

MICROVILLI OF THE HUMAN JEJUNAL EPITHELIAL CELL

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The brush border of the epithelial cells of the small intestine has attracted the interest of microscopists for many years. Controversy concerning its structure remained unresolved until 1950, when Granger and Baker (1, 2), using an electron microscope, described the microvilli which constitute the border. Since then, several reports have appeared describing the intestinal microvilli in animals (3-5) and in man (6-8). Although mention has been made of the variation in the size of the microvilli in relation to their location in the villus, no systematic study of this variance is available. The present work was undertaken to provide this information.

MATERIAL AND METHOD

Jejunal biopsy specimens were obtained during intra-abdominal surgical procedures on five patients. These patients were selected on the basis of clinical information (history, physical examination, and stool examination) which indicated that they had normal intestinal function. In addition, the jejunal mucosa was considered to be normal by light microscopy. The patients had fasted for at least 14 hours prior to the biopsy.

The biopsy specimens were divided into two portions immediately after removal: one portion was cut into blocks approximately 1 mm² and placed in Dalton's fixative (9), and the other was fixed in 10 per cent formalin and used for conventional light microscopy.

The blocks to be studied with the electron microscope were allowed to fix for 1 hour; they were then rapidly dehydrated with alcohol and embedded in a mixture of butyl and methyl methacrylate (4:1). Polymerization was accomplished at 40°C for 12 to 14 hours. Sections were cut with a glass knife on a Porter-Blum microtome. Section thickness was estimated to be between 300 and 400 Å.

An attempt was made during embedding of the tissue blocks to orient them so as to have the plane of section normal to the surface. This was done with the aid of a dissecting microscope. However, the small size of the blocks and their dark color made this procedure difficult.

The epithelial cells of the jejunal mucosa were divided into three groups on the basis of their location in the crypts, on the intervillous surface, or on the villous crest (Fig. 1). Determination of the location of the cell was made by examination of sections 1 μ thick cut from the methacrylate blocks and stained with hematoxylin and eosin. Blocks were not included in this study when any doubt existed as to the precise location of the epithelial cells. Approximately half the blocks were discarded for this reason.

The blocks considered adequate for study included 6 of the villous crest (1 each from four of the patients and 2 from the fifth), 31 of the intervillous space, and 11 of the crypts.

The thin sections were studied in an RCA EMU-3E electron microscope. Initial magnifications were generally about $\times 7500$, with photographic enlargement to about $\times 15,000$.

After a positive identification of the cell locus had been made, the height, diameter, and linear density of the microvilli were measured. The following criteria were used in the selection of the microvilli to be measured: (a) the diameter of the microvillus was constant throughout its length, (b) some portion of the central dense material was present from the reflection of the plasma membrane at the base of the microvillus to its tip, and (c) the plasma membrane was intact over the entire surface of the microvillus. Linear density was defined as the number of microvilli per unit length of cell surface measured at the base of the microvilli. To be included in this measurement, only the lower part of the microvillus was required to be present. At least 5 microvilli were counted in each determination in a crypt cell and 10 in an intervillous surface or crest cell.

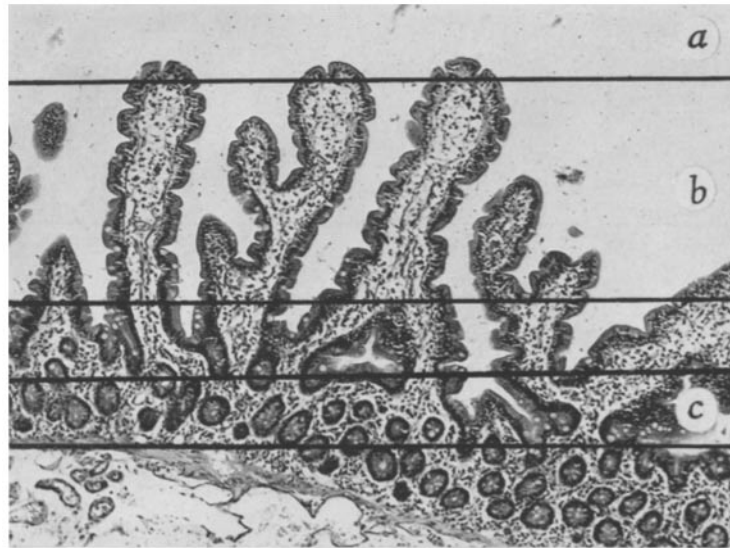


FIGURE 1

Three zones of jejunum in which microvilli were measured. *a*, surface, or villous crest; *b*, intervillous surface; *c*, crypts.

RESULTS

The results of the various measurements are given in Table I. The differences in the mean height, diameter, and linear density of the microvilli are statistically significant (0.05) between the crest and the intervillous lining cells and between the crest and the crypt cells. Although the mean height of the microvilli of the intervillous cells was significantly greater than that of the microvilli of the crypts, the diameter and linear densities approached, but did not exceed, the level established for significance. The mean values of the diameters and linear densities of the microvilli in the intervillous spaces were, however, lower and higher, respectively, than those in crypt cells. Representative microvilli from each of the three areas are shown in Figs. 2, 3, and 4.

The volume and surface area of a "mean" microvillus from each of the three sites, as determined from the mean values in Table I, are given in Table II. A progressive decrease in volume and increase in surface area of the individual microvilli from the crypt to the crest is apparent. The total surface area and volume of the microvillus in terms of the surface area of the cell increases considerably from the crypt to the crest of the villus.

TABLE I

Microvillous Dimensions in Human Jejunal Epithelial Cells

Cell location	Height μ	Diameter μ	Density*
Villous crest:			
Mean	1.36	0.08	10.7
Standard deviation	0.24	0.01	3.4
Intervillous space:			
Mean	1.01	0.10	4.7
Standard deviation	0.23	0.02	1.7
Crypts:			
Mean	0.67	0.15	3.9
Standard deviation	0.24	0.12	2.1

* Microvilli per μ of cell surface.

COMMENT

The data in Table I fall within the range reported by others for these parameters. Except for Dalton's paper (3), however, none of the previous reports specified the locus of the microvilli described. Dalton, studying the small intestine of the rat,

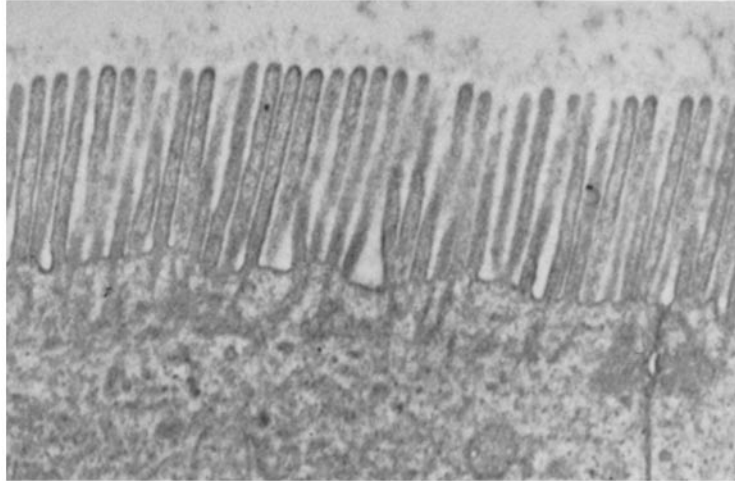


FIGURE 2

Microvilli of crest cell of jejunal epithelium. Measurable microvilli averaged 1.12μ in height, 0.09μ in diameter. $\times 24,000$.

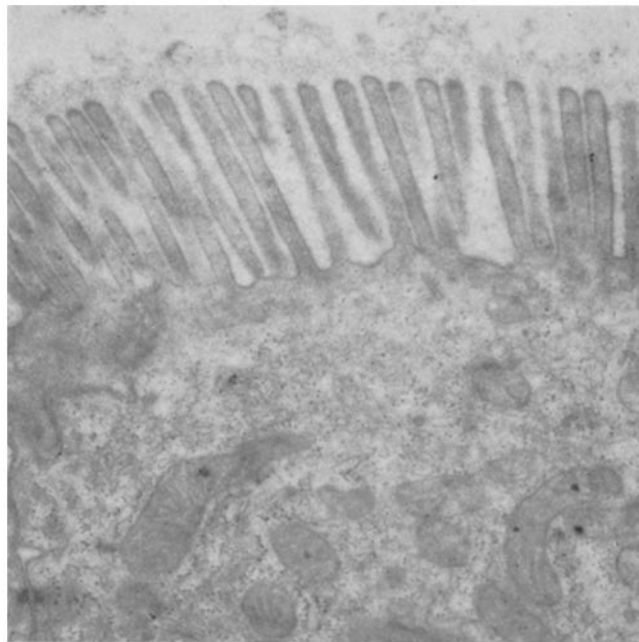


FIGURE 3

Microvilli of an intervillous surface cell. Microvilli averaged 1.04μ in height, 0.11μ in diameter. $\times 23,000$.

reported a height of 0.9μ for microvilli on the villous crest and 0.44μ for those in the crypts. In man, reported values for the height of the microvillus have varied from 0.85μ to 1.60μ (6), 0.8μ to 1.3μ (7), and 1.3μ (8). The values for the diameter in these reports were 0.1μ , 0.12μ , and 0.08μ , respectively.

Estimates of the number of microvilli per unit area of cell surface have also varied considerably. There has been general use of Granger and Baker's (2) value of $15 \mu^2$ as the area of the free surface of the intestinal epithelial cell. When this figure has been utilized, the number of microvilli per cell has varied in experimental animals from

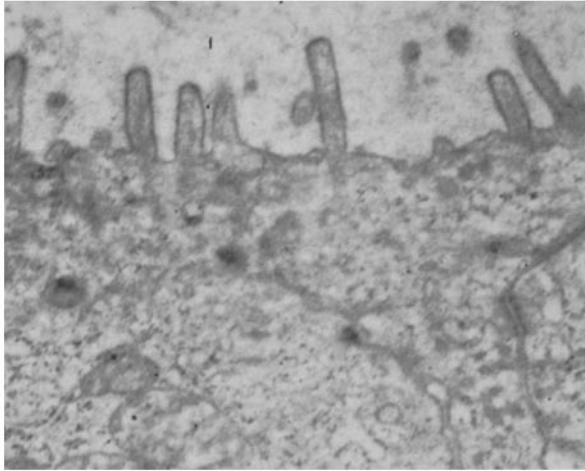


FIGURE 4
Surface of crypt cell showing short, wide microvilli. Average height of microvilli was 0.54μ , diameter 0.14μ . $\times 24,000$.

TABLE II
Volume and Surface Area of Microvilli of Jejunal Epithelial Cells

Cell location	Volume of "mean" microvillus μ^3	Volume of microvilli per μ^2 * of cell surface μ^3	Surface area of "mean" microvillus μ^2	Surface area of microvilli per μ^2 * of cell surface μ^2	Microvilli per cell†
Villous crest	0.0068	0.76	0.342	39.2	1717
Intervillous space	0.0079	0.17	0.317	7.0	331
Crypt	0.0118	0.18	0.316	4.8	225

* Microvilli per μ^2 calculated from microvillous density in Table I.

† Area of free surface of cell taken as $15 \mu^2$.

650 (5) to 1125 (4) to 3000 (2). The last mentioned estimate was considered to have been maximal, the actual value being something less than this. In man, Haubrich and associates (6) reported 735 microvilli per cell, and Ashworth and coworkers (8), 1800. On the basis of the present data, it appears that Haubrich counted microvilli in the intervillous space as well as on the villous crest, whereas Ashworth confined his attention largely to the villous crest.

The morphologic maturation of the intestinal microvilli can be reconstructed in the following manner. In the crypts they are short, broad, and widely spaced. As the cells move on to the intervillous surface, the microvilli are longer and narrower, with little change in their individual volume or surface area but with a slight increase in number per unit area. This change becomes progressively more apparent until the final form is attained on the villous crest. In this area the microvilli individually and collectively present a maximal surface area.

The mechanism by which the number of microvilli per unit area is increased from 15 per μ^2 in the crypts to 114 per μ^2 in the villous crest cannot be answered directly from this study. One way by which this increase could be accomplished is by a progressive decrease in the area of the free surface of the cell between the crypt and the villous crest. If the assumption is made that the free surface of the crest cell is $15 \mu^2$ and contains 1717 microvilli, it can be calculated from the number and cross-sectional area of microvilli found in the crypt cells that these cells would have a free surface of approximately $120 \mu^2$; the actual value could not be found in the literature. The foregoing calculation assumes that no new microvilli develop in the upper part of the crypt and on the intervillous surface. The variation in height and diameter of the microvilli in the crypts is such, however, as to suggest that early forms may not have been recognized and therefore not measured or counted. In this connection it may be pointed out that the cross-sectional area of the

microvilli in the crypts occupies only 28 per cent of the cell surface, while on the villous crest this value is 57 per cent. Both a decrease in the surface area and an absolute increase in microvilli very likely play a part in the "concentration" of the microvilli during the maturation process.

There is general agreement that the microvilli are important structural factors in the digestive function of the small intestine. Furthermore, there is evidence that maximal absorption of material from the intestinal lumen occurs at the villous crest (10). It is not surprising, therefore, that the greatest number of microvilli, each with a maximal surface area, is found on the villous crest. To the extent that microvilli are involved in the absorption of nutrients, it would be expected that this process would decrease progressively along the intervillous surface and into the crypts.

Variations in size of the microvillus were regarded by Granger and Baker (2) as being due to changes in the functional state of these organelles. The relatively uniform appearance of the microvilli of the villous crest was a constant feature of the biopsies in this study. However, none of the patients had taken any food or liquid by mouth for 14 hours prior to removal of the tissue, so that functional activity was probably minimal.

SUMMARY

The microvillous border of the jejunal epithelial cell was studied in five patients with normal gastrointestinal function. The epithelium was divided into three zones: crypts, intervillous surface, and villous crest. Microvilli in each of these zones were measured with regard to height, diameter, and number per unit length of cell surface. The microvilli were short, wide, and relatively few in number in the crypts. In the intervillous area and on the villous crest, the microvilli became progressively higher, thinner, and more numerous. A corresponding increase in the surface area of the microvilli occurred along

with a decrease in volume. Mensuration data for the microvilli in each of the three sites are given.

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REFERENCES

1. GRANGER, B., and BAKER, R. F., Electron microscope investigation of the striated border of intestinal epithelium, (abstract) *Anat. Rec.*, 1949, **103**, 459.
2. GRANGER, B., and BAKER, R. F., Electron microscope investigation of the striated border of intestinal epithelium, *Anat. Rec.*, 1950, **107**, 423.
3. DALTON, A. J., Electron micrography of epithelial cells of gastrointestinal tract and pancreas, *Am. J. Anat.*, 1951, **89**, 109.
4. PALAY, S. L., and KARLIN, L. J., An electron microscopic study of the intestinal villus. I. The fasting animal, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 363.
5. ZETTERQVIST, H., The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum: An Electron Microscopic Study Including Some Experiments Regarding the Problem of Fixation and an Investigation of Vitamin A Deficiency, Karolinska Institutet, Stockholm, 1956.
6. HAUBRICH, W. S., WATSEN, J. H., O'DRISCOLL, W., and VALENTINE, V., Electron microscopy of the free border of the human intestinal epithelial cell, *Henry Ford Hosp. Med. Bull.*, 1959, **7**, 113.
7. HARTMAN, R. S., BUTTERWORTH, C. E., JR., HARTMAN, R. E., CROSBY, W. H., and SHIRAI, A., An electron microscopic investigation of the jejunal epithelium in sprue, *Gastroenterology*, 1960, **38**, 506.
8. ASHWORTH, C. T., CHEARS, W. C., JR., SANDERS, E., and PEARCE, M. B., Nontropical sprue, *Arch. Path.*, 1961, **71**, 13.
9. DALTON, A. J., A chrome-osmium fixative for electron microscopy, (abstract) *Anat. Rec.*, 1955, **121**, 281.
10. PATZELT, V., Der Darm, in *Handbuch der mikroskopischen Anatomie des Menschen*, (W. Von Möllendorff, editor), Berlin, Julius Springer, 1936, **5**, 104.