Reactivation of Intestinal Epithelial Cell Brush Border Motility: ATP-dependent Contraction via a Terminal Web Contractile Ring

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ABSTRACT Various models have been put forward suggesting ways in which brush borders from intestinal epithelial cells may be motile. Experiments documenting putative brush border motility have been performed on isolated brush borders and have generated models suggesting microvillar retraction or microvillar rootlet interactions. The reported Ca^{++} ATP-induced retraction of microvilli has been shown, instead, to be microvillar dissolution in response to Ca^{++} and not active brush border motility. I report here studies on the reactivation of motility in intact sheets of isolated intestinal epithelium. Whole epithelial sheets were glycerinated, which leaves the brush border and intercellular junctions intact, and then treated with ATP, PPi, ITP, ADP, GTP, or δS-ATP. Analysis by video enhanced differential interference-contrast microscopy and thin-section transmission electron microscopy reveals contractions in the terminal web region causing microvilli to be fanned apart in response to ATP and δS-ATP but not in response to ADP, PPi, ITP, or GTP. Electron microscopy reveals that the contractions occur at the level of the intermediate junction in a circumferential constriction which can pull cells completely apart. This constriction occurs in a location occupied by an actin-containing circumferential band of filaments, as demonstrated by S-1 binding, which completely encircles the terminal web at the level of the intermediate junction. Upon contraction, this band becomes denser and thicker. Since myosin, α-actinin and tropomyosin, in addition to actin, have been localized to this region of the terminal web, it is proposed that the intestinal epithelial cell can be motile via a circumferential terminal web contractile ring analogous to the contractile ring of dividing cells.

The idea that the intestinal epithelial cell brush border is contractile is an old one, and claims of visualizing such putative motility in vivo have been made (36, 37). While these claims remain unconfirmed, two key reports of in vitro brush border motility have been described (29, 35). Using isolated demembranated brush borders, Mooseker (29) reported that microvillar core filament bundles are induced to move into the terminal web in a Ca^{++}- and ATP-dependent manner. However, a reinvestigation of this report shows that microvillar core retraction does not occur but that the core of actin filaments is solated in response to free Ca^{++} (8). In another report of in vitro brush border motility, Rodewald et al. (35) reported that membranated brush borders can be induced to contract radially in the region of the terminal web in response to exogenous ATP, but in a Ca^{++}-independent manner.

The presence of an elaborate muscle protein-based cytoskeleton in the brush border argues strongly for some form of motility in this region of the cell. Numerous studies on the chemistry of the brush border have identified the major structural proteins within the fingerlike microvilli and the underlying meshwork termed the terminal web. Within each microvillus is a bundle of unidirectionally polarized actin filaments (3, 32) cross-linked by at least two proteins, villin (6, 10, 26, 30) and fimbrin (16). Each bundle of actin filaments is extensively associated with the plasma membrane by a 110,000-M_r polypeptide (25), likely associated with calmodulin (15, 21), at

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specific sites on actin filaments to electron-dense patches on the membrane (27). The core filament bundle extends into the terminal web region, where it is then termed a rootlet. It is likely that the rootlet has associated with it both tropomyosin (12, 14) and myosin (5, 12, 15, 17, 31).

The terminal web region is organized in a trilayered fashion with each layer composed of different arrangements and types of filaments (22) that correlate with the three levels of junctions present (i.e. tight, intermediate, and desmosome). One interesting feature of the intermediate junction region is the presence of a circumferential ring of thin filaments (22) that, as demonstrated by immunofluorescence or by use of fluorescent probes, is composed of actin, myosin, and α-actinin (5, 8, 11, 14).

Up till now, all attempts to reactivate motility in intestinal epithelial cells have been made using isolated brush borders as the starting material. The present study was undertaken with the premise that attempts to reactivate motility in brush borders should be guided by the fact that the brush border is not an isolated entity but is part of an epithelial cell which is incorporated into an epithelial sheet. Ideally, efforts should be directed to a preparation which preserves, as closely as possible, the in vivo architecture of the brush border, cell, and epithelium.

Therefore, this study focused on efforts to reactivate motility in large sheets of coherent intestinal epithelial cells. Epithelial cell sheets were permeabilized using glycerol and were found to respond to exogenous ATP but not to nonhydrolyzable ATP analogues by contracting radially at the level of the zonula adherens in a manner most consistent with contraction of an adherens level contractile ring. No microvillar retraction was observed.

MATERIALS AND METHODS

Epithelial Sheet Preparation

Sheets of chicken intestinal epithelial cells were collected as described earlier (25). After pelleting at 1,000 *g*, the sheets were incubated in 25% glycerol, 0.1 M KCl, 5 mM EGTA, 3 mM $MgCl_2$, 10 mM PO_4, pH 7.2, at 4°C for 2 to 5 h. Cells were washed twice by gentle centrifugation in 0.1 M KCl, 3 mM $MgCl_2$, 3 mM EGTA, 10 mM PO_4 or Imidazole, pH 6.9, before use. For demembranation, cells were incubated in 0.1% Triton X-100 in 75 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 10 mM Imidazole, pH 6.9, at room temperature for 30 min and then washed twice in the same solution minus the Triton X-100 before further use. The proteolytic enzyme inhibitor or phenylmethylsulfonyl fluoride (PMSF) in dimethyl sulfoxide (DMSO) was added to all preparations at a final concentration of 0.1 mM from a stock solution whereby 1 μl of PMSF in DMSO was added to each milliliter of cell suspension.

Reactivation Conditions

Epithelial sheets were resuspended for 10 min at room temperature in 0.1 M KCl, 3 mM EGTA, 3 mM $MgCl_2$, 10 mM PO_4 or Imidazole, pH 6.9 (solution 1), or in the same solution made 5 mM in ATP, δS-ATP, PPi, ITP, GTP, or ADP. Sheets of cells were also exposed to Solution 1 (Soln 1) containing $CaCl_2$ (with or without ATP, ITP, δS-ATP, PPi) at 0.8, 2.1, and 3.0 mM. The Ca^{++} concentration of the stock $CaCl_2$ solution had been determined by atomic absorption. The free Ca^{++} in Soln 1 was calculated using a program written by Dr. Peter Chantler (Brandeis University) which takes into consideration the temperature, pH, Mg^{++}, EGTA, and ATP which affect the level of free Ca^{++} in solution. At 0.8, 2.1, and 3 mM $CaCl_2$ in Soln 1 containing 5 mM ATP, the free Ca^{++} is 1.6×10^{-7}, 1.0×10^{-6}, and 1.0×10^{-5} M, respectively.

Electron Microscopy

Extracted sheets of cells were incubated for 30 min at room temperature in 1.0 mg/ml or 2.5 mg/ml muscle myosin subfragment-1 (S-1). All samples were fixed and processed for thin sectioning as described earlier (8). Silver sections were cut with a diamond knife on a Sorvall MT-5000 Ultramicrotome (DuPont Instruments–Sorvall Biomedical Div., DuPont Co., Newtown, CT), stained with lead citrate and uranyl acetate, and examined on a JEOL 100CX operated at 60 kV.

Video Enhance Microscopy

Epithelial sheets were mounted for light microscopy between two #0 cover slips in Soln 1, and various experimental solutions were then perfused. Video-enhanced differential interference contrast microscopy (1) was done with a Hamamatsu C-1000 camera and control unit coupled to a Panasonic WJ-810 Time Date Generator, a SONY VO-2610 videocassette recorder and a Panasonic Model WV5310 television monitor. The light microscope was a Leitz Diavert inverted microscope with Smith-T Interference Contrast optics. Images were recorded from the videomonitor with 35mm Plus-X film.

PAGE

Whole cells, glycerinated cells, a 10,000-*g* supernatant from glycerinated cells, and brush borders prepared as in Matsudaira and Burgess (25) were analyzed on microslab gels poured with a 5–20% gradient of acrylamide and a 3–6 M gradient of urea following the method described by Matsudaira and Burgess (24). The 10,000-*g* glycerol supernatant from permeabilized cells was dialyzed and concentrated ten fold against Soln 1 by vacuum dialysis (Micro-Pro Di Con model 315; Bio-Molecular Dynamics, Beaverton, OR) before electrophoresis. Protein was determined by the method of Bradford (4).

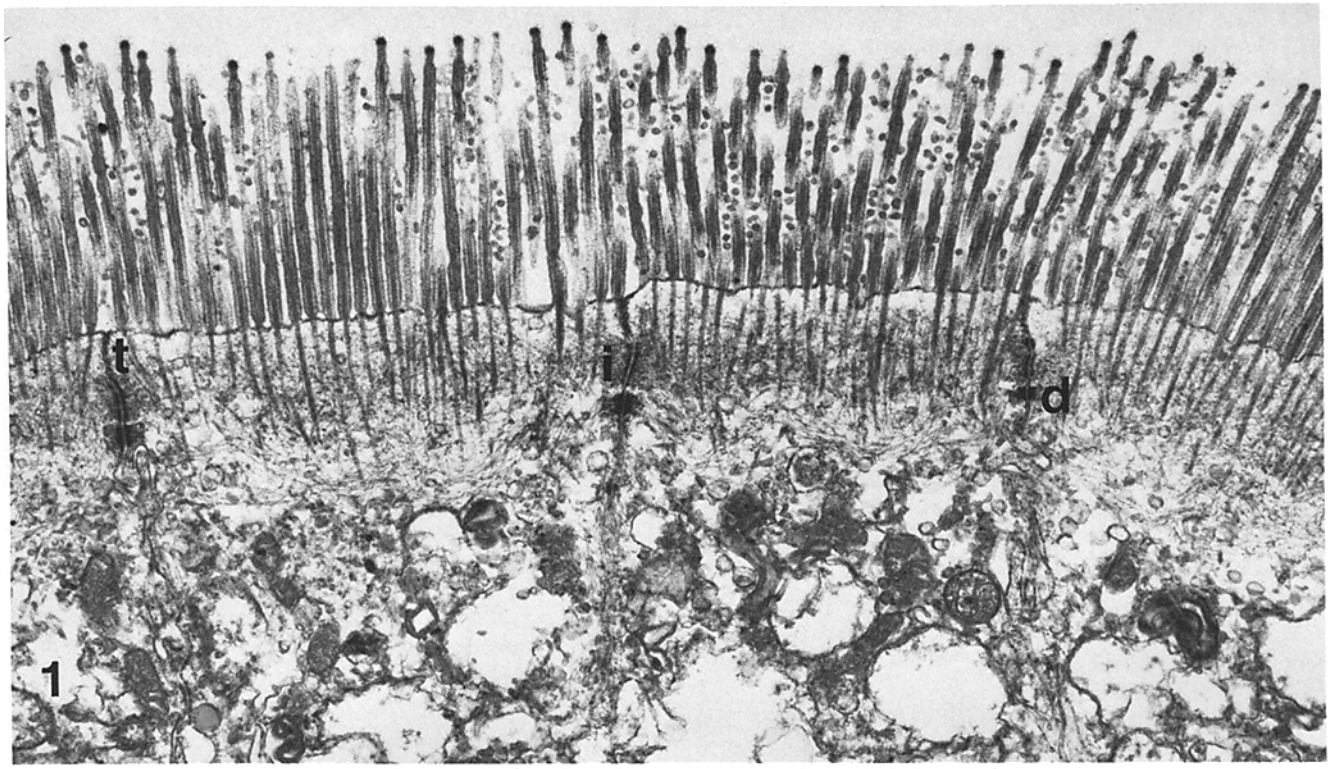
RESULTS

Structure of Glycerinated Epithelial Sheets

Sheets of epithelial cells remain remarkably intact after glycerination (Fig. 1). The most obvious change in cell ultrastructure is a general extraction of the cytoplasmic matrix; however, the nuclei, mitochondria, and basal and lateral membranes remain normal in appearance. The brush border, in-

FIGURE 1 Thin section of glycerinated control sheet of epithelium in Solution 1. The cytoplasmic matrix is significantly extracted, yet mitochondria remain fairly normal in appearance. The brush border region is quite normal in appearance. The microvillar cores with associated cross filaments and the microvillar membranes remain intact. The junctional complex appears normal with the tight (*t*), intermediate (*i*), and desmosome (*d*) junctions remaining intact. The terminal web region with all classes of filaments appears normal. × 15,200.

FIGURE 2 Thin section of a glycerinated control sheet cut tangentially across the brush border region to reveal the organization of the terminal web filaments. In the apical region (*A*), adjacent to the tight junction, the primary structures observed are rootlets cut in cross section. The intermediate region (between bars) can be subdivided into two zones, the apical (*Ia*) and the basal (*Ib*) zones. In the *Ia* zone are found rootlets and a band of thin filaments running parallel to the membrane around the cell. In the *Ib* zone are found rootlets, the band of membrane-associated thin filaments, and 100-Å filaments associated with the rootlets. The basal zone (*B*) of the terminal web, at the level of the desmosome, is a zone where few rootlets penetrate and is dominated by 100-Å tonofilaments (below dashed line). × 21,200. (*Inset*) Cross section of the *Ib* region of the terminal web showing the band of thin and thick filaments associated with the intermediate junction region. × 35,000.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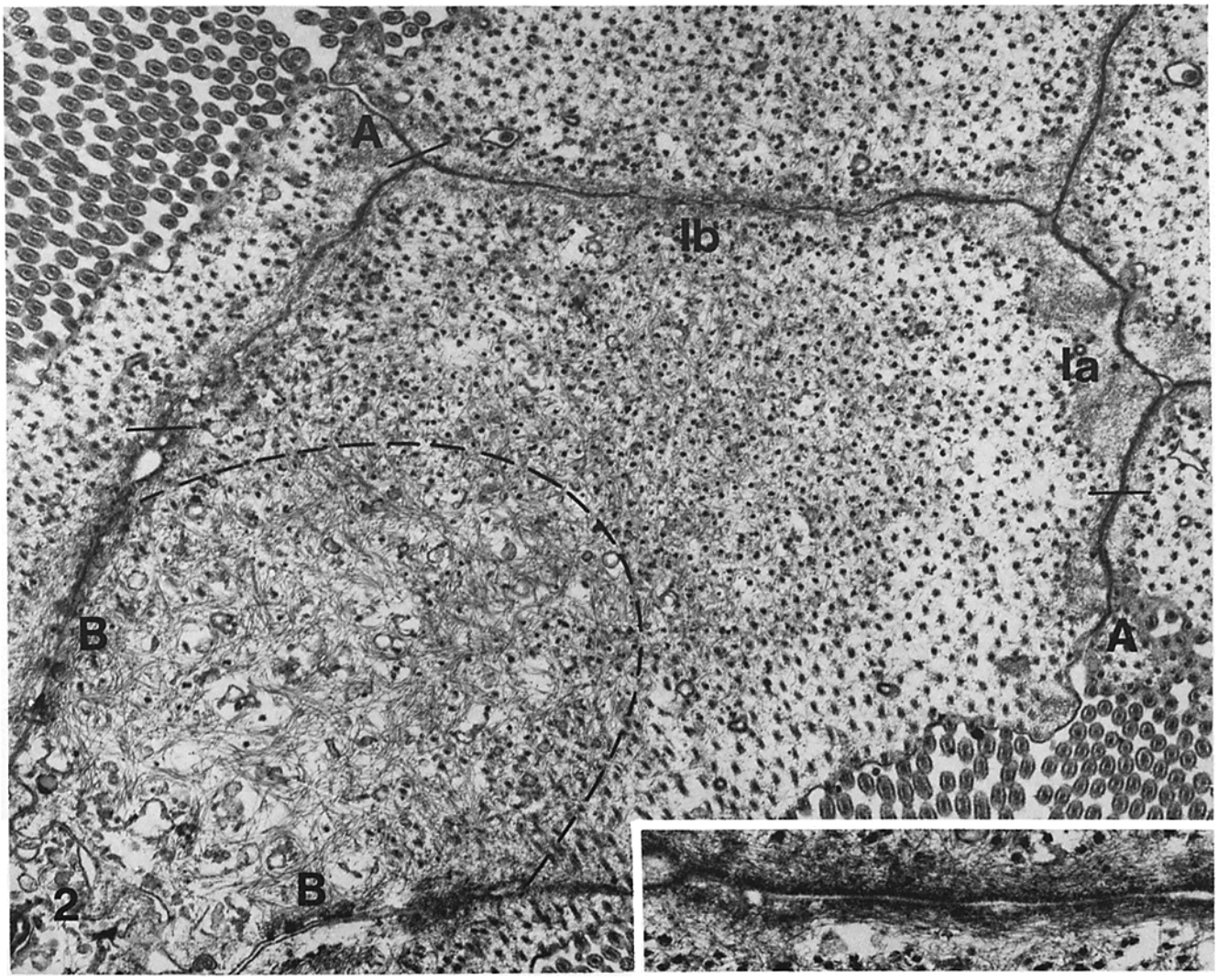
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2

cluding all classes of filaments and junctional complexes, retains its normal ultrastructural organization (Figs. 1 and 2). Occasionally, a desmosome or an intermediate junction appears slightly split apart. Brush border microvilli of adjacent cells within a sheet of cells remain erect and perpendicular to the cell surface. The last cell on the edge of a sheet is characteristically curved in at the level of the junctions on those sides of the cell unattached to any other cell.

In addition to sheets of epithelial cells, the preparation also contains single cells and some clusters composed of only two or three cells. The brush border region of most single cells and of those in small clusters tends to appear radially constricted in the region of the adherens junction; the brush border in these cases assumes the shape of an open fan.

Glycerination of the epithelium does not markedly change the protein composition of the cell sheets as revealed by PAGE (Fig. 3). Approximately 30% of total cellular protein is released into a 10,000-*g* glycerol supernatant. In a typical experiment, when 88.6 mg of cells were extracted in 50 ml of glycerol solution, 26.6 mg of protein was released into the 10,000-*g* supernatant. The polypeptides solubilized by glycerol represent a broad spectrum; however, the released polypeptides are not a mere representation of whole cell or brush border specific polypeptides (Fig. 3).

Cross sections through the terminal web region at the level of the zonula adherens reveal a circumferential band of 8–10 nm in diameter thin filaments running parallel to and closely associated with the membrane (Fig. 2). These filaments form a distinct 200–300 nm wide band which excludes organelles from the area near the membrane. This band of filaments is distinct from the circumferential band of thick tonofilaments at the level of the desmosomes. Exposure of extracted epithelial cells to 1 mg/ml myosin S-1 shows that many filaments within the brush border region become decorated in a periodic fashion (Fig. 4). While higher levels of S-1 demonstrate more dramatic arrowhead decoration of microvillar cores and rootlets, they are highly disruptive of brush border integrity, making recognition of the adherens region problematical (also see reference 3). Lower concentrations of S-1 decorate, but do not splay apart, core filaments and preserve the overall integrity of the brush border including the adherens filaments. After S-1 treatment the adherens zone filaments appear much denser and many filaments appear fuzzy and thicker. The circumferential band itself becomes much broader after S-1 treatment (Fig. 4*c*). Decoration of thin filaments within the adherens circumferential ring is apparent while other filaments appear undecorated or incompletely decorated (Fig. 4).

Video Microscopy and Light Microscopy

Entire sheets of glycerinated epithelial cells are easily observed with video-enhanced differential interference-contrast microscopy. The epithelium from entire villi is released and optical sections of these epithelial sheets show that the microvillar region and the terminal web are easily discerned, thus making definitions between adjacent cells readily apparent. Before perfusion with any reactivating solution, it is clear that brush border microvilli from adjacent cells are perpendicular to the cell surface along their entire lengths (Fig. 5*a*).

Sheets of cells respond in a dramatic fashion to a perfused ATP solution, with or without added Ca^{++} (3 mM EGTA/3 mM $CaCl_2$ or 3 mM EGTA in Soln 1, respectively) in ~75% of the samples over a 20-min observation period. The response typically starts between 3 and 7 min after ATP perfusion and consists of cells within the sheet constricting at the level of the terminal web (Fig. 5). This constriction separates adjacent cells at the terminal bars, reduces the diameter of the apical portion of each cell up to about one-half of its unconstricted diameter, and causes the microvilli to spread apart appearing rather like an open fan. Control Soln 1 alone, PPi, ITP, ADP, δS-ATP, or Soln 1 + Ca^{++} causes no discernible responses in the brush border region at the light microscopic level in a 20-min observation period.

Electron Microscopy of Reactivated Epithelia

The response of glycerinated epithelial sheets to Soln 1 + 5 mM ATP (0 Ca^{++}) is identical to that of epithelia treated with ATP solutions containing varying levels of free Ca^{++} (10^{-5}, 10^{-6}, and 10^{-7} free Ca^{++} in Soln 1). The only major difference noted is that in $> 10^{-6}$M Ca^{++} the microvilli tend to break down. Cells within a sheet are invariably constricted at the zonula adherens region. The degree of constriction ranges from (*a*) slight, where cells are pulled apart only at the zonula adherens (Fig. 6*a*), to (*b*) moderate, where the entire junctional complex region is completely pulled apart in each adjacent cell (Fig. 6*b*), and to (*c*) severe, where constriction at the junctional

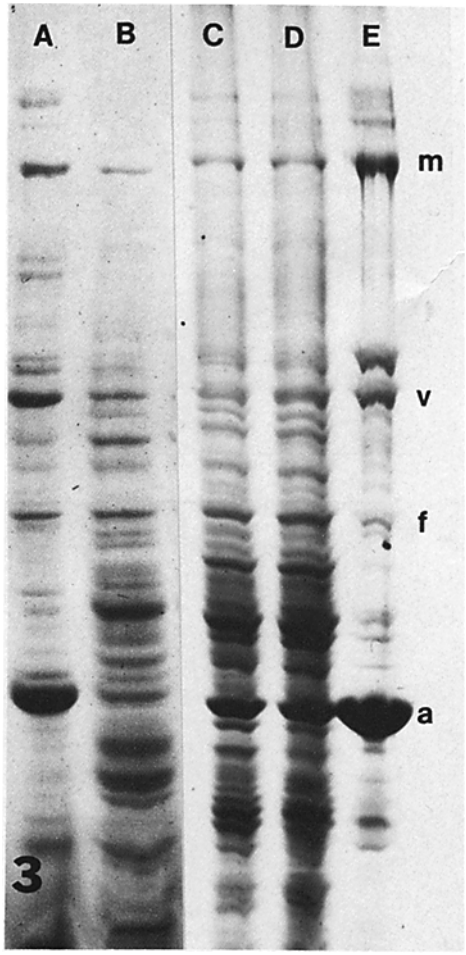


FIGURE 3 Polyacrylamide microslab gel electrophoresis of (*A* and *E*) demembranated brush borders, (*B*) 20 μg of tenfold concentrated glycerol supernatant, (*C*) glycerinated epithelial cells, and (*D*) whole epithelial cells. Near identity is noted between the polypeptides of whole cells and those of glycerinated cells. *m*, Myosin. *v*, Villin. *f*, Fimbrin. *a*, actin.

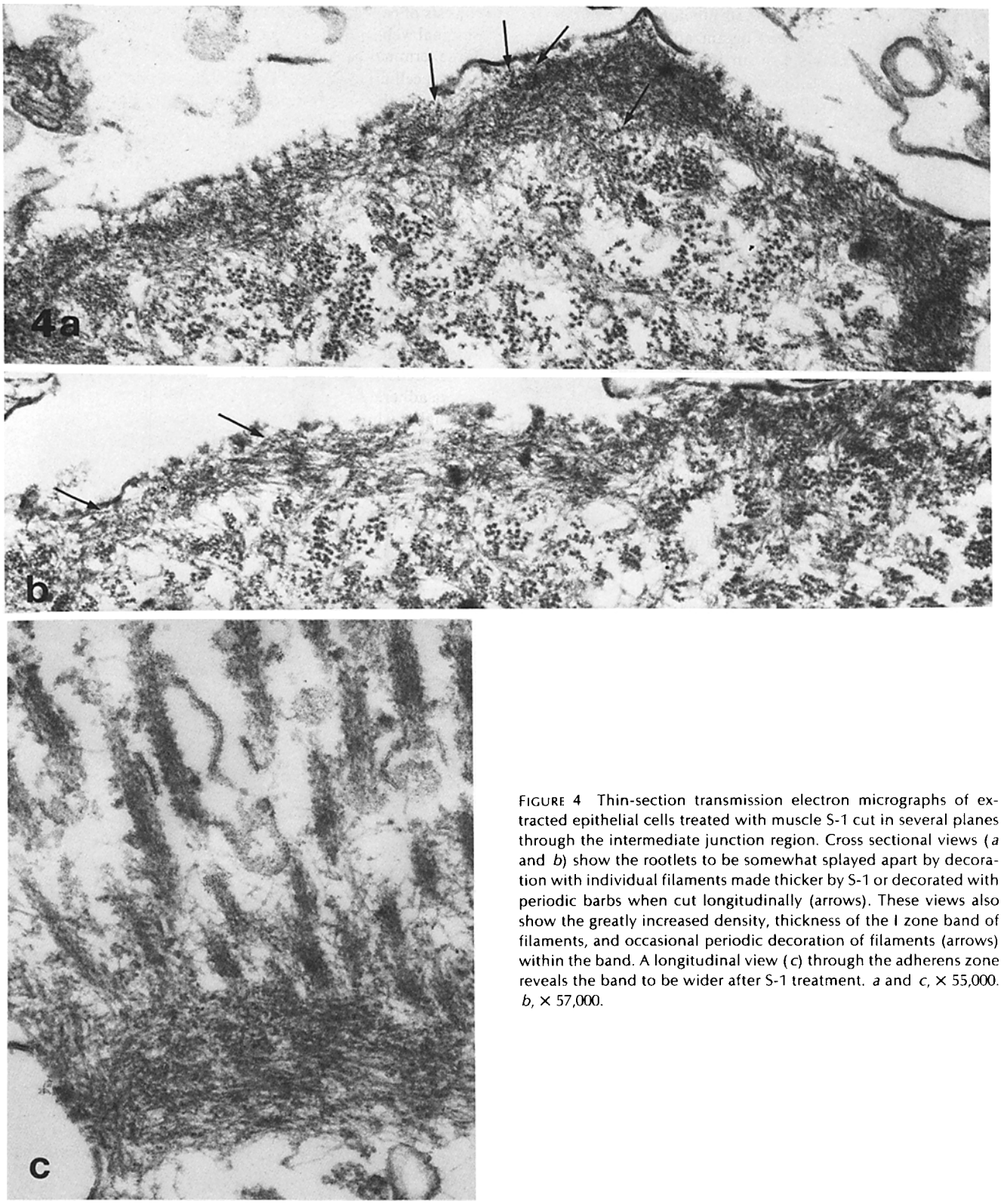


FIGURE 4 Thin-section transmission electron micrographs of extracted epithelial cells treated with muscle S-1 cut in several planes through the intermediate junction region. Cross sectional views (*a* and *b*) show the rootlets to be somewhat splayed apart by decoration with individual filaments made thicker by S-1 or decorated with periodic barbs when cut longitudinally (arrows). These views also show the greatly increased density, thickness of the I zone band of filaments, and occasional periodic decoration of filaments (arrows) within the band. A longitudinal view (*c*) through the adherens zone reveals the band to be wider after S-1 treatment. *a* and *c*, × 55,000. *b*, × 57,000.

complex region is so complete as to leave the microvilli fanned-out and the cell's opposing junctions brought close together (Fig. 6*c*). There is no correlation noted between degree of constriction and level of free Ca^{++}. Treatment of epithelia with δS-ATP also induces constriction at the adherens region, and this apical constriction is slight to moderate as described in the terms above (Fig. 7).

Cross sections of ATP-treated epithelia through the level of the zonula adherens reveal that the circumferential band of thin filaments appears wider and denser than in controls (Fig. 8). Cross sections through this plane reveal that adjacent cells pull themselves apart from one another in a radial fashion and clearly demonstrate a circumferential constriction at the level of the adherens junction (Fig. 8). Microvillar rootlet filaments

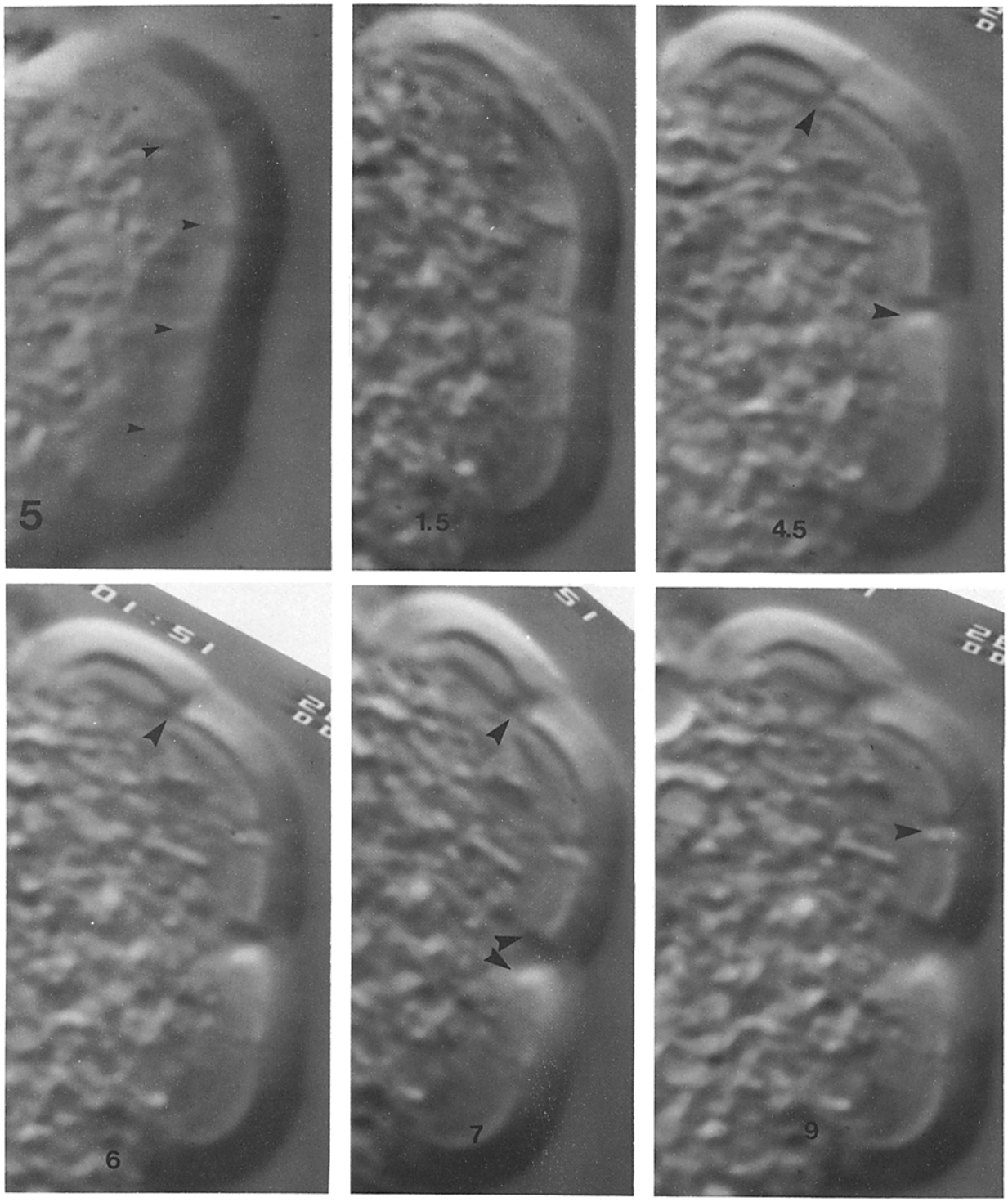


FIGURE 5 Time sequence of video-enhanced differential interference contrast images of an epithelial sheet and its response to perfusion of 5 mM ATP in solution 1. In *a*, sheet is viewed just after initiation of perfusion of ATP. Boundaries between adjacent cells are evident as thin lines (small arrowheads). Sequence *b–f* demonstrates contractions in the terminal web region (arrows) that cause the brush borders to become fanned out. Time after initiation of perfusion is indicated in minutes. Bar, 2 μm. × 3,000.

remain well-bundled and show no tendency to splay apart in response to ATP. In fact, in constricted brush borders, only the microvillar rootlets adjacent to the junctions become tilted away from their normal perpendicular orientation. No microvillar retraction is noted.

The morphology of the tight junctions and desmosomes and their associated filaments undergoes no discernible changes in response to ATP. When the tight junctions pull apart they can separate cleanly or the whole junction can be pulled to one cell causing another cell to be ripped open. When desmosomes are separated by the constriction, they always split apart between cells whereby complete half-desmosomes are formed. No change in the tonofilaments associated with the desmosomes was noted.

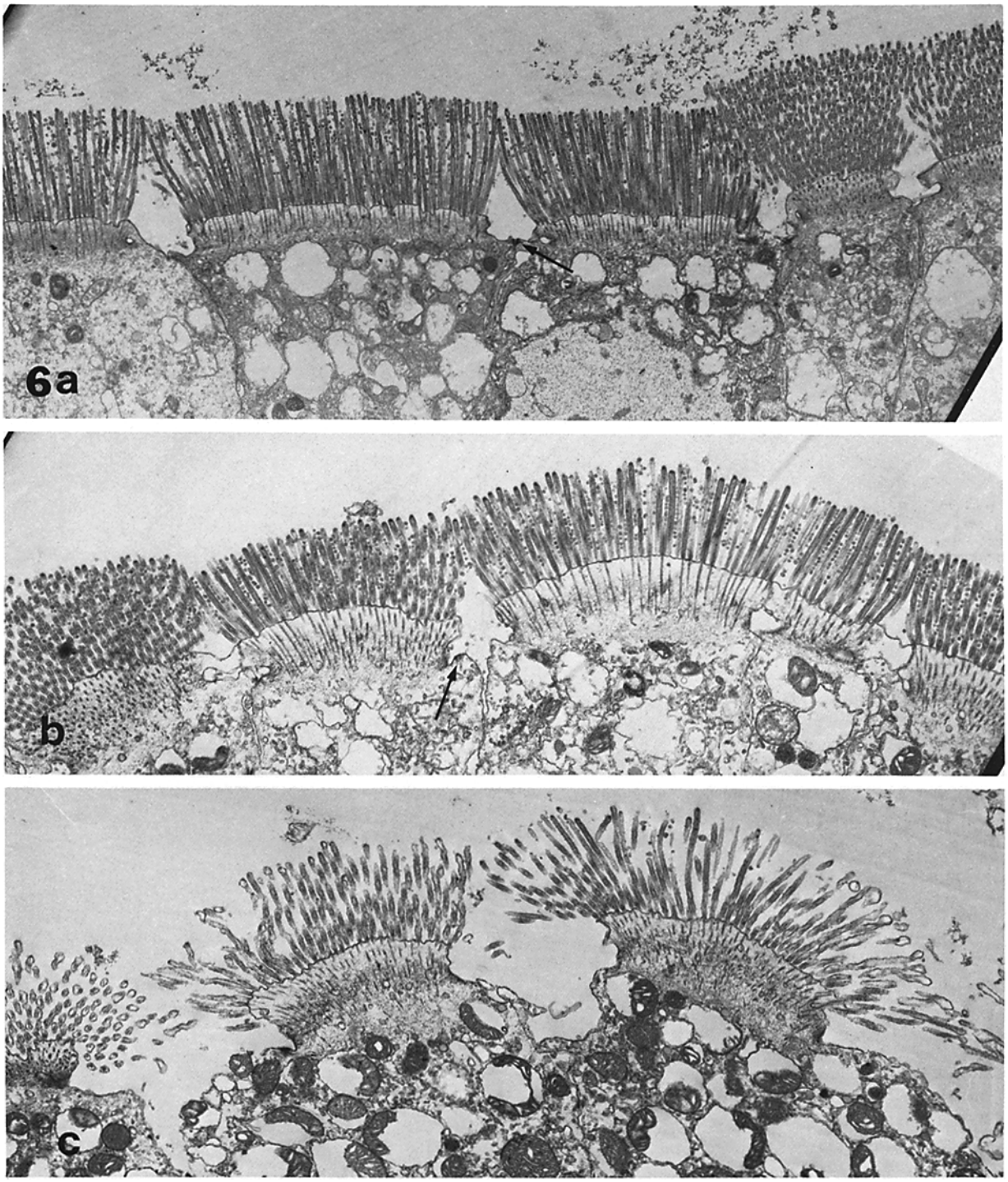


FIGURE 6 Thin-section transmission electron micrographs of representative epithelial sheets in response to 5 mM ATP in Solution 1 (0 Ca^{++}). (*a*) An example of a sheet which has undergone slight contractions at the level of the adherens junction. The desmosomes are left intact (arrows) and microvilli are only slightly fanned apart. (*b*) An epithelial sheet which has undergone moderate contraction at the level of the adherens junction resulting in the spearation of the desmosomes (arrow). (*c*) Part of an epithelial sheet which has undergone severe contractions at the adherens zone resulting in major separation between adjacent cells and the microvilli being fanned apart. *a*, × 6,100; *b*, × 5,600; *c*, × 8,900.

Treatment of epithelia with ADP, GTP, ITP, PPi, or any Ca^{++} solution alone (10^{-5}, 10^{-6}, and 10^{-7}M free Ca^{++} in Soln 1) causes little or no change in intercellular contact between cells in an intact sheet. Constrictions in the zonula adherens region are not commonly observed (Fig. 9) except, as in controls, in single cells and in small clusters of cells where some curving in toward the adherens region was noted.

DISCUSSION

This report provides evidence for a unique form of motility of intestinal epithelial cell brush borders. Glycerinated sheets of epithelia, which remain surprisingly structurally intact, exhibit a form of motility in the brush border region that is most simply explained by a circumferential constriction, mediated

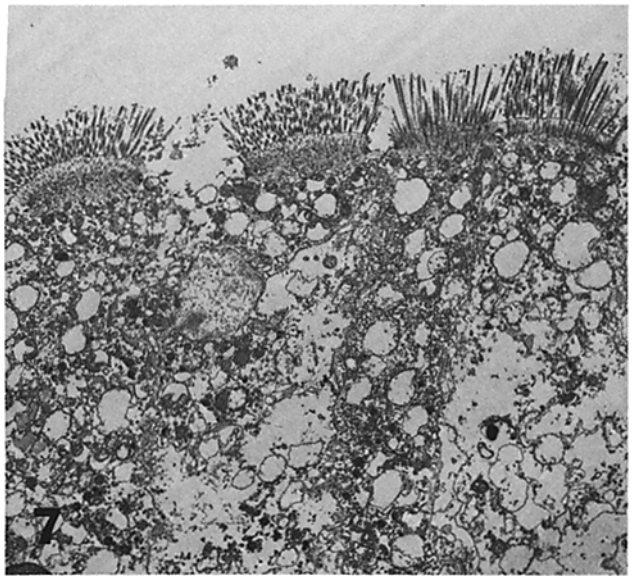


FIGURE 7 An epithelial sheet treated with 5 mM δS-ATP in Solution 1 demonstrating moderate contraction at the level of the adherens zone. × 3,500.

by a contractile ring of microfilaments, at the level of the zonula adherens. Slight constrictions in this region of a cell within an epithelium would tend to induce the microvilli to fan out. Rounds of constriction and relaxation in this region of the cell would facilitate nutrient absorption by gently stirring the luminal microenvironment. This model has the advantage over other models proposed (12, 29, 35) for brush border motility for several reasons: (*a*) it is a simple model; (*b*) it is consistent with all available structural and biochemical evidence; (*c*) it does not require any special features of rootlet or terminal web structure that have yet to be demonstrated; and (*d*) it could occur in the villus epithelium.

Results of the experiments in this study and results of others provide strong arguments in favor of a contractile ring hypothesis. This study and those of others (22) have noted a circumferential ring of thin filaments at the adherens region. This ring is composed, in part, of actin filaments as demonstrated in this report by S-1 decoration and as shown by NBD-phallicidin staining (8). Several other major contractile proteins which would be necessary components for a contractile ring have also been localized to this region. Immunofluorescence and/or immunoelectron microscopy have localized myosin, tropomyosin, and α-actinin at the adherens region of intestinal epithelial cells (5, 8, 11, 14). All of these contractile proteins have been localized in the contractile ring of dividing cells (2, 13). Therefore, ample evidence exists for the spatial relationship between an assembled contractile apparatus (with all necessary component proteins) and an active contractile ring.

The reactivation experiments reported in this study provide support for brush border motility via a contractile ring. As assayed by both video-enhanced light microscopy and electron microscopy, glycerinated sheets of cells are able to respond to added ATP by contractions at the level of the adherens junctions. The slight to moderate constrictions at the adherens zone observed by electron microscopy are probably unobservable by light microscopy because of the very limited tendency of microvilli to fan out in these cases. Since all epithelia treated with ATP are contracted at the adherens region, as assayed ultrastructurally, it is likely that only those epithelia which constrict severely at the adherens zone are apparent with light microscopy. This may explain why only ~75% of epithelia appear to contract at the light microscopic level. Also, the finding that epithelia are not contractile with ADP, GTP, ITP, or PPi supports the hypothesis of an energy-requiring contraction based on an actomyosin system since these reagents either are nonhydrolyzable or are not (or are very poor) substrates for myosin kinase. It is interesting that δS-ATP supports slight to moderate adherens constrictions, an observation perhaps consistent with the fact that this ATP analogue serves as a substrate for myosin light chain kinase (20).

One of the strongest correlative lines of evidence supporting a contractile ring in intestinal epithelial cells is the recent demonstration by Owaribe et al. (34) of a contracting ring, composed in part of actin filaments, in the adherens region of cultured sheets of pigmented retinal epithelial cells. They demonstrated, in glycerinated sheets of these epithelial cells, that the ring of filaments could be induced to constrict by addition of ATP and Mg^{++}, causing groups of cells to become cup-shaped. A similar finding has been reported by Burnside et al. (9) in that detergent-lysed models of teleost retinal epithelia were induced to contract by ATP in mimicking normal light-induced contractions. Also, a ring of adherens microfilaments has been described in the embryonic chick intestinal epithelium and has been implicated as a mediator of cell shape changes responsible for the early folding morphogenesis of this epithelium (7).

Other models for brush border motility suggest that microvilli might be forced to retract into the terminal web region, a contraction induced by splaying rootlet filaments interacting with terminal web myosin which in turn would interact with other splayed rootlet actin (29), terminal web actin (12), or with adherens zone actin filaments (35). All of these models envision the microvillus as one-half of a musclelike sarcomere with only the other half of the analogy differing. One difficulty with these models is the lack of a source for the required equal but opposite force which would pull (or push) the microvilli back out to their resting position. With the contractile ring hypothesis a cell or small group of cells within the villus epithelium could contract, fanning out microvilli in one domain and thus stirring the microenvironment. This contraction would be resisted by all other surrounding cells within the epithelium which are extensively held together by apical junctional complexes. The epithelial sheet itself would then provide the equal but opposite forces required to re-establish the resting place for contracted cells after apical constriction. If such constrictions were localized to a few cells at a time on a villus and if the contractions were slight, then the difficulty of documenting brush border motility in vivo would be explained.

A second difficulty with models suggesting retracting microvilli is the lack of clear demonstration of actin filament splaying from rootlets, and in fact there is recent work, with either conventionally fixed thin-section stereo or quick-freeze deep-etch electron microscopy, suggesting that splaying of rootlet filaments may not occur (18, 28) and that there are few horizontally arrayed actin filaments in the terminal web (19). In addition, no evidence of splaying of rootlets was observed in the present study. Another difficulty with a model for retracting microvilli is the recent finding that the in vitro demonstration of brush border motility via retraction of microvilli into the terminal web (29) is likely the result of microvillar core filament solation induced by the Ca^{++}-induced actin severing properties of villin (8). A final concern for a model

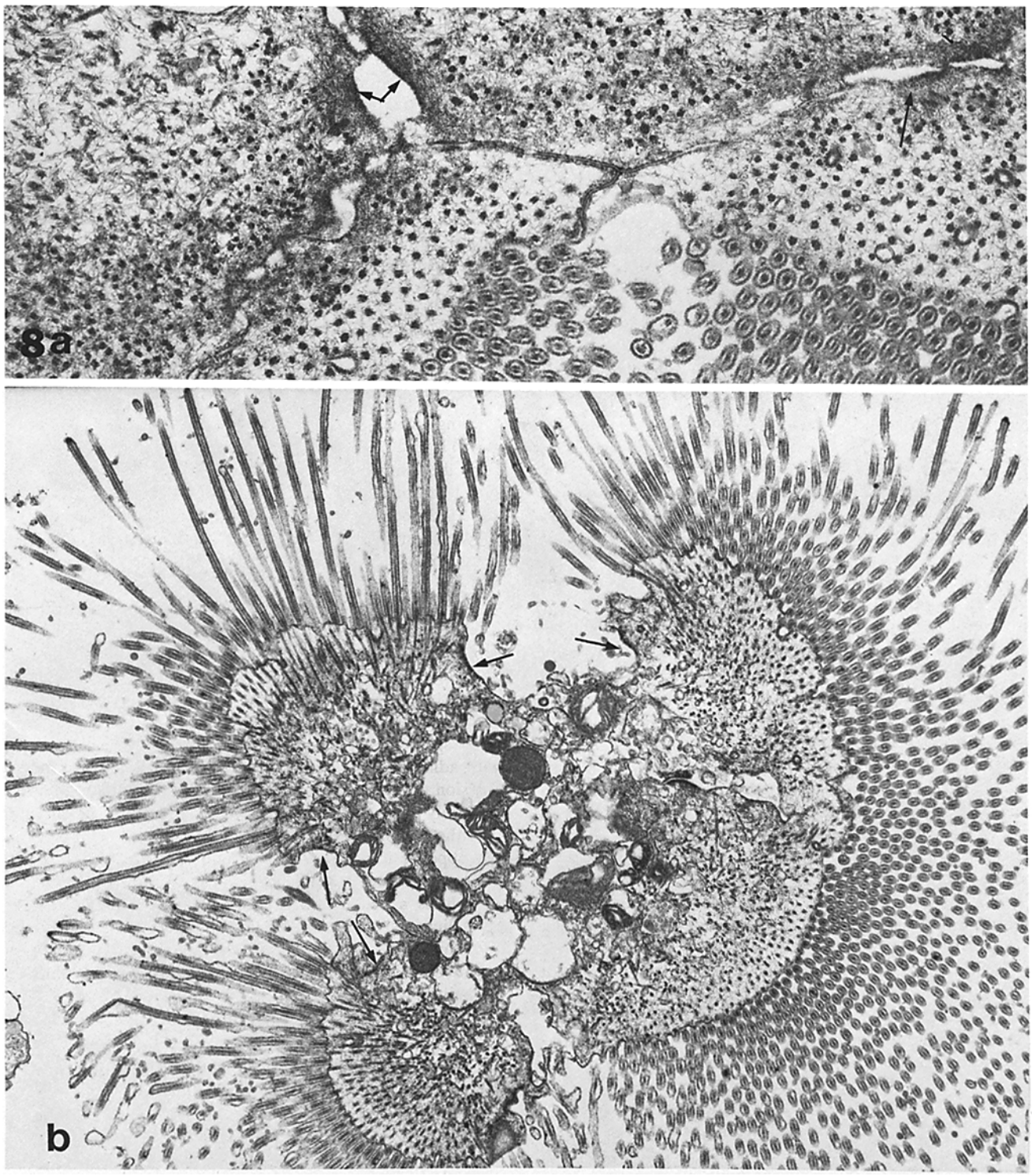
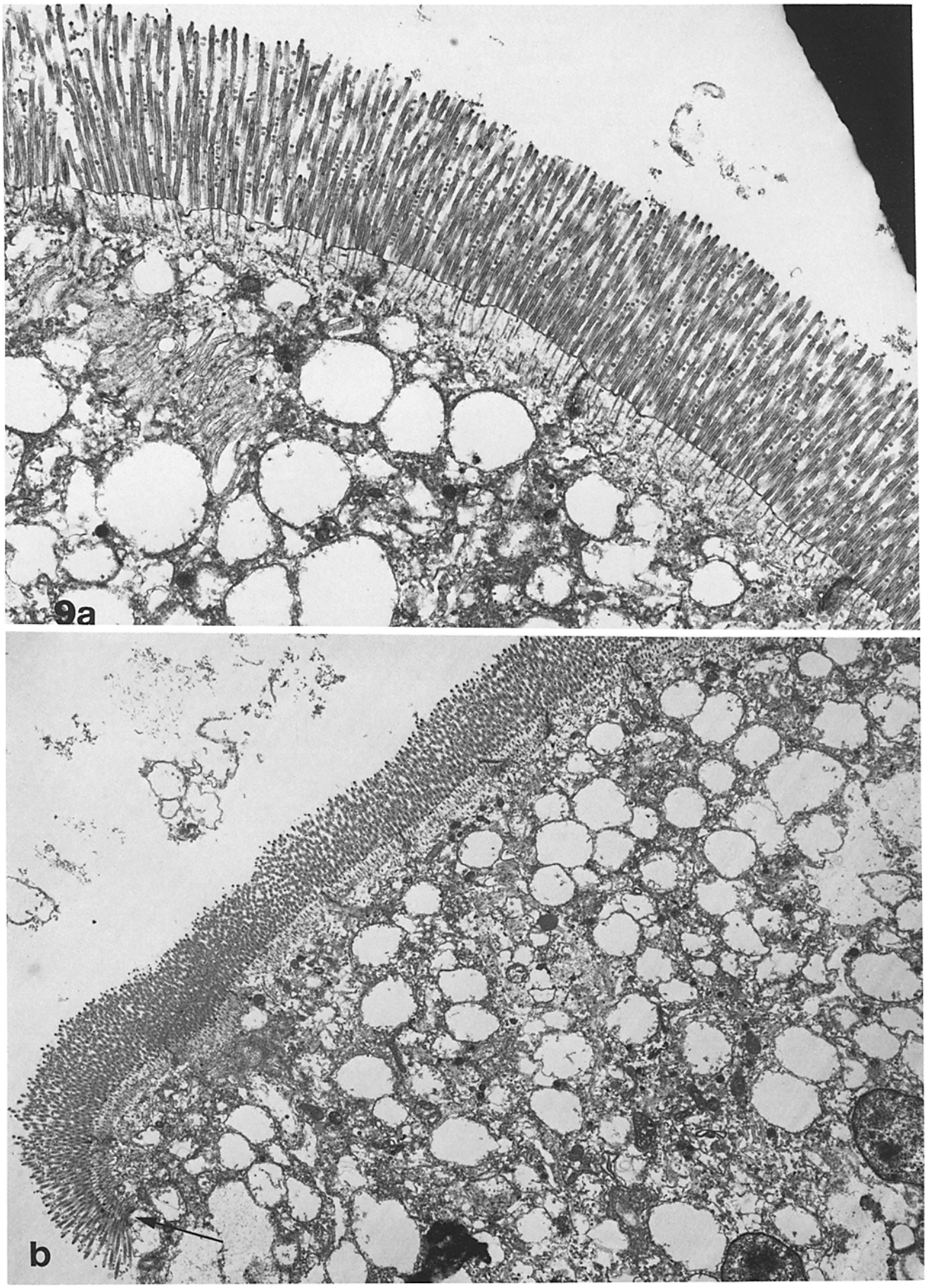


FIGURE 8 Cross sections through the terminal web region of epithelia treated with 5 mM ATP in Solution 1 (0 Ca^{++}). (*a*) An epithelial sheet which has undergone slight constriction in response to ATP shows the adherens zone band of filaments to be denser, wider, and thicker in appearance (arrows) than the band in control epithelial sheets. (*b*) An epithelium which has undergone severe contraction at the level of the adherens zone showing how the cells have pulled themselves apart (arrows) resulting in dramatic fanning out of the microvilli. *a*, × 27,000. *b*, × 15,400.

based on splaying of rootlet filaments is the fact that the rootlets are tightly bundled, probably by villin and/or fimrbin, neither of which loses its bundling abilities in response to ATP. In fact, the rootlets remain well bundled even in the presence of high levels of free calcium and ATP (8). It seems likely that the rootlets are structural elements used to keep microvilli erect by being supported and/or cross-linked by tonofilaments and the very fine filaments which appear to bridge between rootlets (3). Recent antibody localization and structural studies suggest that these fine filaments are not actin but may be composed, in part, of myosin (19). We have suggested that the extensively cross-linked rootlets may be under tensile forces and that the terminal web's main role is to anchor the rootlets in this rigid meshwork (28).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Questions as to the molecular regulation of brush border motility must await further careful study. Brush border myosin may be calcium regulated (23) perhaps by calmodulin. However, one finding in the present study suggests that the intestinal epithelial cell is constantly under some tension from a contractile ring which would normally be prevented from constriction when the cells are integrated into an epithelium. A finding suggesting constant tension by a contractile ring is the observation that whenever single cells, small groups of cells, or the end cell on an epithelial sheet were observed, they were found to be constricted at the adherens region. This finding suggests that without the integrity of a continuous epithelium, where adherens contractile ring constrictions would be resisted on all sides by opposing forces, a cell will constrict at the adherens zone. If, as it appears, cells are under constant tension due to an adherens contractile ring, then studies on brush border reactivation using isolated brush borders are open to question.

A second finding which may bear upon the molecular regulation of brush border motility is the finding here that adherens contractile ring activity is insensitive to Ca^{++} in vitro. Constrictions were as dramatic and as common in the complete absence of Ca^{++} as in solutions where Ca^{++} was buffered to different levels. This finding of a contraction insensitive to Ca^{++} is not unique. Using the same glycerination procedure used here on cultured pigmented retinal epithelial cells, Owaribe et al. (34) found similar ATP-induced apical contractile ring constrictions mediated in a Ca^{++}-insensitive manner. Significantly, Rodewald et al. (35) found that in vitro motility (which appears as though it could be due to an adherens contractile ring) in membranated brush borders, while dependent upon divalent cations and ATP, is Ca^{++}-independent. Lack of Ca^{++} sensitivity in these studies may merely reflect the physiological state of the tissues, cells, or brush borders when isolated and/or permeabilized. Myosin and/or various control proteins could be expected to remain more active in certain preparations. It is also possible that a myosin light chain kinase could lose its Ca^{++} sensitivity through the extraction procedure used here. This question is currently under investigation.

While this report documents the contraction of intestinal epithelial cell brush borders in situ mediated by an adherens zone contractile ring, there is still no clear in vivo demonstration of brush border motility. However, a contractile ring hypothesis is a simple, feasible and likely model for brush border motility. One only hopes that careful observation of intact villi will provide direct support of this hypothesis.

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FIGURE 9 Two examples of epithelial sheets treated with 5 mM ADP in Solution 1. The brush border region throughout the epithelium is normal in appearance with the junctional complexes intact. No separation between adjacent cells at the adherens zone is noted. In *b*, the last cell on the sheet has undergone contraction at the adherens zone (arrow), an occurrence which is quite common to free cells, small clusters of cells, and the cells at the end of a sheet. *a*, × 10,500; *b*, × 5,200.