THE FINE STRUCTURE OF THE MICROVILLI OF ISOLATED BRUSH BORDERS OF INTESTINAL EPITHELIAL CELLS

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INTRODUCTION

Since Granger and Baker's classical paper (8), the fine structure of the microvilli on the luminal face of the intestinal columnar cell has been studied in some detail. There is, however, still considerable uncertainty as to the nature of the contents of the microvillus and in particular whether the central structure, the microvillus core, is composed of filaments (13) or of microtubules (12).

Recently, cell fractionation techniques have been developed that allow the isolation of the brush border pole of the epithelial cell from homogenates of mucosal scrapings (1, 6, 14). Isolated brush border preparations allow certain nonconventional techniques to be used in investigating the fine structure of the microvillus. We here report on our findings with such brush border preparations.

MATERIAL AND METHODS

Isolated brush borders, prepared from the small intestinal epithelial cells of adult pigeon by the method of Boyd, Parsons, and Thomas (1), were treated in one of two ways. For sectioned material, a pellet of the preparation was fixed in glutaraldehyde (2.5 ml in 100 ml phosphate buffer, pH 7.25) and then postfixed in Dalton's chrome-osmium (pH 7.2) (4); the pellets were dehydrated in graded alcohols and embedded in Araldite (Ciba, Duxford, Cambs.). Ultramicrotome sections were cut with a diamond knife and subsequently double stained with saturated aqueous uranyl acetate and lead citrate (20) to enhance contrast.

For negatively stained preparations, brush border suspensions were prefixed in phosphate-buffered glutaraldehyde and then exposed to periods of ultrasonication at 0°C for between 30 and 120 sec at 100 w output in an ultrasonic disintegrator (Measuring and Scientific Equipment, Ltd., London, England). The suspensions were then negatively stained (3) with isotonic 1% buffered phosphotungstic acid, and small amounts of specimen material were gathered onto Formvar-coated grids which were then air-dried.

Grids were examined in a Siemens Elmiskop I electron microscope operating at 80 kv. Electron micrographs were taken at magnifications varying between 1,500 and 80,000.

RESULTS AND DISCUSSION

The isolated brush border is composed of microvilli and the subjacent terminal web; a variable amount of lateral cell membrane is also often present, but the preparation is strikingly free from nuclei and cytoplasmic inclusions such as mitochondria. The fine structure of the microvilli is remarkably well maintained during the brush border isolation procedure. In cross-section the hexagonal pattern of the array of microvilli appears unaltered, and individual microvilli exhibit good preservation of the plasma membrane and the surrounding glycocalyx (10). Indeed, in some cross sections through the microvilli of the isolated brush border, threads of glycocalyx can be seen crossing from one microvillus to the next, forming a network (Fig. 1). Such an arrangement has also been seen with the glycocalyx surrounding microvilli on the surface of the placenta (2). The excellent preservation of the structure of the microvillus after isolation of the brush border preparation permitted more detailed investigation of unsectioned material after negative staining. Fig. 2 shows the edge of a single brush border, not exposed to ultrasound, which has been negatively stained with phosphotungstic acid. The considerably distorted profiles of several microvilli are seen; some of them appear in clumps joined at their bases by darkly staining threads which are clearly visible within the profiles of all the microvilli.

Since the intact brush border usually proves too large to be successfully stained in this way, it was therefore further broken down by means of ultrasonication. By exposing brush border preparations to brief periods of ultrasound, clumped microvilli such as are seen in Fig. 2 are separated into small isolated clusters while longer exposure yields single isolated microvilli (Fig. 3). This procedure allows examination of the remaining core structure of the microvillus in some detail in material which has been treated differently than that in embedded, sectioned specimens.

Surrounded on both sides by electron-opaque

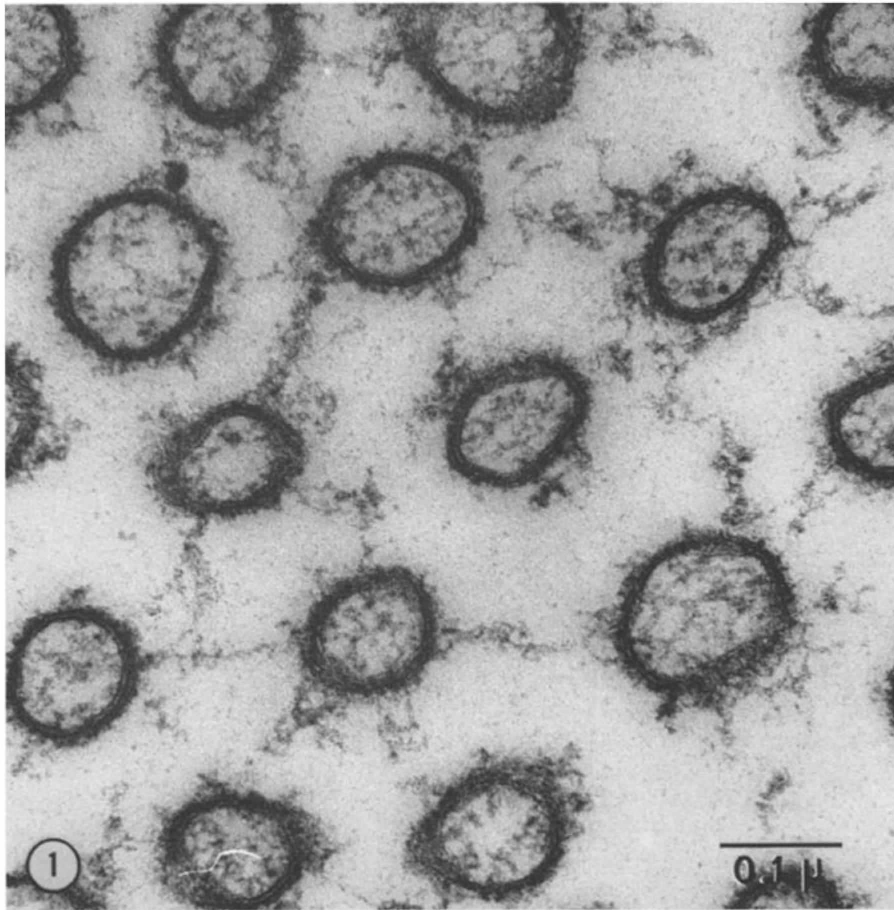

FIGURE 1 Cross section through the microvilli of brush border isolated from pigeon. Note strands of glycocalyx, up to 200 mμ in length, connecting adjacent microvilli. This section cuts near the base of the microvilli. Glutaraldehyde and osmium. ×150,000.

stain, the plasma membrane that defines the microvillus appears as a continuous pale line 88 ± 5 (7) A thick (Figs. 2 and 3), only slightly thinner than that seen in fixed embedded material (21, 16). It encloses a variable number, 4–15, of longitudinally arranged elements. These threadlike structures, which sometimes show bending, may extend beyond the base of the microvillus into the region that in the intact brush border is occupied by the terminal web. When these core elements extend out of the microvillus for some distance (Fig. 3), separate threads from different microvilli can be seen to come into close relationship. In Fig. 3 the threads appear as dark-light-dark structures 100 ± 7 (5) A in over-all thickness: each dark line of phosphotungstic acid stain is 30 ± 4 (5) A thick, leaving a central light band 42 ± 5 (5) A wide.

Are these structures related either to the microfilaments or to the microtubules found in the cytoplasm of other cells? Porter (19), in a review, points out that microtubules are often found in the cytoplasm of aldehyde-fixed cells; they are some 250 A in over-all diameter with a center 100 A or so in width which is of low electron density. They are straight, nonbranching structures surrounded by low density zones from which cytoplasmic particles, particularly ribosomes, are usually excluded. The microtubule wall itself appears to be composed of up to 13 longitudinally orientated filamentous subunits showing 50 A center-to-center spacing. Recently Gall (7), using

FIGURE 2 Electron micrograph of pigeon microvilli in a brush border preparation, not exposed to ultrasound. Within the rather distorted microvilli, notice the many thin threads that appear to join groups of the microvilli together. Negatively stained with 1% phosphotungstic acid. ×36,000.

unsectioned and unfixed negatively stained material, has reached similar conclusions about the fine structure of microtubules in the amphibian red blood cell.

Microfilaments are common cytoplasmic components often appearing to act in a structural capacity; in sectioned material they are usually 40–50 A thick (5). Such filaments appear to be the structural basis for the terminal web of the intestinal epithelial cell (6, 18, 22).

However, as a review of the literature concerned with the structure of the core of the microvillus of the intestinal epithelial cell reveals (Table I), there is some disagreement about the nature and arrangement of the core structure. Since the material presented here has been subject to homogenization and ultrasonication, care is needed in interpreting the arrangement or, indeed, the number of threads composing the core. Such information is possibly more easily derived from micrographs of cross-sectioned material, e.g., Fig. 7 in Mukerjee and Williams (16). However, Fig. 3 in this paper provides good evidence that the core threads are filaments about 40 A in diameter, the dark surround to the thread in this negatively stained image being the electron-opaque stain. Some of these filaments are closely connected to the tip of the microvillus plasma membrane. Within the microvillus they may be closely related, for short distances, to the inner face of the cell membrane, while at the base of the microvillus they extend out into the terminal web. This conclusion is in agreement with the findings of McNabb and Sandborn (13), although the filaments in our material appear to be somewhat thinner; this may be a species difference. Overton et al. (17) examined, by shadowing and negative staining, a fraction separated from hamster brush borders, disrupted with 1 M Tris, tris(hydroxymethyl)aminomethane. This fraction was thought to be composed predominantly of microvillus core material; the core was considered to be composed of helically interwoven strands some 350 A in diameter which in turn appear to be formed from particles some 60 A in diameter which are aligned as fibrils. Although in our ultrasonicated material we have found no evidence for such strands, the "fibrils" described by Overton et al. (17) appear to resemble our filaments.

Finally, one can consider the possible functions of elements such as these which appear to be common in the microvilli of many different epithelial cells, e.g., those described in the microvilli of the mouse kidney proximal tubular cell by Hanssen and Herman (9). Microvilli are often described as specializations for increasing the surface area of absorbing cells. The presence of microvilli clearly increases the surface area of the cell membrane and also the total activity

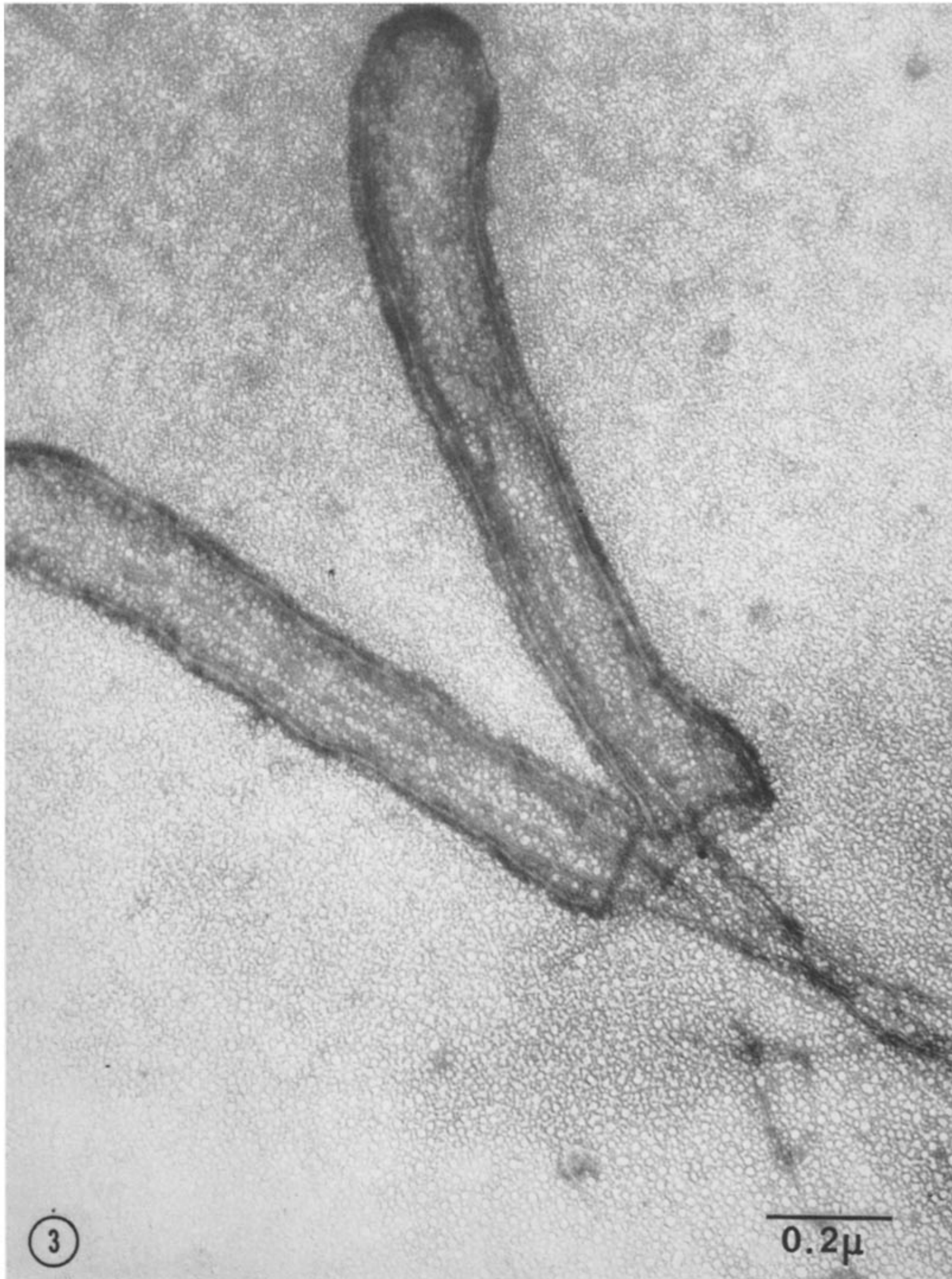

FIGURE 3 Electron micrograph of two negatively stained microvilli separated by 30-sec ultrasonication from a glutaraldehyde-fixed pigeon brush-border preparation. Note the pale continuous plasma membrane surrounded by a darkly staining sheath which is especially marked at the tip of the microvillus. Also, note the core elements, which extend far out of the microvilli and show a dark-light-dark appearance. Negatively stained with 1% phosphotungstic acid. ×90,000.

TABLE I

Internal Fine Structure of Intestinal Epithelial Cell Microvilli

Author	Specimen	Species and segment	Elements composing core	Elements	Arrangement of elements	Dimensions of elements
				No.		
Millington and Finean (15)	Section	Rat jejunum	Straight elements: ? tubules. ? paired fibrillar strands	Variable	In center giving 400 A diameter core	?
Kjaerheim (11)	Freeze-substituted section	Mouse jejunum	"Dark dots and circular profiles;" ? filaments/tubules	Many	Some subunit profiles paired, some circular	30 A diam.
McNabb and Sandborn (13)	Section	Rat duodenum	Straight, nonbranching filaments	Up to 50	Hexagonal; 100–150 A center-to-center spacing	60 A diam.
Laguens and Briones (12)	Section	Human duodenum	Straight, parallel-orientated microtubules	6–10	Hexagonal	60–150 A outside diam. 30 A wall thickness
Mukerjee and Williams (16)	Section	Mouse jejunum	Microfilaments, some appearing tubular	10–50	Hexagonal; 180–240 A center-to-center spacing	60–110 A outside diam. 30 A wall thickness
Overton et al. (17)	Negatively stained and shadowed Tris-disrupted hamster brush borders		Large strands composed of parallel fibrils	2 strands; many fibrils	In loosened rod, strands in ? double helix	Strands 350 A diam. Fibrils 60 A diam.
This paper	Negatively stained ultrasonicated pigeon brush borders		Flexible nonbranching filaments	4–15	Not fixed; sometimes appears helical	42 ± 5 A diam.

of enzymes associated with this region of the plasma membrane. However, all substances moving from the core of the microvilli into the cell body have to move across the base of the microvilli. Thus, suppose that the presence of the microvilli amplifies the plasma membrane area of the mucosal face of the cell by a factor of 20, then substances moving from the microvillus core into the cell body will have to diffuse across an area only one-twentieth of the microvillus membrane surface area. Movement out of the microvillus core into the cell body across this limiting region would clearly be assisted if the microvilli underwent cycles of contraction and relaxation. The fibrils of the core of the microvilli would evidently be candidates for the role of contractile elements, if it is ever proved that microvillous pumping does occur.

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