BRUSH BORDER MOTILITY

Microvillar Contraction in Triton-Treated Brush Borders Isolated from Intestinal Epithelium

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ABSTRACT

The brush border of intestinal epithelial cells consists of an array of tightly packed microvilli. Within each microvillus is a bundle of 20–30 actin filaments. The basal ends of the filament bundles are embedded in and interconnected by a filamentous meshwork, the terminal web, which lies directly beneath the microvilli. When calcium and ATP are added to isolated brush borders that have been treated with the detergent, Triton X-100, the microvillar filament bundles rapidly retract into and through the terminal web region. Biochemical studies of brush border contractile proteins suggest that the observed microvillar contraction is actomyosin mediated. We have shown previously that the major protein of the brush border is actin (Tilney, L. G., and M. S. Mooseker. 1971. *Proc. Natl. Acad. Sci. U. S. A.* **68:**2611–2615). The brush border also contains a protein with the same molecular weight as the heavy chain subunit of myosin (200,000 daltons). In addition, preparations of demembranated brush borders exhibit potassium-EDTA ATPase activity of 0.02 μmol phosphate/mg-min (22°C); this assay is diagnostic for myosin-like ATPases isolated from vertebrate sources. Other proteins of the brush border include a 30,000 dalton protein with properties similar to those of tropomyosin, and a protein with the same molecular weight as the Z band protein, α-actinin (95,000 daltons). How these observations bear on the basis for microvillar movements in vivo is discussed within the framework of our recent model for the organization of actin and myosin in the brush border (Mooseker, M. S., and L. G. Tilney. 1975. *J. Cell Biol.* **67:**725–743).

Absorption by intestinal epithelial cells may be facilitated by movements of the microvilli comprising the "brush border" surface of these cells. Preliminary observations of *in situ* microvillar movements have been reported by two laboratories (24, 30). These reports suggest that this motility consists of rapid microvillar "beating," although the exact nature of the movement has not been determined. It is not known whether microvilli bend, shorten, vibrate, or move in some other way.

The ultrastructure of the brush border also reveals a potential for movement. Each microvillus contains a bundle of actin filaments (10, 16, 32) and the polarity of these filaments suggests a functional similarity in their organization to that of

actin filaments in striated muscle (16). The highly organized ultrastructure of this organelle makes the brush border a particularly favorable model system for analyzing the molecular and structural basis of cellular movements which are actomyosin mediated.

In the present study, I have investigated the nature and mechanism of microvillar motility by characterizing the motile properties of the isolated brush border. In addition, I have extended earlier work (32) on the characterization of brush border contractile proteins in the hope of establishing the biochemical basis for microvillar motility.

The results presented here indicate that the isolated brush border is a calcium-regulated motile apparatus. The addition of calcium and ATP to isolated, detergent-treated brush borders causes rapid retraction of the microvilli into and through the terminal web. This contraction is presumably actomyosin mediated since the results of preliminary biochemical studies indicate that the brush border contains, in addition to actin, other contractile proteins including myosin, "nonmuscle" tropomyosin (30,000-dalton subunit; see references 5, 7, 8), and α-actinin.

The significance of these results with regard to possible mechanisms for the generation of in vivo microvillar movements is discussed within the framework of our recent model for the functional organization of actin and myosin in the brush border (16).

The results presented here have appeared previously in abstract form (15).

MATERIALS AND METHODS

Brush Border Isolation

Brush borders were isolated from the small intestines of chicken, rat, rabbit and pig as described in Mooseker and Tilney (16). This procedure is summarized briefly below.

Epithelial cells were isolated from segments of intestine by a modification of the method of Evans et al. (6). Brush borders were prepared from these isolated cells by procedures adapted from Forstner et al. (9): pellets of isolated cells were suspended in 10–20 vol of 4 mM EDTA, 1 mM EGTA, 10 mM imidazole buffer, pH 7.3, to which 10 mM tosyl arginine methyl ester (TAME; Sigma Chemical Co., St. Louis, Mo.) and 0.1 mg/ml soy bean trypsin inhibitor (SBTI; Sigma) were added to inhibit proteolysis. The suspended cells were homogenized for 10–15 s in an Omnimixer (Dupont Instruments, Sorvall Operations, Norwalk, Conn.) at setting 7. A lower mixer setting and shorter homogenization time could be used to obtain larger cell fragments consisting of the apical brush border surface and variable amounts of underlying cytoplasm. Brush borders (or cell fragments) were isolated from this homogenate by centrifugation at 800 *g* for 5 min. The pellets of brush borders were resuspended in 10 vol of solution A: 75 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM dithiothreitol (DTT; Sigma), 10 mM imidazole buffer, pH 7.3, and 10 mM TAME. Centrifugation and resuspension of the isolated brush borders in solution A were repeated several times. Brush border preparations isolated by this procedure are contaminated by nuclei. Reduction of this nuclear contamination was unnecessary if the preparations were to be used for cytological studies, but, for biochemical studies, nuclei were removed by centrifugation on a sucrose step gradient. Brush border pellets were suspended in 1 vol of Solution A and mixed with 9 vol of solution A containing 55% (wt/vol) sucrose (Schwartz Mann Div. Becton, Dickinson & Co., Orangeburg, N. Y.). This suspension was layered onto a vol of 55% sucrose solution equal to about half the volume of the brush border suspension. The gradient was centrifuged at 7,000 *g* for 25 min. Nuclei were pelleted and the purified brush borders collected at the interface between the sucrose solutions. The purified brush borders were removed from the gradient with a Pasteur pipet, diluted in 10 vol of Solution A and collected by centrifugation to remove residual sucrose.

Characterization of Brush Border Contraction

Triton Treatment of Isolated Brush Borders

Brush borders must be exposed to the detergent Triton X-100 to exhibit the contraction described here. Pellets of isolated brush borders were suspended in solution A containing 1% Triton X-100 (wt/vol; Schwartz Mann) and collected by centrifugation (800 *g* for 5 min). Several washes with Triton are required to completely remove the membrane from isolated brush borders (16), but a single wash was sufficient to allow contraction. For most of the experiments reported here, a single wash procedure was used. To determine the minimum concentration of detergent required to allow microvillar contraction, a range of Triton concentrations was used (0.0001%–1.0%) to treat brush border preparations before addition of contraction solutions.

Assay for Determination of Contraction Conditions

A simple light microscope assay was used to determine whether various test solutions elicited contraction. Triton-treated brush borders were suspended in each test solution for 30 s at room temperature and then fixed by addition of glutaraldehyde to a final concentration of about 1%. Phase contrast microscopy

was used to determine whether a given test solution was effective in generating microvillar contraction. I tested a number of parameters including calcium concentration, nucleotide requirement, pH, temperature, and high magnesium concentration (above 15 mM). Each test was made by varying one of the above parameters in the following contraction medium: 75 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 10 mM TAME, 0.5 mM ATP, 0.2 mM $CaCl_2$, and 10 mM imidazole buffer, pH 7.3 at room temperature.

CALCIUM CONCENTRATION: Calcium buffers were prepared by the method of Portzehl et al. (22). Contraction solutions containing free calcium ion concentrations on the order of 10^{-7} M (3 mM EGTA, 1 mM $CaCl_2$), 10^{-6} M (3 mM EGTA, 2.4 mM $CaCl_2$), and 10^{-5} M (2 mM EGTA, 2 mM $CaCl_2$) were tested.

NUCLEOTIDE REQUIREMENT: To test for nucleotide requirement, endogenous ATP was removed by preincubation of brush border preparations with hexokinase and glucose. Preparations of Triton-treated brush borders were incubated for 30 min at room temperature, or for 6 h in the cold, in solution A to which 1 mg/ml hexokinase (type III from yeast; Sigma) and 5 mM glucose were added (see Taylor et al. [29] for a similar application of this technique). After washing with solution A, the brush border preparations were used to test the effectiveness of contraction solutions containing calcium alone (0.2 mM) or calcium plus 0.5 mM added nucleotide (ATP, ADP, ITP, or GTP).

EFFECTIVE PH RANGE: Contraction solutions with a pH range of 5.5–9.0 were tested, at 0.5 pH-unit intervals. Three different buffers were used; potassium phosphate (5.5–6.5), imidazole (7.0–7.5), and Tris (8.0–9.0). All buffers were used at a concentration of 10 mM.

TEMPERATURE DEPENDENCE: To test the effect of low temperature on contraction, pellets of Triton-treated brush borders were suspended in cold contraction solution for 30 s on ice, and then fixed.

Perfusion Techniques for Light Microscope Observations of Brush Border Contraction

Direct observations of brush border contraction were made by perfusing highly flattened microscope slide preparations of isolated brush borders with contraction solutions. Either brush border preparations were Triton-treated before placement on the slide or membrane-intact brush borders were used and detergent was included in the contraction solution. To select for brush borders that would remain attached to the slide or coverslip during perfusion, most preparations were first perfused with control solution (solution A which contained 0.1 mM EGTA rather than 1 mM) before perfusion with contraction solution. A wide variety of contraction solutions were used, as this technique was used in parallel with the "in test tube" assay for contraction described above. (The perfusion technique was not used to assay for effectiveness of a given contraction solution because I observed considerable variation in the effectiveness of the *same* contraction solution from preparation to preparation.) Generally, best results were obtained by using a higher calcium concentration (0.5 mM–1 mM) than for the "in test tube" contraction experiments.

These observations were made with a Zeiss light microscope using Zeiss (plan) 40× or 100× oil immersion, phase contrast objectives. Movies of microvillar contraction were made with a 16-mm Bolex movie camera (Paillard Bolex, Sainte-Croix, Switzerland) equipped with a motor drive. Film speeds of 18–32 frames per second were used. Illumination was provided by a 100 W mercury arc lamp with a green light interference filter (broad band; Zeiss) and Corning heat-cut filter no. 4602 (Corning Glass Works, Corning, N. Y.). Movies were made on Kodak Plus X reversal film and were processed commercially.

Electron Microscopy of Contracted Brush Borders

Pellets of Triton-treated brush borders were suspended in contraction solution and, after incubation for 30 s at room temperature, an equal volume of cold contraction solution containing 2% glutaraldehyde (Electron Microscope Sciences, Fort Washington, Pa.) was added. The fixed brush borders were immediately brought to 4°C and then collected by centrifugation. The pellets were resuspended in 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3 at 4°C and incubated for 45 min. The brush border preparations were postfixed with 1% OsO_4, 0.1 M cacodylate buffer, pH 7.3 (4°C). At this point, some preparations were *en bloc*-stained with 0.5% uranyl acetate for 2–3 h. (in the cold). All preparations were rapidly dehydrated with acetone and embedded in Araldite. Thin sections were cut on a Sorvall Porter-Blum ultramicrotome I or II, stained with uranyl acetate and lead citrate and viewed with a Philips EM 200 electron microscope.

The isolated brush borders in Figs. 5 and 6 were processed for electron microscopy using fixatives buffered with phosphate as described by Mooseker and Tilney (16).

Characterization of Brush Border Contractile Proteins

Isolation of Brush Border Tropomyosin

An attempt to isolate tropomyosin from the brush border was carried out using a procedure based on that of Cohen and Cohen (5) as adapted by Fine et al. (8) for the isolation of tropomyosin from brain. This procedure is summarized briefly below.

Pellets of demembranated brush borders isolated from pig intestine were extracted with ethanol and ether. The resulting powder (about 2 g) was extracted overnight, in the cold, with 1 M KCl, 1 mM DTT, 10 mM TAME,

and 10 mM imidazole buffer, pH 7.0. The extract was cleared by centrifugation (50,000 *g* for 30 min), and the supernate was placed in a boiling water bath for 10 min, a procedure which relied on the remarkable heat stability of tropomyosin (2). The extract was centrifuged at 80,000 *g* for 1.5 h, and an enriched fraction of (presumptive) tropomyosin was isolated from the supernate by an ammonium sulfate fractionation procedure as described by Fine et al. (8). Inhibitors of proteolysis were included in all aqueous stages of this procedure (either SBTI or TAME).

Potassium-EDTA ATPase Assay

Samples (0.2 ml) of isolated, demembranated brush borders isolated from chicken intestine were pipetted into 5-ml of assay mixture consisting of 2 mM ATP, 0.6 M KCl, 5 mM EDTA, 1 mM DTT, 20 mM imidazole buffer, pH 7.3 at 22°C. Samples (1.8 ml) were taken at 0- and 30-min time intervals, and enzymatic activity was stopped by addition of 0.2 ml of 50% trichloroacetic acid. Inorganic phosphorus was determined by the method of Taussky and Schorr (28). Protein concentrations of the brush border preparations used for this assay were determined by the method of Lowry et al. (14).

Preparation of Muscle Protein Standards

Protein samples of skeletal, cardiac and smooth muscle were prepared for SDS polyacrylamide gel electrophoresis as follows: tissue minces of chicken breast, heart, and gizzard were each suspended in a solution containing 1% Triton X-100, 70 mM KCl, 5 mM $MgCl_2$, 5 mM EGTA, 1 mM DTT, 20 mM imidazole buffer, pH 7.3 at 4°C, and then homogenized for 20–30 s in an Omnimixer at top speed. The homogenates were centrifuged at 5,000 *g* for 10 min., and the resulting pellets were resuspended in the above-described solution and collected by centrifugation several additional times. The pellets were then solubilized in SDS for gel electrophoresis as described below.

Skeletal muscle tropomyosin was prepared from chicken breast muscle by the method of Bailey (2). Partially purified skeletal muscle myosin was prepared from chicken muscle by the method described by Tilney et al. (31). α-Actinin isolated from pig skeletal muscle was a gift from Doctors D. E. Goll and J. V. Schollmeyer, Muscle Biology Group, Iowa State University, Ames, Iowa.

SDS Gel Electrophoresis

Brush border preparations and muscle protein standards were run on 5%, 7.5%, and 10% polyacrylamide gels containing 0.1% SDS with a running buffer consisting of 10 mM phosphate buffer, pH 6.8 and 0.1% SDS (33). Muscle protein standards and brush border protein samples were prepared for electrophoresis by boiling for 2–5 min in 1.5% SDS, 1.5% mercaptoethanol, 10 mM phosphate buffer, pH 6.8. Some brush border protein samples were concentrated after this boiling step by the following procedure: dissolved brush border proteins were precipitated from the SDS solution by addition of 9 vol of cold (−20°C) acetone. The precipitants were redissolved in a smaller volume of SDS gel sample solution. In addition to concentrating the samples, it was found that this acetone precipitation procedure reduced background staining on the gels without any noticeable effects on band population or intensity. Gels were stained with Coomassie blue.

RESULTS

Microvillar Contraction

Light Microscope Observations

The isolated brush border consists of a fanlike array of microvilli which extends from a band of phase-dense material called the terminal web (Fig. 1). After addition of contraction solution containing calcium and ATP, the microvilli of brush borders which have been treated with the detergent, Triton, plunge rapidly toward the terminal web (Fig. 2). (One can partially disrupt or completely remove the plasma membrane from isolated brush borders with Triton without damaging the underlying filamentous structure [16].) From direct observations and cinematographic recording of this contraction using slide perfusion techniques, I have determined that this contraction can occur rapidly (in less than 1 s) or can occur over a longer time period (up to about 5 s). There may be several causes for variation in contraction time.

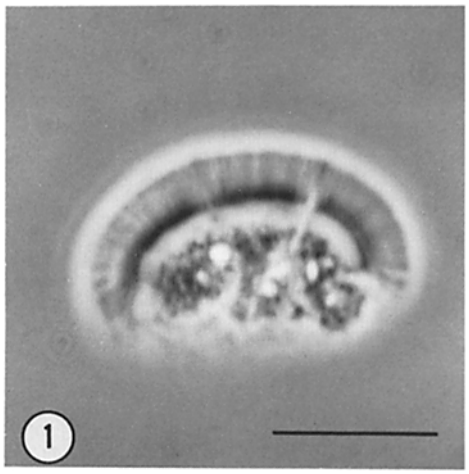

FIGURE 1 Isolated brush borders from chicken intestinal epithelium. The microvilli extend from a band of phase-dense material called the terminal web. Phase-contrast light micrograph. Scale is 10 μm. × 2,200.

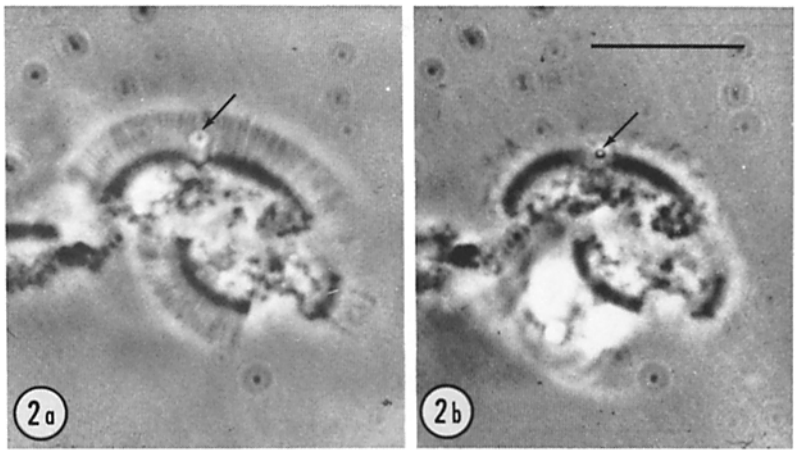

FIGURE 2 Light micrographs from a 16 mm film of microvillar contraction. Triton-treated brush borders before (*a*) and after (*b*) perfusion with contraction solution containing calcium and ATP. Time elapsed between (*a*) and (*b*) is 3 s. Most of the microvilli have moved toward and presumably into the terminal web regions. Note the particle (arrow) which has been carried toward the terminal web as a result of contraction. There is an increase in phase density and thickness of the terminal web regions after contraction. There is also a change in morphology of the region beneath the terminal web. These micrographs are from a film shown at the 14th Annual Meeting, American Society for Cell Biology, San Diego, Calif., November, 1974. Scale is 10 μm. × 2,000.

Brush borders treated with low concentrations of Triton take longer to contract than those treated with more concentrated detergent solutions. Adhesion to the slide or cover slip also increases the time required for contraction. Since reliable observations of contraction can only be made on brush borders which are stuck to the slide or cover slip, the actual time required for contraction of brush borders in suspension may actually be less than that reported here. Direct observations indicate that this longer time is mainly the result of the fact that the onset of contraction is not synchronous for all the microvilli of a given brush border. However, some decrease in the rate of retraction of individual microvilli has also been observed.

Since microvilli are very thin (less than 0.1 μm in diameter) and also densely packed, it was difficult to discern the "behavior" of individual microvilli during contraction. However, in instances where contraction occurred slowly, and onset of microvillar retraction was not synchronous, I could at least detect, if not resolve, the movement of individual microvilli toward the terminal web. In one observation, lateral movement of microvilli during retraction could be detected. As is the case in Fig. 2, I could sometimes observe debris, which was attached to the microvilli, move toward the terminal web during contraction (see arrow, Fig. 2).

Microvillar contraction has been induced in both isolated brush borders and in larger cell fragments which contain considerable amounts of attached cytoplasm beneath the terminal web region. Brush borders devoid of attached cytoplasm are relatively unstable after contraction, and often disintegrate if not immediately fixed with glutaraldehyde. Brush borders with considerable underlying cytoplasm are more stable after contraction and because of this they have been useful cytological material for determining the fate of the retracted microvillar material after contraction. In such cell fragments, an increase in the thickness and phase density of the terminal web region is often seen after contraction (Figs. 3 and 4). These changes in the morphology of the terminal web are variable and, in some instances, one can actually observe what appear to be microvilli within the terminal web of these contracted brush borders (Fig. 4). These observations indicate that a contraction consists of microvillar retraction *into* rather than bending onto the terminal web.

ELECTRON MICROSCOPE OBSERVATIONS

For the sake of comparison, I have included electron micrographs of uncontracted brush borders, both before and after detergent treatment (Figs. 5 and 6; see Mooseker and Tilney [16] for a report on the morphology of the brush border).

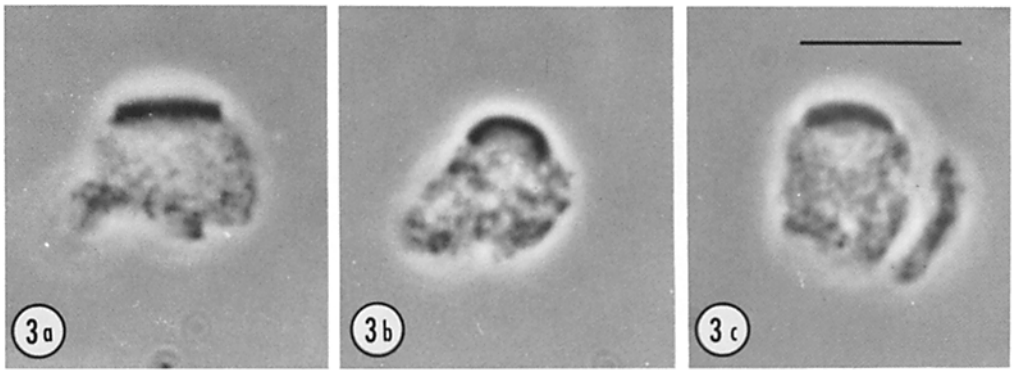

FIGURE 3 Examples of contracted brush borders in Triton-treated epithelial cell fragments (*a–c*). Contraction was induced by calcium and ATP. Note the marked phase density of the apical surfaces of these cell fragments, suggesting that the microvilli have retracted into the apical cytoplasm of the fragments. Phase-contrast light micrographs. Scale is 10 μm. × 2,200.

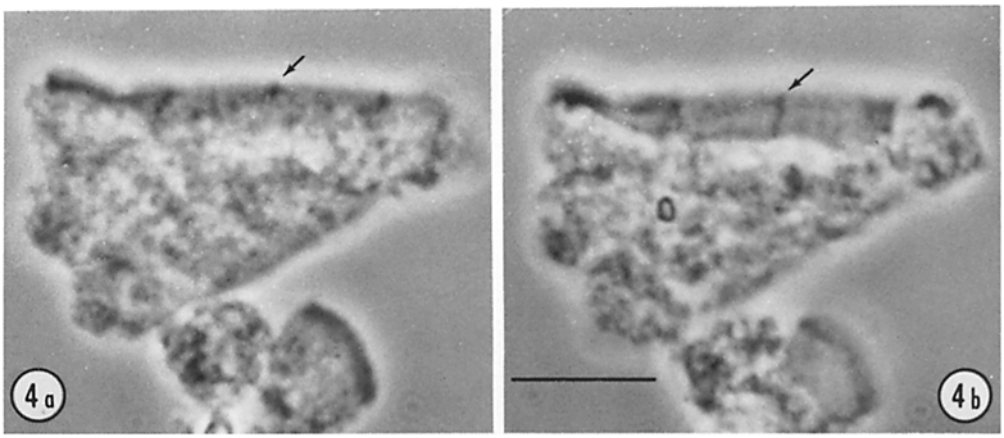

FIGURE 4 Optical sections (*a* and *b*) of at least three connected cell fragments, suggesting the presence of retracted microvilli within the terminal web regions of the fragments. Microvillar contraction was induced by the addition of calcium and ATP. The arrows indicate the region of an intercellular junction between two of the cell fragments. This junction marks the apical boundary of the terminal web, indicating that the microvilli have retracted into the interior of the cell fragments. Phase-contrast light micrographs. Scale is 10 μm. × 2,000.

Electron microscopy of contracted brush borders confirms the light microscope observations that microvillar contraction consists of a retraction of the microvillar actin filament bundles into and through the terminal web (Figs. 7 and 8). The retracted microvillar filament bundles remain straight and the bundles now extend completely below the apical boundary of the terminal web (Fig. 7). Since the membrane at the base of the microvilli resists solubilization with detergent, there often remains a "line" of membrane fragments which serves as a marker for this apical boundary. Remnants of membrane junctional complexes also help locate the terminal web zone (Fig. 7). There are at least two other indications that contraction consists of penetration of the microvillar filament bundles into the terminal web. Fragments of microvillar membrane associated with filament bundles often appear to be carried below the apical boundary of the terminal web as a result of contraction (Figs. 7 and 8). The membrane at the distal tips of the microvilli also resists detergent treatment, and, when present, these membrane fragments often appear to have been pulled toward the terminal web with the retracting microvillar filament bundles (Fig. 7). Although

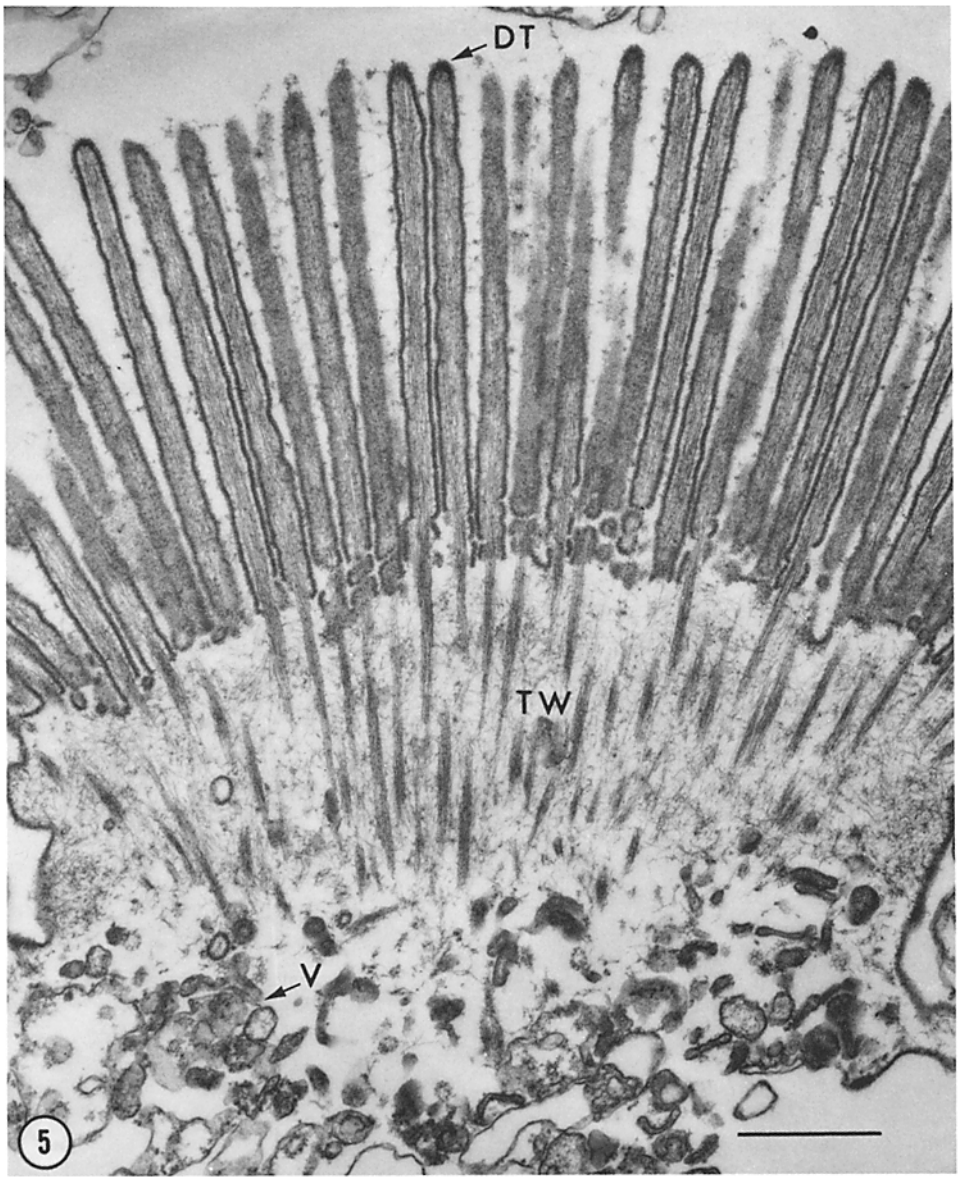

FIGURE 5 Electron micrograph of an isolated brush border. Each microvillus contains a bundle of actin filaments which extends the entire length of the microvillus and below it into the terminal web (*TW*). Each bundle of actin filaments is embedded in a dense matrix (*DT*) at the tip of the microvillus which presumably effects the attachment of the filaments to the plasma membrane. Numerous vesicles (*V*) lie directly below the terminal web region. Scale is 0.5 μm. × 38,000.

the microvillar bundles remain essentially intact after contraction, there is a considerable increase in the amount of filamentous material between the microvillar filament bundles. This increase appears to be the result of lateral splaying of filaments from the periphery of the microvillar bundles. The average length of the filament bundles in contracted bursh borders is about 25% less than in uncontracted brush borders (this measurement includes that region of the filament bundles, in uncontracted brush borders, within the terminal web region). We cannot determine whether bundle

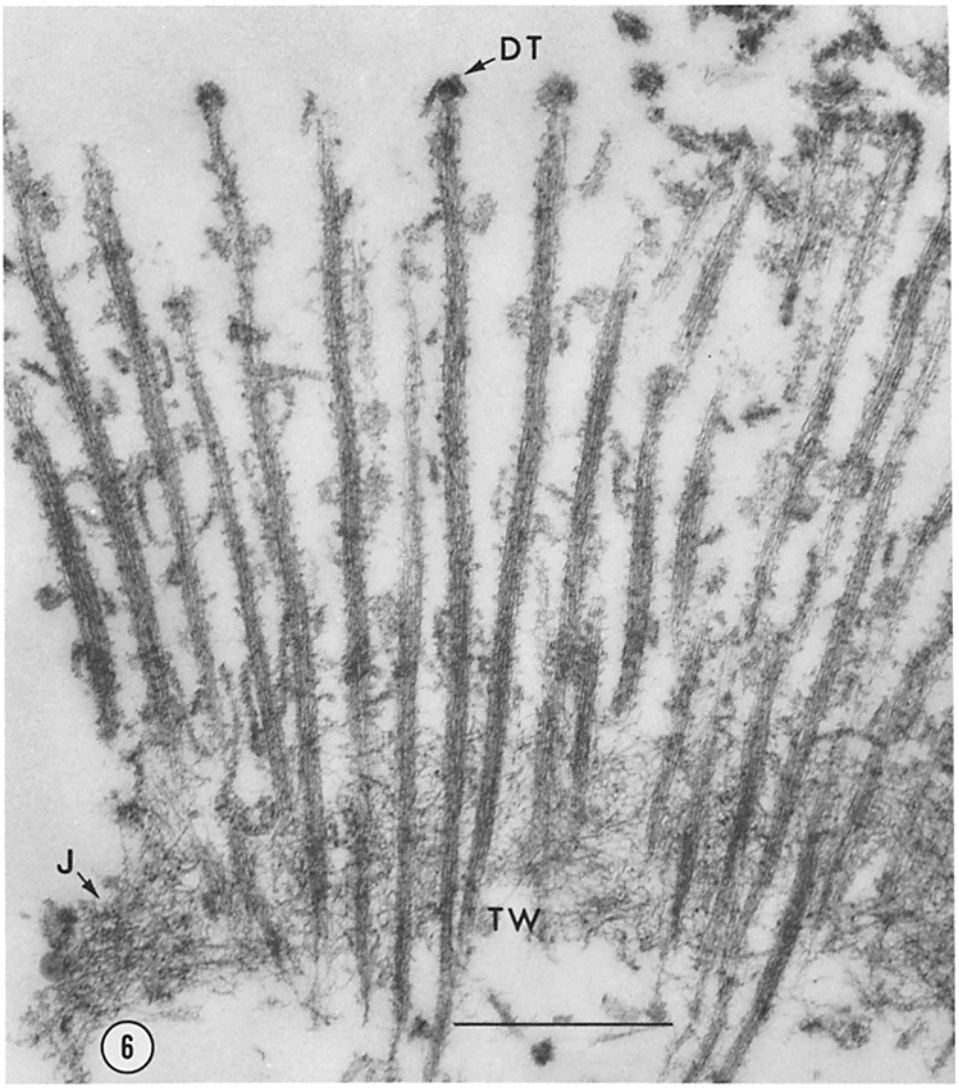

FIGURE 6 Isolated brush border demembranated with the detergent Triton X-100. The terminal web (*TW*), actin filament bundles, and dense tip material (*DT*) are not disrupted by detergent treatment. Remnants of an intercellular junctional complex (*J*) remain associated with the lateral boundary of the terminal web. Note the numerous cross bridges along the length of the microvillar actin filament bundles. Scale is 0.5 μm. × 58,000.

shortening is a true feature of the contractile event or whether it is due to the instability of contracted brush borders after contraction. Another interesting feature of contracted brush borders is that the microvillar filament bundles are closer to each other at their basal ends than they are along the rest of their lengths.

REQUIREMENTS FOR CONTRACTION

The contraction of isolated, demembranated brush borders requires conditions similar to those established for the contraction of muscle and isolated amoeba cytoplasm (29). All of these contractions require calcium and ATP. A satisfactory medium for generating contraction in Triton-treated brush borders consists of 75 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM $CaCl_2$, (or alternatively, 2 mM $CaCl_2$ and 2 mM EGTA; free calcium ion concentration 10^{-5} M), 0.5 mM ATP, 10 mM imidazole buffer, pH 7.3 (room temp.) and 10 mM TAME. Except for the calcium and ATP, the contents of this medium reflect condi-

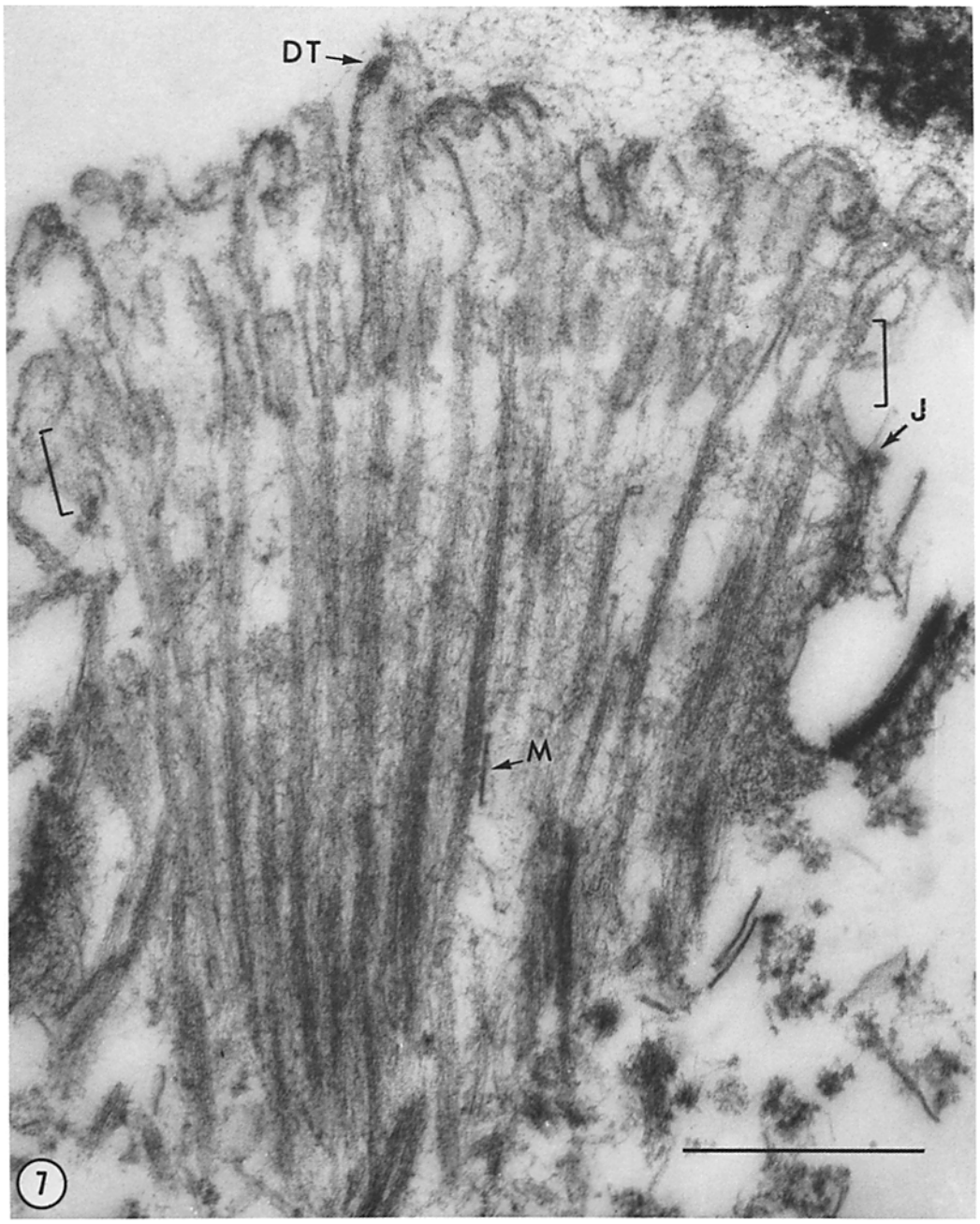

FIGURE 7 Electron micrograph of contracted brush border. The microvillar filament bundles have retracted into and through the terminal web. The apical boundary of the terminal web, above which the microvilli used to extend, is marked by a "line" of membrane fragments (brackets) and remnants of a membrane junctional complex (*J*). A fragment of microvillar membrane (*M*) appears to have been carried into the terminal web region as a result of contraction. Fragments of plasma membrane associated with the dense tip material (*DT*) also appear to have been "pulled" toward the terminal web. The microvillar bundles remain essentially intact although there is considerable filamentous material between the filament bundles, possibly the result of shredding of the peripheral filaments of the bundles during contraction. Scale is 0.5 μm. × 68,000.

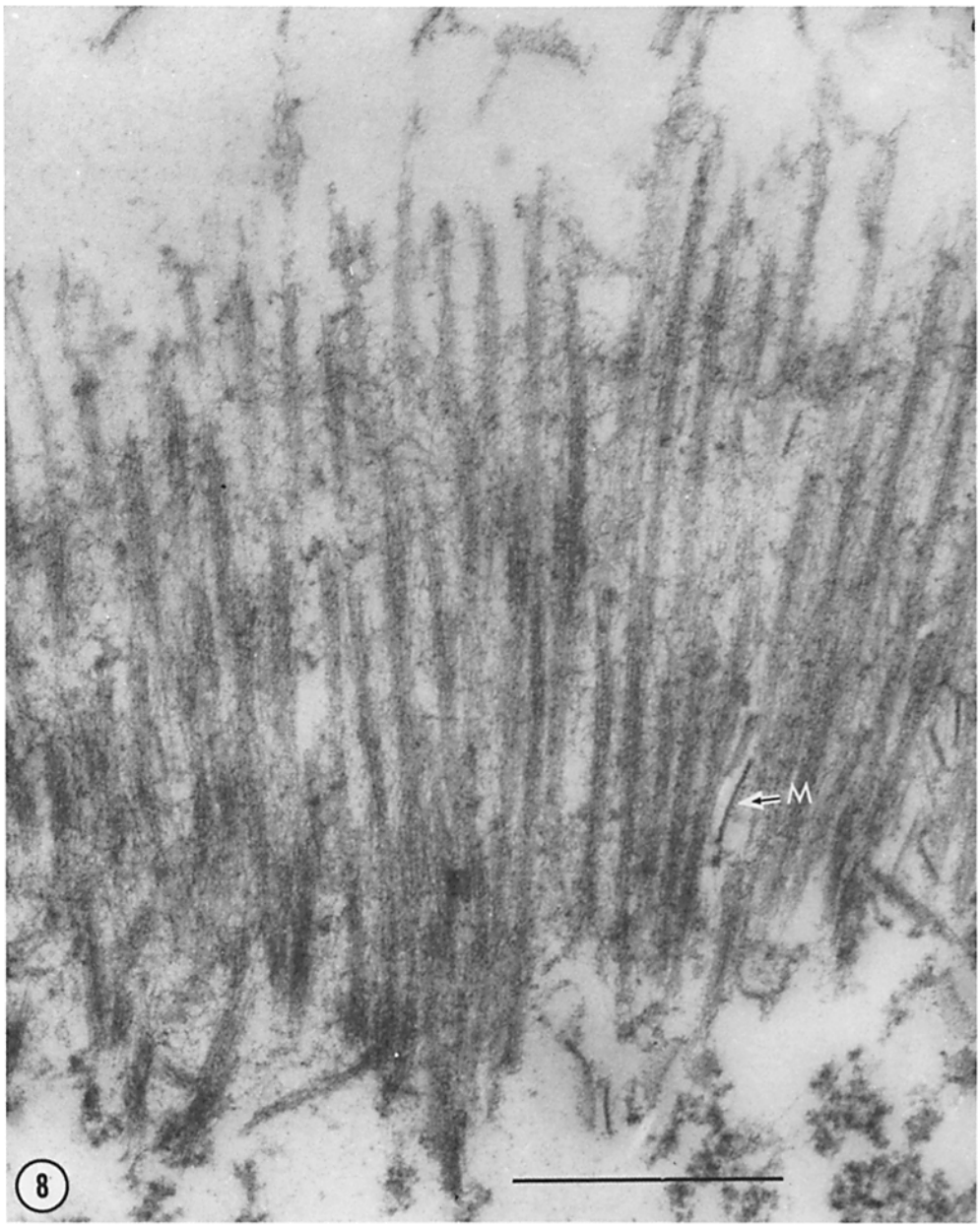

FIGURE 8 Electron micrograph of a contracted brush border. As in Fig. 7, fragments of microvillar membrane (*M*) appear to have been carried into the terminal web as a result of contraction. The basal ends of the microvillar filament cores are more tightly associated at their basal ends than along the rest of their lengths. As is usually the case, the dense tip material no longer remains attached to the apical ends of the microvillar filament bundles. Scale is 0.5 μm. × 72,000.

tions determined from a large number of experiments, which are favorable for maintaining "healthy" brush border morphology, at both the light and the electron microscope level.

CALCIUM: Using calcium buffers, I determined that the minimum concentration of calcium ion required to elicit contraction is on the order of 10^{-6} M. At this threshold concentration, most of the brush borders in the preparations tested were contracted.

NUCLEOTIDE: The nucleotide requirements for contraction were established using brush border preparations depleted of endogenous ATP by pre-incubation with hexokinase and glucose, since we observed that freshly isolated preparations of brush borders often contracted in the presence of calcium alone. ATP-depleted brush borders depend on added ATP, ITP or GTP, although fewer brush borders per preparation contracted with ITP or GTP than with ATP. ADP was ineffective.

MAGNESIUM: I did not attempt to determine whether magnesium was required for contraction since Triton-treated brush borders are unstable in the absence of this ion. It is interesting to note, however, that magnesium concentrations greater than about 15 mM inhibit microvillar contraction. This concentration of magnesium causes the microvillar actin filament bundles to form paracrystals *in situ* (16).

pH: Contraction occurs in the pH range between 6 and 8. Demembranated brush borders are not stable beyond the extremes of this range.

TEMPERATURE: Microvillar contraction does not occur within 30 s at 0°C, but I have observed that contraction does occur in brush border preparations stored on ice for longer time periods (1–2 h).

TRITON CONCENTRATION: Disruption of the brush border membrane with detergent is required for calcium and ATP to elicit contraction. The minimum effective concentration of Triton X-100 is about 0.01%.

Contraction solution added to brush borders exposed to more dilute concentrations of Triton (0.0001–0.005%) has a very interesting effect. In some cases, I have observed "reactivation" of microvillar motility. This reactivation consists of rapid vibration of microvilli without detectable microvillar shortening. This movement is arrested upon addition of EGTA at a concentration (5 mM) sufficient to reduce the calcium ion concentration below that needed for microvillar contraction. I have not yet determined, however, whether this motility is active movement or simply induced Brownian movement (e.g., the microvilli may become flaccid in contraction solution, and as a result, more likely to exhibit Brownian movements).

Contractile Proteins of the Isolated Brush Border

Some time ago, we demonstrated that the major protein of the brush border is actin (32). There is now evidence that the brush border contains other contractile proteins including myosin, tropomyosin, and α-actinin.

Preparation Purity

One problem with characterizing the contractile proteins of the brush border is the possibility that the brush border preparations used are contaminated with smooth muscle. The conventional technique for isolating brush borders (9) is particularly open to this criticism. In this procedure, brush borders are isolated from intestinal mucosa obtained by scraping the luminal surface of the small intestine with a glass slide. The procedures used in this study greatly reduce the possibility of smooth muscle contamination since brush borders are prepared from isolated cells rather than intestinal scrapings. Preparations of excellent purity (Fig. 9) can be obtained by this method.

Rough estimates of brush border preparation yield in terms of wet pellet volume per intestine are as follows: chicken, 0.5 ml; rabbit, 0.2 ml; rat, 0.01 ml; pig, 20 ml/20 feet of intestine. Brush borders isolated from chicken were used for most of the experiments reported here in order to parallel the cytological studies.

SDS Gel Electrophoresis

5% SDS gels comparing the proteins of membrane-intact and demembranated brush borders isolated from chicken with proteins of chicken skeletal, cardiac, and gizzard muscle are shown in

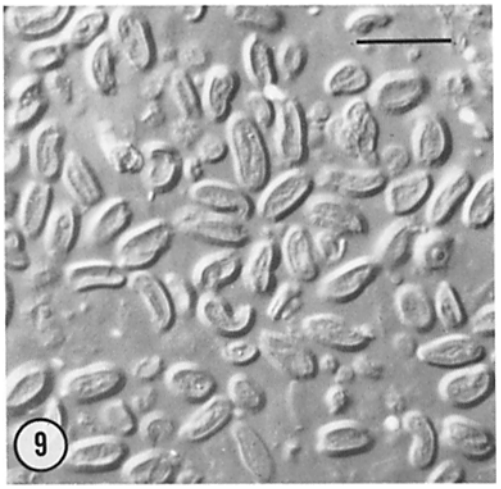

FIGURE 9 Low magnification light micrograph of a brush border preparation isolated from rat intestine. Nomarski differential interference optics. Scale is 20 μm. × 500.

Fig. 10. This comparison suggests a similarity in the proteins of the brush border to those of muscle.

Although the membrane-intact and Triton-treated brush border gels have the same prominent bands, there is much less background staining and fewer minor bands on the gel of demembranated brush border proteins. The brush border gels contain a number of prominent bands with the same electrophoretic mobility as muscle proteins. The most prominent band is a 43,000-dalton protein which we have previously identified as actin (32). Another prominent band co-electrophoreses with the 200,000-dalton subunit of myosin. The 95,000-dalton band co-electrophoreses with α-actinin isolated from pig skeletal muscle. In addition, we have shown that antibody to α-actinin reacts *in situ* with the brush border, and also forms a single precipitant band on agar diffusion plates when tested against solubilized preparations of isolated brush borders (reference 25; Schollmeyer, unpublished observation).

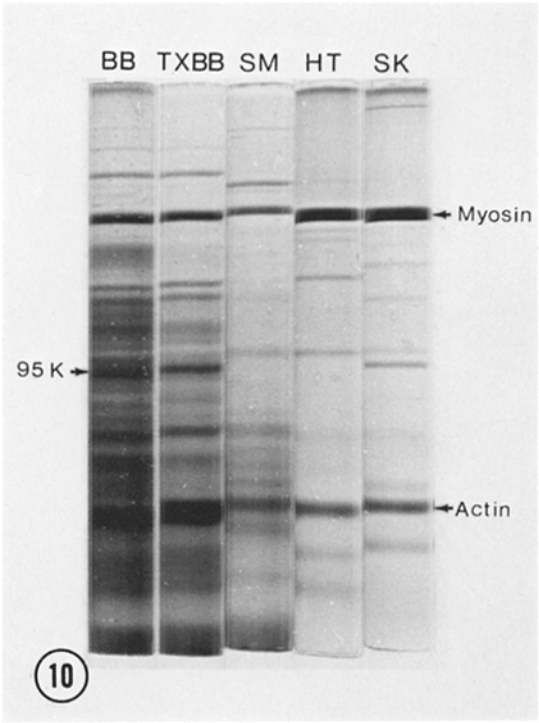

FIGURE 10 Comparison of brush border and muscle proteins by SDS-gel electrophoresis. 5% gels of isolated brush borders (*BB*) and Triton-treated brush borders (*TXBB*) are compared with gels of Triton-treated homogenates of muscle tissue from chicken gizzard (*SM*), heart (*HT*), and breast (*SK*). The brush border gels have prominent bands which co-migrate with actin (43,000 daltons) and myosin (200,000 daltons). The 95,000-dalton band on the brush border gels co-electrophoreses with purified α-actinin.

Other protein bands on the Triton-treated brush border gel, for which there is no identification, include the following: 280,000, 150,000, 140,000, and 70,000 daltons. A 70,000-dalton band is also present on all the muscle gels.

A comparison of the same protein samples run on 10% SDS gels (minus the membrane-intact brush border) is shown in Fig. 11. There are numerous low molecular weight bands on the brush border gel. Prominent bands correspond to molecular weights of 30,000, 18,000, and 16,000 daltons. The 30,000-dalton band may contain "nonmuscle" tropomyosin; see results below.

SDS gels of intestinal brush borders isolated from other sources including rat, pig, rabbit, and even teleost fish all have protein band patterns similar to those reported here for chicken brush borders with respect to major protein constituents. All have prominent bands with the same electrophoretic mobility as actin, myosin heavy chain, and α-actinin (Mooseker, unpublished observations).

Potassium-EDTA ATPase Activity

This ATPase assay is diagnostic for myosin-like ATPases isolated from vertebrate sources. Preparations of isolated demembranated brush borders exhibit potassium-EDTA ATPase activity of 0.02 μmol/mg-min at 22°C.

Brush Border Tropomyosin

The brush border probably contains a tropomyosin-like protein similar in molecular weight (30,000 daltons) to tropomyosins isolated from other nonmuscle sources including platelets (5), brain (7, 8), pancreas, and fibroblasts (7).

I attempted to isolate tropomyosin from preparations of brush borders isolated from pig intestines using the procedures developed for isolation of tropomyosin from nonmuscle sources (5, 8). These procedures are highly selective for tropomyosin-like proteins. This resulted in an enrichment of a 30,000-dalton protein. This presumptive brush border tropomyosin, like other "nonmuscle" tropomyosins, has a faster electrophoretic mobility on SDS gels than skeletal muscle tropomyosin (Fig. 12).

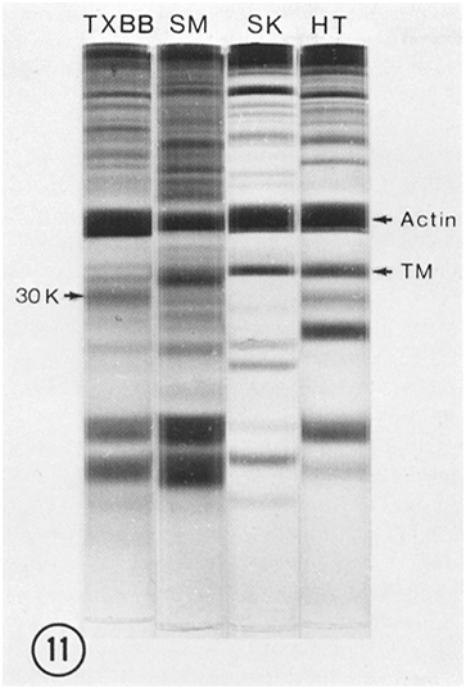

FIGURE 11 10% SDS gels of brush border and muscle proteins (the same protein samples as in Fig. 10). The Triton-treated brush border gel (*TXBB*) has three prominent low molecular weight bands below actin (30,000, 18,000, and 16,000 daltons). The 30,000-dalton band on the brush border gel (30 K) may contain a tropomyosin-like protein which has an electrophoretic mobility faster than that of the tropomyosin (*TM*) in muscle. See Fig. 12.

DISCUSSION

I have presented observations which establish that the isolated brush border is a motile apparatus activated by calcium and ATP. Preliminary characterization of brush border contractile proteins suggests that the contraction of microvilli in vitro is an actomyosin-mediated phenomenon. The conclusion drawn from these results is that the brush border is a motile apparatus that functions in vivo to generate microvillar movements, and that this motility, like the in vitro contraction reported here, is actomyosin mediated and calcium regulated.

The conclusion that the brush border is a motile structure is also supported by the recent results of Rodewald et al. (23). They have observed a form of motility in brush borders isolated from intestines of neonatal rat that is different from the microvillar contraction I have described. The contraction they have observed is induced by addition of ATP and magnesium *or* calcium, and is characterized by a pinching in of the plasma membrane at the zonula adherens. As a result, the brush borders round up into tight balls. The contraction is restricted to the terminal web region and movements or shortening of microvilli do not occur. Rodewald et al. suggest that the contraction is mediated by myosin interactions with actin filaments attached to the plasma membrane at the zonula adherens.

Many of the differences in the morphology of contraction in the two experimental systems may be explained by the fact that the brush borders in the preparations of Rodewald et al. were not treated with detergent before addition of contraction solution. For example, movements or shortening of microvilli may have been prevented by the extensive attachments of the core filaments

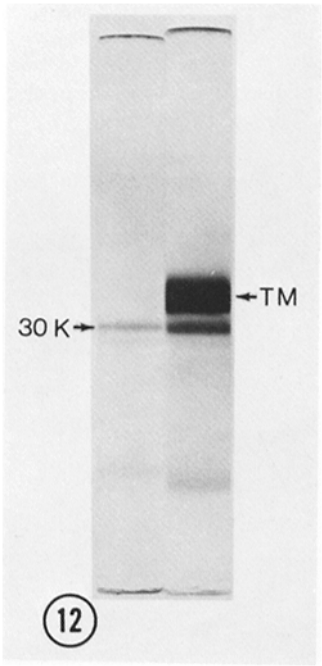

FIGURE 12 Comparison of a tropomyosin preparation from pig brush borders with muscle tropomyosin by SDS-gel electrophoresis (7.5% gels). The brush border tropomyosin preparation (left gel) contains a single prominent band at 30,000 daltons and two minor bands. In the right gel, the same preparation is co-electrophoresed with tropomyosin (*TM*) isolated from chicken skeletal muscle.

to the plasma membrane. The main inconsistency in the two sets of observations is that the results of Rodewald et al. suggest that brush border motility is not mediated by calcium. The reasons for this difference in calcium sensitivity remain unexplained. One exciting possibility is that brush borders isolated from neonatal rats have not yet developed a calcium regulatory system. Differences in experimental procedure (e.g., species of animals used, brush border isolation methods employed, ionic contents of solutions used to elicit contraction) may also account for the lack of calcium sensitivity in the preparations of Rodewald et al. I should point out that the contraction solution I have used to induce microvillar contraction in Triton-treated brush borders does not produce terminal web contractions in membrane-intact brush borders isolated from chicken intestine.

A Model for the Organization of Contractile Proteins in the Brush Border

The dynamics of the microvillar contraction reported here are consistent with our recent model for the functional organization of contractile proteins in the brush border (Fig. 13). The main experimental basis for this model is the determination that all the actin filaments in a microvillus have the same polarity (16). The polarity of these filaments is such that an interaction with myosin would result in movement of the actin filaments in only one direction, toward the terminal web. This, of course, is exactly the phenomenon observed in the in vitro contraction of isolated, Triton-treated brush borders.

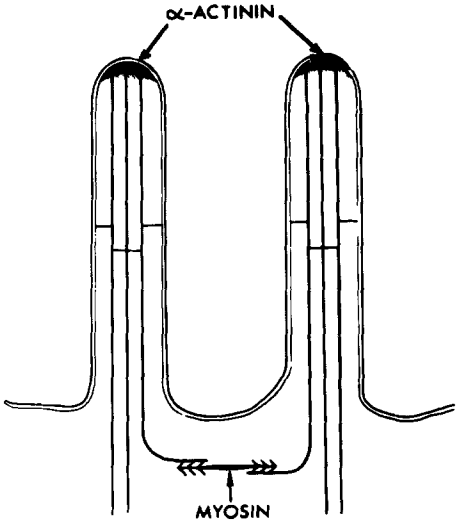

FIGURE 13 A model for the functional organization of actin and myosin in the brush border (from Mooseker and Tilney [16]).

The lateral splaying of peripheral microvillar actin filaments as a result of contraction is also consistent with the model we presented. In the model, bi-polar myosin "units" are localized in the terminal web and function by interacting with adjacent microvillar filament bundles. (There is morphological evidence for the presence of such filaments in the terminal web [16]). If in vitro microvillar contraction is caused by myosin "units" pulling on peripheral filaments of adjacent microvillar bundles, then one might expect the observed shredding of the filament bundles to occur.

Another aspect of our model is that displacement of a microvillar actin filament core is effected by myosin interactions with only a limited number of filaments in the core. This is presumably facilitated by the fact that actin filaments in the microvillus are linked to other filaments within the bundle by cross bridges along their lengths (16, 17). Experimental evidence for this kind of actin bundle-myosin interaction is provided by the nature of microvillar contraction in vitro since the filament bundles contract as units into the terminal web. This observation is also of general significance since actin filament bundles are found in a wide variety of nonmuscle motile systems. Examples include cytokinesis, (21, 26), cytoplasmic streaming in *Nitella,* (12, 19), amoeboid movement (29), motility of cultured fibroblasts (4, 10, 20), platelet contraction (1, 34, 35), and retinal cell elongation in teleost fish (3).

Recently, Rodewald et al. have proposed a similar model for the functional organization of actin and myosin in the brush border (23). The main difference between the two models is in the proposed site of myosin interaction with actin. Although their model includes myosin interactions between actin filaments of adjacent microvilli (as depicted in Fig. 13), they suggest that the main site of actomyosin interaction is between filaments of the microvilli and the actin filaments attached to the plasma membrane at the zonula adherens (23). Although the observations of Rodewald et al. (23) suggest that the terminal web filaments associated with the zonula adherens are somehow involved in microvillar movements in vivo, I question their relative importance because the model

of Rodewald et al. does not readily explain the dynamics of microvillar contraction in vitro. A key feature of their model is that the contractile system is anchored, in part, by the actin filaments attached to the plasma membrane at the zonula adherens. Since the attachment site of these filaments can be completely disrupted by detergent treatment, yet microvillar contraction can still occur, the filaments associated with the zonula adherens are probably not required for the basal movement of the microvillar filament cores in either microvillar contraction in vitro or motility in vivo. On the other hand, basal movements of microvillar actin filaments could be generated by the kinds of myosin interactions I have proposed (Fig. 13) as long as there is some restraint system to prevent the bipolar myosin units from "crawling up" the microvillar cores rather than pulling the cores down. Such a restraint system could be provided by structural elements of the terminal web. Either the filamentous meshwork of the terminal web is sufficient to trap the myosin in that region or the myosin could be anchored in some other way.

How Cyclic Movements of Microvilli are Generated In Vivo

Although the nature of in vitro contraction is consistent with our view of the organization of contractile proteins in the brush border, we are still left with the central question of how the observations reported here relate to the structural basis of microvillar movement in vivo. We are severely handicapped in this regard because the exact nature of that movement is not known. For this reason, the model which I have discussed is one of static organization, rather than a model for motility. Regardless of whether microvilli bend, bend and shorten, or simply vibrate like the prongs of a tuning fork, it is quite likely that the basal contraction of actin filaments toward the terminal web is one aspect of in vivo microvillar movement. However, we can establish from the available reports of *in situ* movement (24, 30) that dramatic changes in microvillar length do not occur. If these reports are accurate, a difference between microvillar contraction in vitro and motility in vivo may be that the magnitude of the basal contraction of actin filaments is much less in vivo. Another difference between microvillar contraction and *in situ* motility is that in vivo movements are cyclic. Therefore, one obvious interpretation of the in vitro contraction is that it represents the contractile phase of a contraction-relaxation cycle which has proceeded to an extreme degree.

The idea that microvillar motility consists of a contraction-relaxation cycle is, of course, an extrapolation from what is known about the motility of skeletal muscle. I have presented evidence which indicates that the basis for the contraction phase of the cycle is an actomyosin interaction. The mechanical basis for relaxation is open to speculation. However, as in muscle, relaxation is probably not actomyosin mediated since the microvillar actin filaments have the wrong polarity.

Regardless of the specific mechanism involved in the relaxation phase of motility, I postulate that there are at least three general requirements for relaxation. The first two are mechanical. There must be a restraint system to prevent the complete retraction of microvillar actin filaments into the terminal web during contraction. There must also be a mechanism to return basally displaced filaments to their original positions. These mechanical requirements for relaxation may be met by tensile elements in the terminal web and/or by the plasma membrane through its extensive attachments to the microvillar actin filament cores. The mechanical involvement of the plasma membrane would require that it have some degree of elasticity or rigidity. However, such mechanical properties, if not intrinsic to the lipid bilayer, might be supplied by the extensive extracellular glycocalyx associated with the brush border membrane (Fig. 5; reference 11).

A third requirement is suggested by the demonstration that calcium is required for the in vitro contraction of Triton-treated brush borders. That is, there may be a control mechanism to regulate the concentration of free calcium ion so that cyclic activation and relaxation of actomyosin complexes can occur. One exciting possibility is that the calcium binding and transport activity of the brush border membrane is somehow linked to the regulation of microvillar motility (see Simkiss [27] for a brief discussion of calcium transport in the brush border). There are a number of possible sites for the regulation of calcium ion concentration in the brush border. For example, the cytoplasmic surface of the brush border membrane may contain specific loci for the binding of calcium. This is suggested by the localization of calcium precipitates associated with the plasma membrane at the bases of microvilli and along the cytoplasmic surfaces of intercellular junctions (18). Alterna-

tively, the numerous vesicles that lie directly beneath the terminal web (Fig. 5) might also be involved in calcium regulation. If our model is accurate (Fig. 13) in the sense that motility is mediated by actomyosin interactions in the terminal web, then either of the above possibilities (the plasma membrane or the vesicles beneath the terminal web) would be ideal candidates for a calcium-sequestering system, given their close proximity to the terminal web.

I am making the assumption that calcium regulation does, in fact, work at the level of the interaction of myosin with actin. This has not yet been shown. If actomyosin isolated from the brush border is shown to be calcium sensitive, then the locus of regulation will also have to be determined; i.e., actin- or myosin-linked control (see Lehman and Szent-Györgyi, reference 13). The presence of tropomyosin in the brush border establishes the possibility for troponin-like (actin-linked) regulation, but certainly does not preclude the possibility of myosin-linked control. It is also possible that the brush border contains regulatory proteins unlike those found in muscle systems.

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