slides were immediately examined with a Zeiss Photomicroscope II fitted with differential interference-contrast (Nomarski) optics.

Electron Microscopy

For electron microscopy, brush borders were centrifuged briefly at 450 *g* and were fixed as a small pellet in either 2% glutaraldehyde or 1% glutaraldehyde and 1% formaldehyde (12) in 0.1 M phosphate buffer, pH 7.3, for 1 h at room temperature. Fixed pellets were rinsed in buffer alone for 30 min at 0°C and were then postfixed in 2% OsO_4 in 0.1 M phosphate buffer at pH 7.3 for 1 h at 0°C. To improve preservation of actin filaments, brush borders from some experiments were postfixed with 1% OsO_4 in phosphate buffer at pH 6.0 instead of pH 7.4 and then were stained *en bloc* with 0.5% uranyl acetate in distilled water for 2 h at 0°C (9, 19). Pellets from both procedures were dehydrated in a graded series of ethanols and were finally embedded in Epon (15). Silver to pale gold thin sections were cut with a diamond knife on a Porter-Blum MT-2 microtome (Dupont Instruments, Sorvall Operations, Newtown, Conn.). Sections were stained sequentially 5 min in uranyl acetate (46) and 5 min in lead citrate (30) and were then examined with a Philips 200 electron microscope. Measurements of filament diameters were made from negatives with a Nikon Model 6C profile projector (Ehrenreich Photo-Optical Industries, Garden City, N. J.).

HMM Binding

Isolated brush borders were incubated for 30 min at 0°C with approximately 2 mg of HMM per ml of incubation medium composed of 0.1 M phosphate buffer, pH 7.3, 1 mM $MgSO_4$ and 1 mM $CaCl_2$. As controls, borders were suspended in the same HMM solution with the addition of either 10 mM ATP or 10 mM pyrophosphate (PP_i). After incubation, samples were fixed, dehydrated, and embedded in the normal manner for electron microscopy.

OBSERVATIONS

Morphology of Brush Borders by Light Microscopy

Fig. 1 *a* illustrates the normal appearance of the isolated brush borders as viewed by Nomarski light optics. Most borders were uniform in size and shape and appeared as prolate ellipsoids approximately 25 x 10 μm in axial dimensions. The outer microvillar surface appeared as a sharply defined band 1- to 2-μm thick which at higher magnification exhibited the fine striations of individual microvilli. The microvilli extended over nearly the entire surface, an observation which suggested that the microvillar surface folded or curled over itself during isolation. The interior of the borders included the terminal web and a variable amount of cytoplasm with a finely granular appearance. The preparation was notably free of most nuclei and other cell fragments and organelles.

Conditions for Contraction

When brush borders were added to BSS which contained 6 mM ATP, pH 7.3, the borders instantaneously contracted into small spheres 10–15 μm in diameter (Fig. 1 *b*). Although a decrease in overall size was clearly evident, movement or shortening within the outer band of microvilli could not be detected at this level of resolution. To determine whether contraction might be an osmotic effect caused, for instance, by ATP-dependent extrusion of solute from closed vesicles, brush borders were suspended in hypertonic medium composed of 200 mM sucrose added to BSS without ATP. Under these conditions, no contraction occurred.

The incubation medium was modified in several different ways to explore the conditions necessary for contraction. The results of these experiments are summarized in Table I. Contraction required ATP; neither AMP, ADP, nor GTP could substitute for ATP. Contraction also required divalent cations. When brush borders were placed in Mg^{2+}/Ca^{2+}-free BSS to which had been added 10 mM EDTA, pH 7.3, no contraction was produced upon further addition of ATP. To determine whether Ca^{2+} was necessary for contraction, as is the case for contraction of actin and myosin in skeletal muscle, borders were suspended in 0.1 M potassium phosphate buffer, pH 7.3, which contained 10 mM EGTA, a strong chelator of Ca^{2+}, but not Mg^{2+}. Addition of 6 mM ATP caused normal contraction and indicated that Ca^{2+} was not an obligatory requirement. In this experiment, other divalent cations such as Mg^{2+} were presumably present as contaminants in the ATP (38) in concentrations sufficient to fulfill the general requirement for divalent cations which was demonstrated by the previous experiment with EDTA. To minimize effects caused by metal ion contamination of the ATP, a subsequent series of experiments was performed in which brush borders were suspended in phosphate buffer which contained 1 mM EDTA and 6 mM Tris-ATP, an ATP salt with low concentrations of metal ions. Under these conditions, the borders did not contract. However, full contraction was observed upon further addition of either 2 mM Mg^{2+} or 2 mM Ca^{2+}. The lack of calcium dependence did not appear to result from an effect of EDTA in the solutions used to isolate the brush borders from intestinal cells. If EGTA was substituted for the EDTA in these solutions, the resultant brush borders were still able to contract when resuspended in buffer which contained 10 mM EGTA, 6 mM ATP, and 2 mM Mg^{2+}.

Because of the strong possibility that actin filaments were involved in the contractility of the brush borders, an attempt was also made to block contraction with cytochalasin B, a strong inhibitor of certain other forms of cell movement in which actin-like filaments have been implicated (16, 36, 47, 49). The borders were preincubated in BSS which contained 20 μg/ml of cytochalasin B for up to 1 h at either 0°C or room temperature. During this time, the brush borders retained their normal morphology and contracted normally if ATP was added.

Morphology of Brush Borders by Electron Microscopy

Brush borders, as observed by electron microscopy, were membrane-bounded structures composed primarily of the microvilli and terminal web region of the epithelial cells (Figs. 2 and 3) and in general closely resembled the brush borders prepared by others (4, 8, 23, 34, 42, 45, 48). The microvilli usually covered more than half the outer surface of each border. The plasma membrane appeared continuous where it formed the outer surface of the microvilli but invariably exhibited large discontinuities where it extended into the microvillus-free region (Fig. 2). The brush borders were therefore open vesicles which presumably would be osmotically inactive.

Remnants of the tripartite junctional complex frequently appeared at the boundary of the microvillar surface of the brush borders (Fig. 4). Closest to the microvilli, the zonula occludens, or tight junction, could often be recognized with a small membrane fragment adherent to the outside of the plasma membrane as if the fragment had been torn from an adjacent cell during isolation of the brush borders. Farther away, bundles of microfilaments were found in close association with the inner surface of the plasma membrane in locations which would correspond to the sites of the zonula adherens and macula adherens.

The most noteworthy feature of the internal contents of the brush borders was the large number of the microfilaments. Three groups of filaments could be differentiated: core filaments of the microvilli, terminal web filaments, and tonofilaments (Fig. 4). The core filaments were similar to those core filaments described by other investigators in similar preparations (18, 31, 32, 45). These bundles of 20–24 parallel fibrils were 5–7 nm in diameter and extended from the tips of the microvilli into the terminal web up to a depth of about 1 μm. The filaments could be seen inserted into small, electron-dense areas on the surface of the plasma membrane at or immediately adjacent to the tips of the microvilli.

The terminal web filaments appeared as a loosely arranged network of fibers which surrounded and intermingled with the core filaments in the terminal web but which showed no obvious preferred orientation (Figs. 4 and 5). Most terminal web filaments were similar in diameter to the core filaments, although thicker filaments were

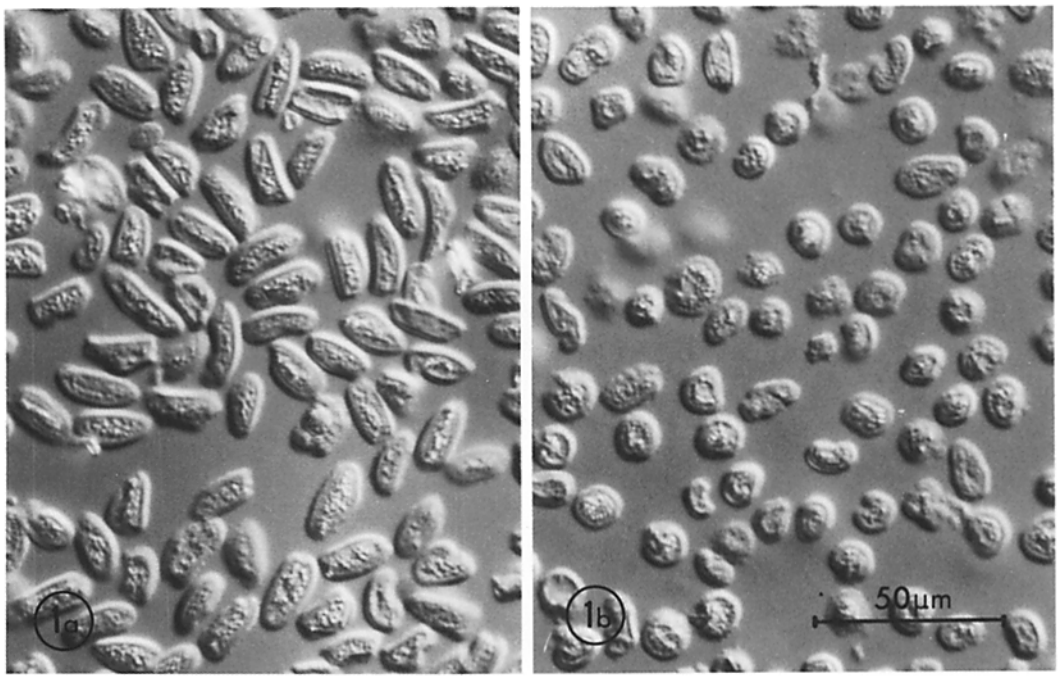


FIGURE 1 Isolated brush borders viewed by Nomarski light optics. (*a*) Brush borders suspended in BSS. (*b*) Same preparation suspended in BSS containing 6 mM ATP. × 500.

TABLE I

Requirements for Brush Border Contraction

Incubation conditions	Contraction
BSS	–
BSS + 6 mM ATP	+
BSS + 6 mM ADP	–
BSS + 6 mM AMP	–
BSS + 6 mM GTP	–
Ca^{2+}/Mg^{2+} free BSS + 10 mM EDTA + 6 mM ATP	–
0.1 M phosphate buffer + 10 mM EGTA	–
0.1 M phosphate buffer + 10 mM EGTA + 6 mM ATP	+
0.1 M phosphate buffer + 10 mM EGTA + 6 mM ATP + 2 mM Mg^{2+}	+
0.1 M phosphate buffer + 1 mM EDTA + 6 mM Tris-ATP	–
0.1 M phosphate buffer + 1 mM EDTA + 6 mM Tris-ATP + 2 mM Mg^{2+}	+
0.1 M phosphate buffer + 1 mM EDTA + 6 mM Tris-ATP + 2 mM Ca^{2+}	+

All incubations were performed at pH 7.3 and room temperature.

infrequently found with diameters of 10–20 nm (Fig. 5). The terminal web filaments were characteristically most concentrated at the margin of the microvillar surface in the region of the zonula adherens where irregular, electron-dense plaques could often be visualized on the inner surface of the plasma membrane (Figs. 5 and 6).

The third filament population was composed of thicker fibrils, approximately 10 nm in diameter, which resembled the tonofilaments found in intact epithelial cells (2, 7, 13). These filaments were randomly arrayed throughout most of the remaining interior of the brush borders. However, clusters of these filaments could often be found in the region of the disrupted macula adherens (Fig. 4), the structure into which tonofilaments normally insert in the intact cell (2, 7). In addition to the microfilaments, the brush borders contained a large number of small vesicles, usually 0.2–0.4 μm in diameter, occasional dense bodies, and infrequent membrane-bounded lipid droplets and mitochondria.

Comparison of Normal and Contracted States

Uncontracted brush borders, when visualized in thin section, appeared circular to oval in profile, their shape presumably dependent on their orientation to the plane of section (Fig. 2). Maximum

overall dimensions conformed to the dimensions observed by light microscopy.

When fixed in the contracted state, brush borders appeared uniformly rounded with almost no oval profiles evident in sectioned material (Fig. 3). The most dramatic change in the contracted preparation was the severe pinching-in of the plasma membrane in the region of the zonula adherens of many but not all of the brush borders (Fig. 3). The deep pocket formed in the surface was frequently more than 1 μm in depth and often gave the appearance that much of the brush border contents was being extruded away from the curled microvillar surface. No consistent change in the length of the microvilli or the thickness of the terminal web was observed upon contraction. However, there did appear to be a characteristic increase in the density of packing of terminal web filaments compared to the uncontracted state. The increase was particularly striking at the margin of the microvillar surface near the remnants of the junctional complex (Figs. 5 and 6). In contrast to the terminal web filaments, the underlying tonofilaments showed no obvious increase in concentration in the contracted borders.

HMM Binding

Brush borders were incubated with HMM to determine which of the filament populations could bind this molecule and hence might be involved in the mechanism of contraction. Since the brush borders appeared to be open vesicles, no pretreatment was deemed necessary to disrupt the membranes further to allow access of HMM to the interior of the borders.

After exposure to 2 mg/ml HMM for 30 min, both the core filaments of the microvilli and the terminal web filaments were found to bind HMM (Fig. 7). Typically, these filaments exhibited a fuzzy, frequently serrated coating which increased their diameter to over 20 nm. The arrowhead configuration, characteristic of HMM binding with other actin systems (10, 13, 26, 27, 33, 40, 45), was only infrequently identified. The 10-nm tonofilaments exhibited no binding or change from the untreated state. No binding was observed on any filaments when HMM incubation was performed in the presence of 10 mM ATP or 10 mM PP_i.

The binding of HMM to the core filaments showed a certain degree of variation among individual brush borders. In most cases, binding was limited to those portions of the fibers which extended into the terminal web, and little apparent staining was found within the microvilli proper (Fig. 7). However, in other brush borders where the plasma membrane of the microvilli appeared to separate from the core filaments, the entire length of these core filaments bound HMM. In the latter cases, arrowhead complexes were often recognized on the core filaments, the polarity of which was invariable with the arrowheads pointing toward the terminal web as observed by others (13, 19).

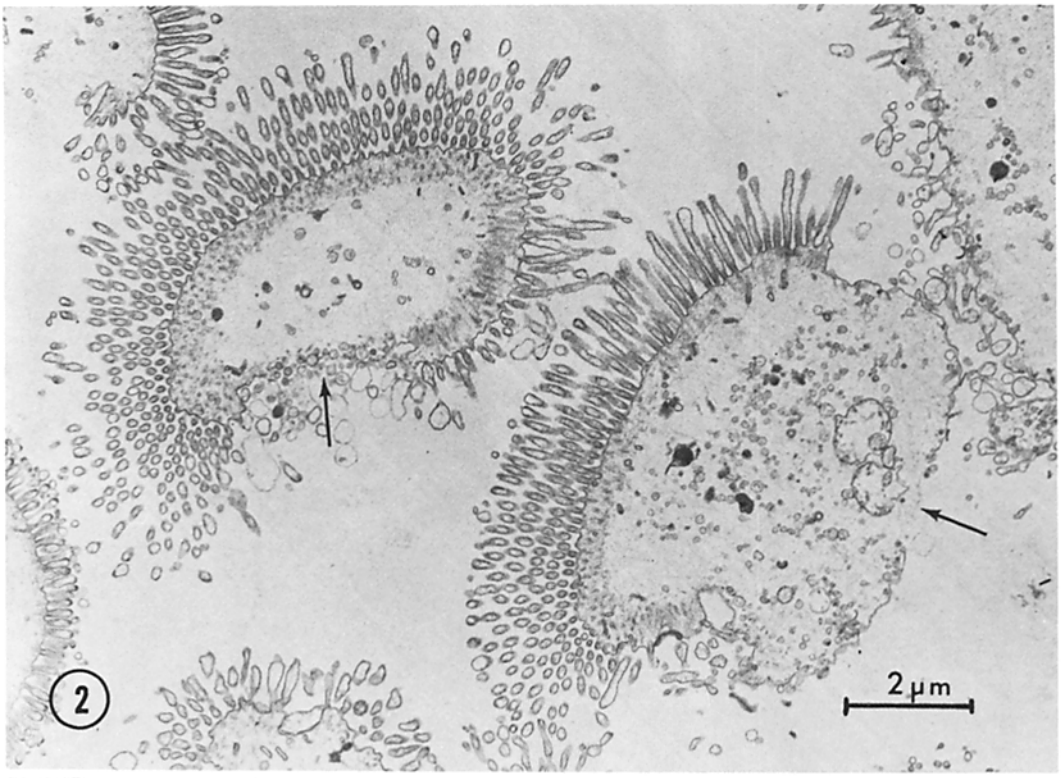
The pattern of HMM binding was more consistent with the loosely arrayed terminal web filaments. Both those filaments which surrounded the core filaments and those which were densely arrayed in the area adjacent to the zonula adherens exhibited a characteristic coating and increased diameter (Fig. 7). However, arrowhead structures could be identified only rarely due to the random orientation of these filaments in the plane of section. As a result, it was impossible to establish the polarity of those filaments which appeared to insert into the zonula adherens.

DISCUSSION

General Features of Brush Border Contraction

Our observations document an ATP-dependent contraction of isolated brush borders which has not been described by other investigators. This contraction is characterized by a rounding and overall decrease in size of the brush borders and, as evident by electron microscopy, is associated with an increased packing of actin filaments in the terminal web.

The brush borders exhibited distinct membrane discontinuities which allowed us to alter readily the internal environment of the brush borders and thus to determine some of the cofactors necessary for contraction. The contraction was specific for ATP and required divalent cation. As indicated by the experiments with EGTA, either Mg^{2+} or Ca^{2+} was sufficient to satisfy this requirement. In this respect, brush border contraction appears distinctly different from contraction in skeletal muscle where Ca^{2+} is an obligatory requirement which initiates contraction (5, 11). As yet, the factors that regulate contraction in nonmuscle systems are only poorly understood (for review, see reference 28). However, at least some of these systems contain contractile proteins whose ATPase activity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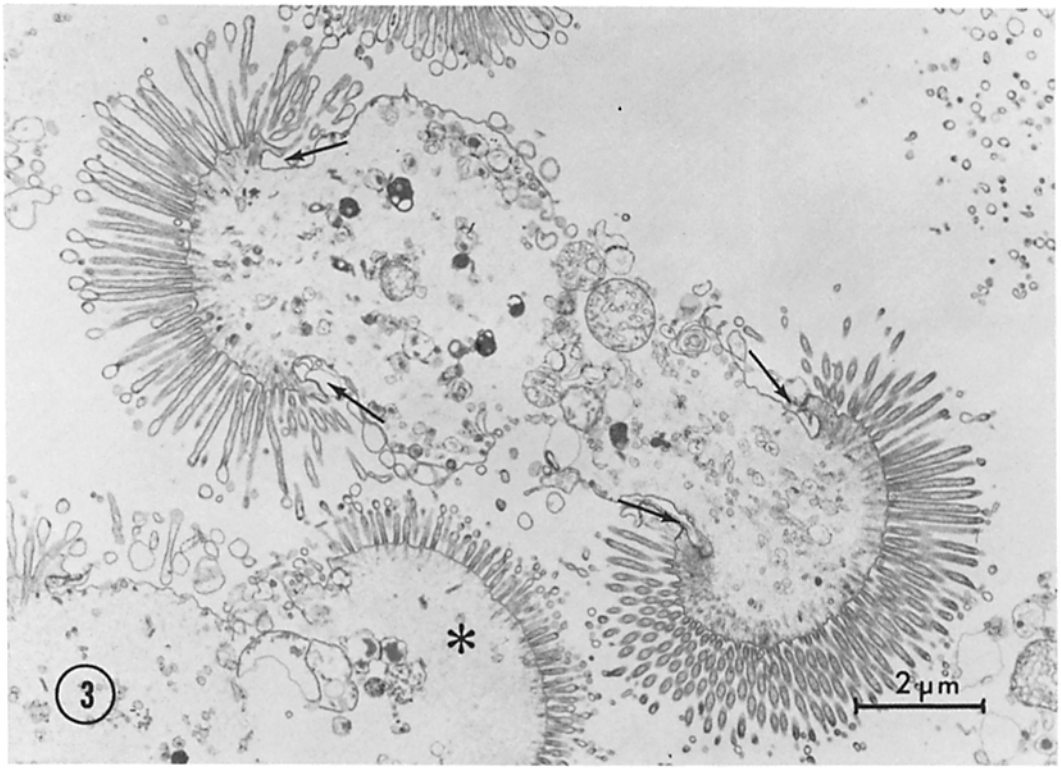
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or contractility does appear dependent on the presence of Ca^{2+} (3, 21, 29, 39, 41). It is possible that contraction of brush borders will prove an exception to those actin and myosin systems regulated by calcium. On the other hand, Mooseker (18) has reported that the inward movement of core filaments in demembranated brush borders from the intestinal cells of the chicken does require Ca^{2+}. The lack of calcium sensitivity in brush borders from neonatal rats may reflect a developmental phenomenon, a species difference, or may be the result of a difference in the methods of isolation. Possibly, cofactors which would normally impart calcium dependence have been lost during the isolation procedures we have employed.

The form of contraction exhibited by the brush borders we isolate also differs significantly from previous descriptions of brush border motility. Thuneberg and Rostgaard (43) used phase-contrast microscopy to describe rapid movements of microvilli in intact cells from the proximal tubules of rat kidney. They noted that the movements appeared unlike ciliary motion and suggested that the movements were caused by changes in the length of microvilli. In contrast, Sandström (35), studying intact cells in the intestinal epithelium of the chicken, described microvillar movement as a rapid, coordinated bending motion. The recent observations of Mooseker (18) on the movement of core filaments into the terminal web of demembranated brush border fragments appear more compatible with shortening of intact microvilli as suggested by Thuneberg and Rostgaard (43).

We did not observe any consistent change in length or movement of microvilli during contraction of the brush borders in our preparation. Instead, contraction appeared to be limited entirely to the terminal web region. The difference between this contraction and that observed by Mooseker (18) may, again, be due to the respective isolation procedures or to inherent differences between the brush borders from chickens and neonatal rats. We are inclined to believe that the different form of contraction in our system was caused by the inclusion of a large portion of apical plasma membrane with these brush borders. As suggested by Mooseker and Tilney (19), the microvillar membranes probably provide a certain degree of mechanical rigidity to the microvilli, an idea in accord with the observation that at least some of the core filaments are anchored along their length to the lateral membrane of the microvilli by short cross-bridges (17, 19, 20). This rigidity might then seriously inhibit any movement of the core filaments into the terminal web and explain why no microvillar shortening is observed with isolated brush borders unless the microvillar membranes are removed.

Role of Actin Filaments in the Terminal Web

Several investigators have documented the existence of actin filaments in brush borders (13, 19, 31, 32, 45). In particular, HMM has been shown to bind to the core filaments of the microvilli and form periodic arrowhead complexes identical in structure to HMM bound to muscle actin (10, 13, 19). In our studies, we see that HMM binds not only to the core filaments but also to the 5- to 7-nm diameter microfilaments associated with the zonula adherens in the terminal web. By virtue of their diameter and ability to bind HMM, these filaments are also actin and thus similar to the core filaments. Previous workers have clearly documented the existence of these terminal web filaments and pointed out their close association with the core filaments (20, 24). In fact, Mooseker and Tilney (19) have suggested that many if not all of the terminal web filaments may represent splayed ends of microvillar core filaments. However, the aggregation of the terminal web filaments in the region of the zonula adherens and their apparent attachment to this structure, as also noted by others (7, 20, 24), argues strongly that many of these

FIGURE 2 Uncontracted brush borders fixed and embedded for electron microscopy. Borders are oval in profile. Discontinuities in the plasma membrane in the microvillus-free regions are indicated by arrows. × 7,800.

FIGURE 3 Contracted brush borders fixed and embedded for electron microscopy. Borders appear rounded in profile. The pinching-in of the plasma membrane is clearly evident in the region of the zonula adherens (arrows) of two brush borders. An apparently uncontracted brush border is also shown (*). × 7,800.

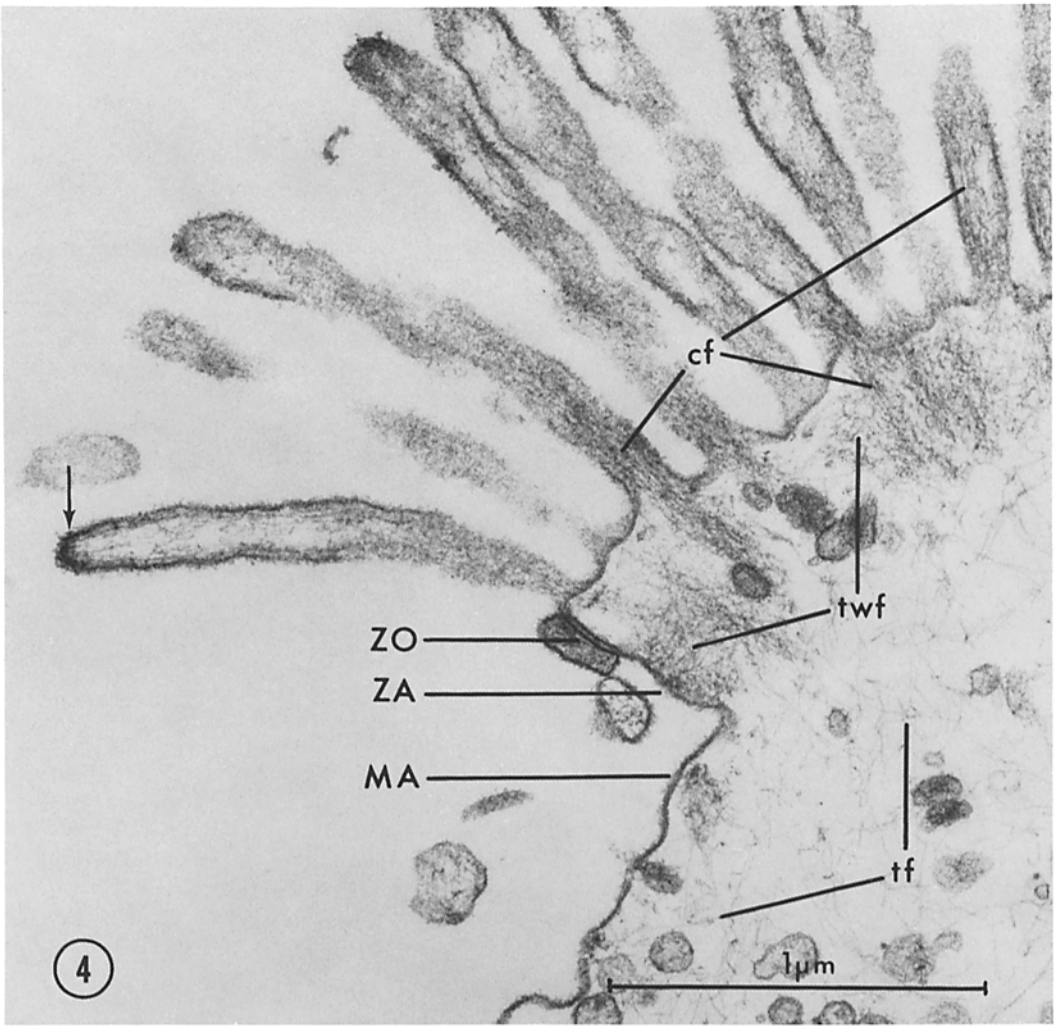
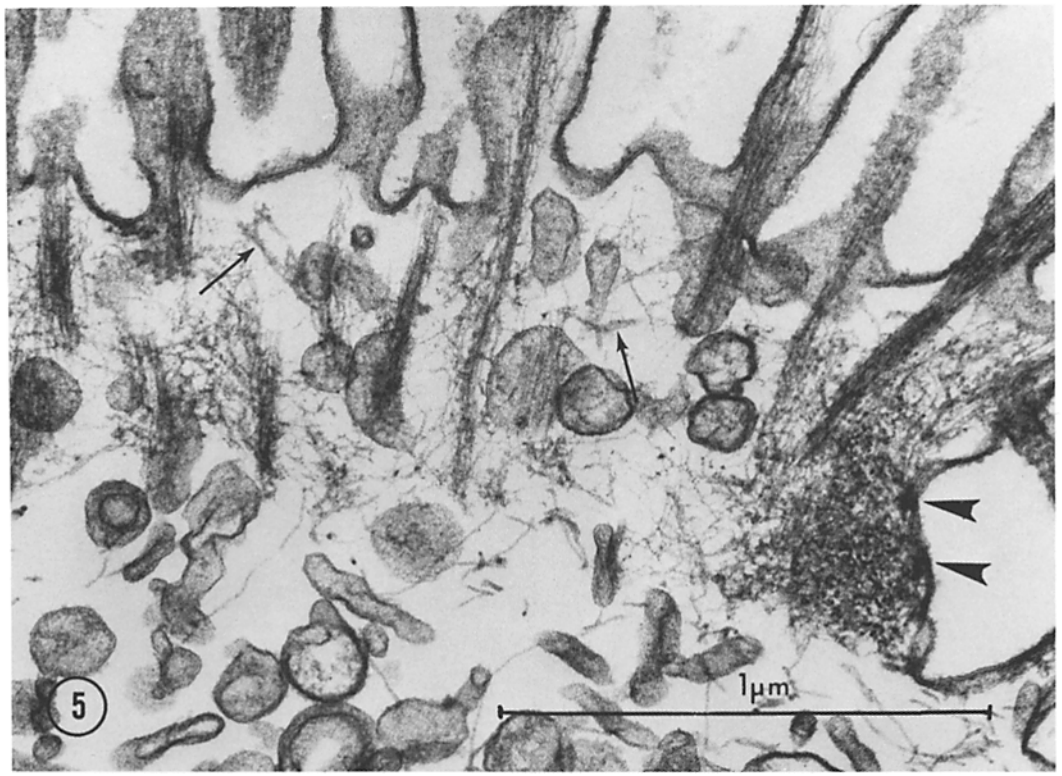


FIGURE 4 Portion of an uncontracted brush border. Three populations of filaments can be recognized: microvillar core filaments (*cf*), terminal web filaments (*twf*), and tonofilaments (*tf*). Core filaments attach to the tops of microvilli as in the region indicated by the arrow. Terminal web filaments are associated with the plasma membrane in the region of the zonula adherens (*ZA*). Probable remnants of the zonula occludens (*ZO*) and macula adherens (*MA*) are also indicated. × 50,000.

FIGURE 5 Terminal web region near the junctional complex of an uncontracted brush border. Infrequent thick fibrils are seen among the terminal web filaments (arrows). Note also the electron-dense plaques evident on the cytoplasmic surface of the cell membrane in the region of the zonula adherens (arrowheads). Stained *en bloc* with uranyl acetate. × 64,500.

FIGURE 6 Terminal web region of a contracted brush border. The terminal web filaments which surround the core filaments in this region near the zonula adherens are more densely packed than in the uncontracted state. Membrane densities in the zonula adherens are indicated by arrowheads. Stained *en bloc* with uranyl acetate. × 64,500.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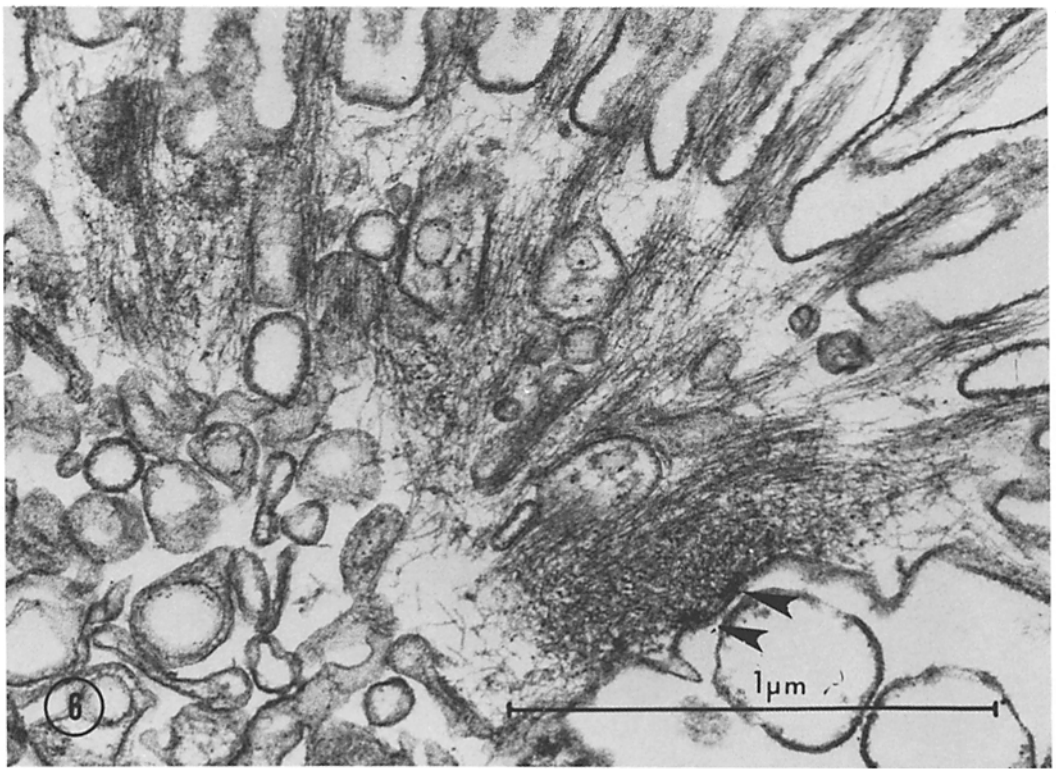
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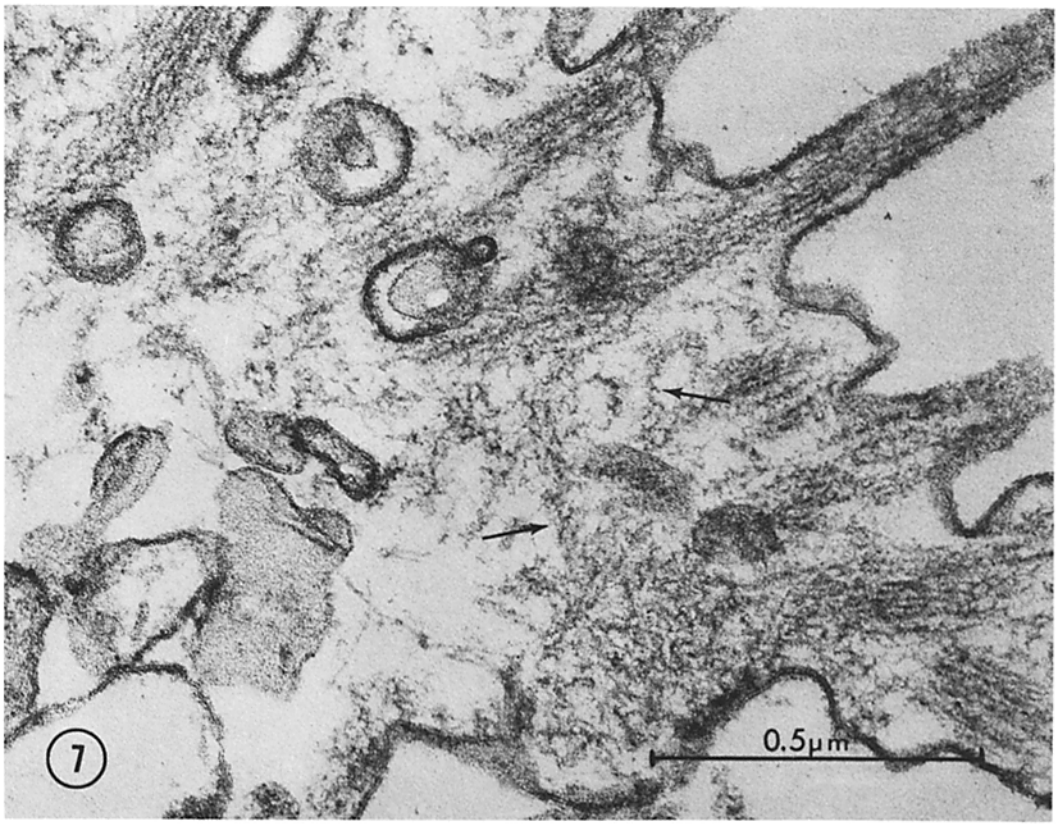

FIGURE 7 Brush border treated with HMM. Several terminal web filaments in the region of the zonula adherens show a fuzzy, serrated coating typical of HMM-binding (arrows). These filaments should be compared with untreated terminal web filaments as shown in Figure 5. Core filaments show little or no HMM-binding within the interior of the microvilli. × 87,000.

filaments represent a separate population of actin filaments discrete from the core filaments.

In ascribing a function to the terminal web filaments, most investigators have suggested that the fibrillar meshwork provides merely a passive, mechanical support for the core filaments embedded in it (1, 2, 20, 25). Our results indicate that, in addition, the terminal web filaments may interact with the core filaments as part of an active contractile system. Of particular importance in the contraction of the brush borders which we have observed is the marked pinching-in of the plasma membrane at the zonula adherens. This observation indicates that the contractile apparatus is firmly affixed to this point of the cell membrane. In intact tissue, the zonula adherens is a bandlike structure which completely encircles the cell and which is held rigidly in place by the zonula occludens and macula adherens on either side (7). It has been often supposed that all three structures form a junctional complex whose main function is to join adjacent cells tightly to each other (7). Although this is probably a principal function of the zonula occludens and macula adherens, we believe that, in analogy with the Z line of the muscle cell (11), the zonula adherens acts primarily as an intracellular site of attachment for actin filaments. The zonula adherens then acts as a *second* site in addition to the tips of the microvilli for which a similar function has already been postulated by others (1, 19, 44). In support of this analogy with the Z line, Schollmeyer et al. (37) recently have found that α-actinin, a protein found in the Z band of muscle (11), could also be demonstrated by means of a specific fluorescent antibody both within the junctional complex and within the microvilli of epithelial cells. The staining in the junctional complex was thought to be at

the zonula occludens although the zonula occludens and zonula adherens probably could not be resolved from each other in this light microscope study.

In the case of the microvilli, the polarity of the actin core filaments which insert into the microvillar tips has been clearly established (13, 45). As with actin filaments attached to the Z line in the muscle sarcomere (10), arrowhead complexes formed by core filaments and HMM point away from the site of attachment, which in this case is at the microvillar tips. Unfortunately, we could not determine with HMM the polarity of the much more randomly oriented terminal web filaments which insert into the zonula adherens. Nevertheless, we believe it is very likely that these filaments have the same polarity as the core filaments with respect to their sites of attachment to the cell membrane.

We rarely identified in our preparation any obvious thick filaments within the terminal web which might correspond to myosin, presumably a necessary component in most contractile systems involving actin (11, 28). Mooseker (18), however, has recently been able to identify a protein from brush borders which migrates in the same manner as muscle myosin when subjected to electrophoresis on acrylamide gels with sodium dodecyl sulfate. In addition, Mooseker and Tilney (19) have been able to visualize thick, myosin-like filaments in the terminal web of demembranated brush borders when incubated in the presence of high concentrations of Mg^{2+}. We presume that myosin is also present within our brush borders either at a concentration too low to be easily recognized or possibly as filaments too thin to be distinguished from actin as is believed to be the case in several other nonmuscle contractile systems (22, 28).

A Model for Brush Border Contraction

Mooseker and Tilney (19) have recently proposed a model to account for the shortening of microvilli in demembranated brush borders which involves the interaction of myosin and microvillar core filaments. In their scheme, the splayed ends of the core filaments from adjacent microvilli are aligned antiparallel to each other within the terminal web and thus are able to interact with myosin to cause contraction in a manner analogous to contraction in the muscle sarcomere. However, if the actin filaments are attached exclusively to the membranes within the microvilli, either at the tips or on the sides of the microvilli as proposed in this model, it is difficult to see how microvillar contraction could occur unless there is some additional attachment site or restrictive element within the terminal web against which the actin filaments could pull. It is unlikely that myosin molecules within the terminal web could serve as second sites of attachment unless they, in turn, are attached to some other structure within the terminal web.

We believe that this problem is overcome if the second population of actin filaments, those anchored at the zonula adherens, can also participate in the contractile mechanism. Our model for brush border contraction which involves both populations of filaments is presented in Fig. 8. If we assume that the core filaments of the microvilli and the terminal web filaments associated with the zonula adherens exhibit the same polarity with regard to their respective attachment sites on the plasma membrane, then interaction of these filaments with myosin molecules in the terminal web would generate tension between the zonula adherens and the tips of the microvilli. The participation of the terminal web filaments, however, does not exclude interaction of some of the core filaments with each other as postulated by Mooseker and Tilney (19). With isolated brush borders, the tension thus created would cause a contraction confined primarily to the terminal web, with a movement of the disrupted zonula adherens toward the interior of the brush border, just as we have observed in this study. A stable contracted state of the microvilli would not occur, perhaps because of the mechanical rigidity imparted to these structures by the microvillar membrane. Microvillar contraction would occur only when this membrane is removed, as in the brush borders isolated by Mooseker (18). However, in intact tissue the zonula adherens, by virtue of its location within the junctional complex between cells, is most likely restrained from appreciable movement. We believe that, under this normal condition, the tension exerted within the terminal web might be sufficient to overcome the rigidity of the microvillar membranes and cause shortening of the microvilli. Membrane rigidity could nevertheless play an important role in any relaxation phase after contraction to restore the microvilli to their original shape and position. We agree with the suggestion of others (35, 43) that any cyclic shortening or other movement of the microvilli would cause stirring of

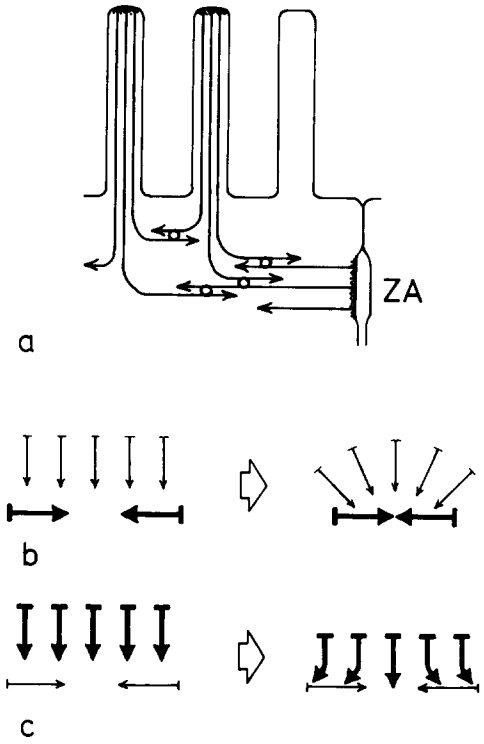

FIGURE 8 Model for brush border contraction. (*a*) Schematic representation of the probable relationships among the actin filaments within the terminal web. The core filaments, which insert at the tips of the microvilli, and the terminal web filaments, which insert at the zonula adherens (*ZA*), have the same polarity with respect to their sites of attachment. Where the filaments align antiparallel to each other, they can interact with myosin (arbitrarily represented by small circles) to cause contraction. (*b*) Contraction in isolated brush borders results primarily in the inward movement of the zonula adherens (heavy arrows) while the microvilli (light arrows) show little or no movement. (*c*) In intact tissue, the zonula adherens is restricted from appreciable movement during contraction, and instead there is a shortening of the microvilli.

the surrounding extracellular fluid and would be a significant factor in increasing the absorptive efficiency of this specialized cell surface.

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