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MORPHOMETRIC STUDY OF ULTRASTRUCTURAL RESPONSE OF MICROVILLI OF RAT SMALL INTESTINAL ENTEROCYTES DURING NATURAL FEEDING

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The problem of changes in the microvilli during performance of its basic function of absorption by the intestine has recently come up for discussion. Previously, when functions of the microvilli were reduced simply to enlargement of the absorbing surface of the intestine, they were considered to be stable formations. Recent investigations have shown that microvilli can contract [10, 13, 14]. Immunofluorescence and immunochemical identification of the actomyosin complex in microvilli demonstrated clearly that the microvilli of enterocytes are active dynamic structures [9, 11]. The study of the response of the microvilli during absorption of individual nutrients confirms the lability of these structures, but gives contradictory results [7, 8, 15]. Under natural conditions a wide range of different nutrients undergoes simultaneous absorption from the chyme; the response of the microvilli during activation of digestive processes, however, has not hitherto been described.

The aim of this investigation was to study the dynamics of changes in size of the microvilli of absorptive enterocytes located in the active zone of the villi of the three principal parts of the intestine, during digestion and absorption of a combination of natural food products.

EXPERIMENTAL METHOD

Mature Wistar albino rats weighing about 200 g were used. Before the experiment the animals were kept on the ordinary animal house diet. The rats were deprived of food (water *ad lib.*) for 36 h before the experiment, and kept in cages preventing coprophagy. After starvation, the animals were fed for 15 min on sunflower seeds and white bread soaked in milk, after which the residual food was removed from the cages.

The animals were killed 25, 50, 100, and 200 min and 8 h after the beginning of feeding. Rats deprived of food for 36 h served as the control. Pieces of duodenum, the middle part of the jejunum, and the distal part of the ileum were removed for investigation.

After decapitation of the animals, a 4% solution of paraformaldehyde in Hanks' buffer (pH 7.3) was injected slowly into the intestinal lumen. After 3-5 min pieces of the three parts of the small intestine were excised and immersed in the same fixative as that in which the tissues were kept for 3 h at 4°C. After rinsing three times in buffer, the pieces were fixed in 2% OsO_4 for 30 min, washed in buffer, incubated for 30 min in a 1% aqueous solution of thiocarbohydrazide, washed, and postfixed for 1 h in OsO_4 [6]. After dehydration in ace-

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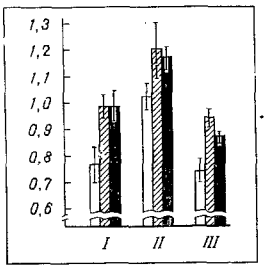

Fig. 1. Height of microvilli of absorptive enterocytes of a hungry rat. Abscissa: unshaded columns — apex of villus; obliquely shaded — upper third of villus; shaded black — middle third of villus; ordinate, height of microvilli (in μ). I) Duodenum, II) jejunum, III) ileum.

tones of increasing concentration, the pieces of tissue were embedded in a mixture of epoxide resins (Epon—Araldite).

Ultrathin sections were studied in the N-300 electron microscope without additional contrasting and photographed under a magnification of 10,000. For subsequent measurement of the linear dimensions of the microvilli, photographs of enterocytes from the apical pole of the villus (apex), the middle of the upper third, and the middle of the middle third of the villi were used. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Microvilli of absorptive enterocytes located in different zones of the villi and in different parts of the intestine differed clearly in length (Fig. 1). The shortest microvilli were found on enterocytes at the apex of the fillus, and this was characteristic of all parts of the small intestine. The length of the microvilli at the level of the upper and middle thirds of the villi was about equal, and it differed significantly only in the ileum. Microvilli were longer in the jejunum, where the absorptive function is most marked [4]. The intestinal villi reach their greatest length here also [5]. This structure results in maximal enlargement of the surface for absorption of digestive enzymes [3] and absorbable substrates. These observations are in full agreement with results obtained on human villi [12].

The dynamics of changes in length of the microvilli of absorptive enterocytes at the apex of the villus during absorption of natural food obeyed the simplest rule. Absorption of food in the intestine led to marked shortening of the microvilli of these cells (Fig. 2a). These changes were most marked in the duodenum and jejunum, and appeared as early as 25 min after the beginning of feeding. Maximal changes were observed 100 min after taking food. In the ileum, shortening of the microvilli of the apical enterocytes was less marked and became significant only 50 min after the beginning of feeding. Later a gradual increase in length of the microvilli was observed. The length of the microvilli 8 h after feeding in the duodenum was close to its initial value, in the jejunum it was a little lower than initially, but in the ileum it exceeded the control.

Enterocytes of the upper third of the villus are known to play the most active part in absorption of nutrients entering the lumen of the intestine. It will be clear from Fig. 2b that as early as 25 min after feeding marked shortening of the microvilli was observed in the duodenum and jejunum, more especially in the duodenum. In the ileum no response of the microvilli appeared at this time. After 50 min the length of the microvilli reached its shortest value, and this was found in all parts of the small intestine. After 200 min the length of the microvilli of the enterocytes in the upper third of the villi was close to its initial value in all parts of the small intestine. Changes in length of the microvilli 8 h after feeding differed in different parts of the intestine. In the duodenum and jejunum some shortening of the microvilli was observed, whereas in the ileum there was an increase in their length.

The most complex time course was observed in the middle third of the villus (Fig. 2c). Soon after feeding (25 min) slight shortening of the microvilli took place in the duodenum and jejunum. In the ileum no significant shortening of the microvilli was discovered. In the duodenum shortening of the microvilli continued until 100 min, after which they became longer, and remained longer until the end of the period of study. In the jejunum the length of the

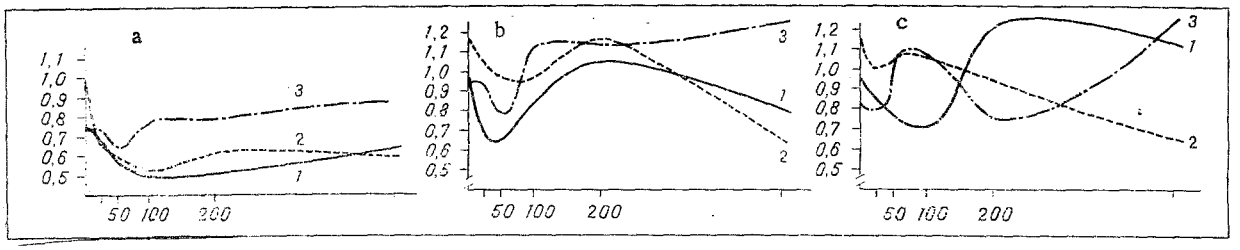

Fig. 2. Time course of response of microvilli after a single feeding. Abscissa, time after feeding (in min); ordinate, height of microvilli (in μ). a) Apex, b) upper third, c) middle third of villus. 1) Duodenum, 2) jejunum, 3) ileum.

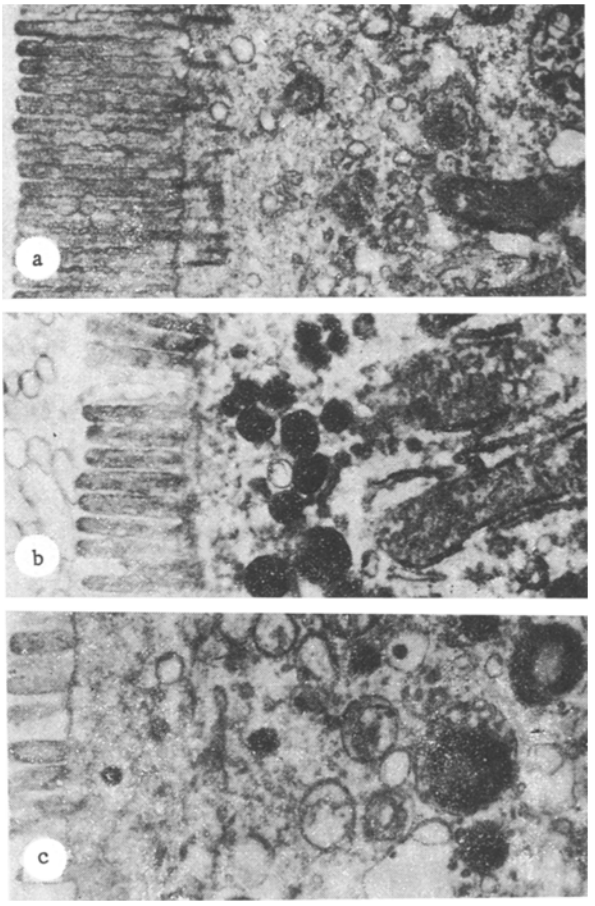

Fig. 3. Structure of microvilli of absorptive enterocytes of jejunum (20,000×). a, b) Upper third of villus 8 h and 25 min, respectively, after feeding; c) apex of fillus 25 min after feeding.

microvilli remained approximately constant from the 25th to the 200th minute, and then decreased until 8 h. Microvilli of the middle third of the villus became longer in the ileum than initially after 50 and 100 min. Shortening to approximately the control value occurred by the 200th minute, after which the microvilli lengthened again until 8 h after feeding.

Analysis of the time course of the length of the microvilli revealed the identical character of the response at different stages after natural feeding, and this was particularly evident in the functionally most active, apical portions of the intestinal villi. A delay in the change in length of the microvilli, observed in the ileum, took place as a result of the later entry of chyme into it.

The considerable shortening of the microvilli which was observed was accompanied by consumption of the apical membrane of the absorptive enterocytes. Meanwhile the mechanisms of realization of the basic function of enterocytes, namely absorption, such as diffusion and

transport with the aid of a carrier, which have been accepted most frequently in the literature, do not envisage, neither do they explain the consumption of membrane material. The earlier hypothesis of a single mechanism of nutrient absorption by pinocytosis [1] interprets this consumption of the apical membrane during absorption of nutrients satisfactorily. The decrease in surface area of the apical membrane in satiated animals compared with that in hungry animals also has been observed in other investigations [15]. Consumption of membrane material of the microvilli also takes place during excretion of substances from enterocytes into the intestinal lumen [2]. Shortening of the microvilli takes place as a result of contraction of the actomyosin complex located along the axis of the microvilli. The membrane of the microvilli becomes corrugated in outline, and this evidently stimulates the formation of pinocytotic vesicles at the base of the microvilli and of secretory vesicles from their lateral surface. During contraction the actin filaments are apparently buried deep inside the cytoplasm of the enterocyte (Fig. 3). In an attempt to explain the causes of periodically observed shortening of the microvilli, possible alternation of contraction and relaxation of the actomyosin complex and the terminal network, which are probably essential for normal functioning of the enterocyte, must not be forgotten. It will also be noted that cells whose microvilli were shortened during absorption move with the course of time toward the apex of the villus. They are replaced by new enterocytes, playing no part in absorption of nutrients and, consequently, possessing longer microvilli. The processes described above were manifested most clearly in the present experiments because food was taken only once and over a short period of time.

Changes in the length of the microvilli observed in response to a food stimulus thus indicate their active participation in absorption of nutrients, the most probable mechanism of which is pinocytosis.

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