

INTRAVITAL DETACHMENT OF INTESTINAL VILLI AND OF THEIR DISTAL FRAGMENTS

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The phenomenon described in this paper is evidence that views on the sources of epithelium in the enteral medium evidently require correction.

EXPERIMENTAL METHOD

The material for examination under a binocular loupe consisted of 205 samples of the contents of different portions of the small intestine of dogs with multiple fistulas and 167 biopsy specimens of mucous membrane obtained from different parts of the small intestine by means of forceps from dogs with multiple fistulas and from intact dogs during operations to form fistulas and for resection of the intestine, all of which were fixed in 12% neutral formalin solution. Biopsy specimens of intestine were straightened under the binocular loupe, fixed with 12% neutral formalin solution, and studied whole, using methods of nontraumatic microdissection to allow a complete investigation, morphometry, and counting of the villi. Microscopic preparations of individual forms of villi were obtained by a special technique [2] from serial longitudinal and transverse paraffin sections 3-5 μ thick.

EXPERIMENTAL RESULTS

Morphological examination of the intestinal contents revealed, in 5% of the samples, villi with streamlined ends, i.e., with no sign of injury such as must inevitably arise in the case of detachment from the mucous membrane; in 49% of intestinal samples these villi were smaller than usual and were denuded of epithelium (Fig. 1a).

During the study of whole intestinal biopsy specimens, attention was drawn to the similarity of the "free" villi from the intestinal samples to villi which were usually taken to be detached during excision of the piece of mucous membrane. In fact, it was found that in all cases, irrespective of the region of the intestine or the method of biopsy, besides detached surplus epithelial structures, "free" villi of different shapes and sizes and also round smooth bodies of uncertain nature were chaotically distributed in the mucous deposits on the surface of the mucous membrane (Fig. 1b). Some such villi had the typical apex and a round or indeterminate, but streamlined basal end with only a local defect of the covering layer, and frequently with branching of a narrow fold or film close to it; others were nonpolar because both ends were atypical. Among these two types there were other forms with a constriction band in the middle or near one end (Fig. 1c). The region of the constriction was usually marked by a thin fold or film, and it varied in distinctness: from a hardly perceptible to a deep furrow and from partial to maximal constriction with rounding of the neighboring parts of one or different sides.

Serial microscopic preparations of the "free" villi and the round bodies from the deposits were characterized by the typical structure of intestinal villi without any marked inflammatory reaction (Fig. 2c). Villi from the intestinal contents in some cases preserved the typical microstructure, but in others they showed signs of autolysis (Fig. 2a, b). The streamlining of the atypical ends of the "free" villi was due to clear demarcation of the local defect of the epithelial cover by the basement membrane (Fig. 2f); the epithelium adjacent to this region was lower than the remainder, with crowding of the enterocytes and nuclei at the same level (Fig. 2d). The branches on the atypical ends and in the region of the constriction of the "free" villi were

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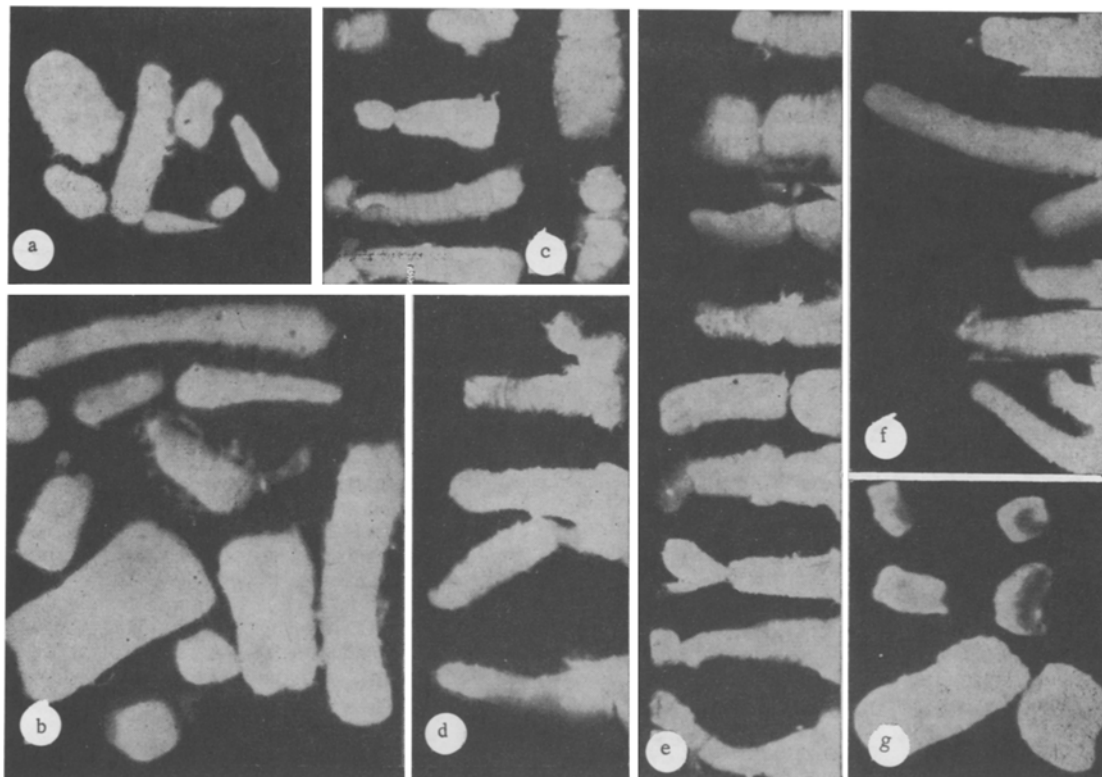


Fig. 1. Shapes of whole villi with direct and indirect features of the phenomenon: a) "free" villi from intestinal samples; b, c, g) "free" villi from mucous deposits on biopsy specimens: of different sizes (b), with signs of a constriction ring (c), before and after remaining 5 and 9 days in chyme in vitro (g); d, e, f) villi from mucous membrane of biopsy material: with signs of detachment in one piece (d), detachment of distal fragment (e), and shortening after detachment of distal fragment (f). 20 \times .

formed by flattened epithelium, denuded of basement membrane, with small nuclei, i.e., they were identical with the surplus epithelial structures which develop in the crypts and in the region of their mouths [1]; at the sites of branching of the flattened epithelium, at the level of the constriction, the basement membrane appeared "vacuolated," and an agglomeration of such vacuoles or spaces joined the rounded parts when a deep constriction was present (Fig. 2e, g).

Counting the total number of villi in the mucous membrane and the total number of "free" villi and round bodies in deposits in 18 intestinal biopsy specimens showed that the number of structures in the deposits of each biopsy specimen varied considerably, but at different stages of digestion the number did not differ significantly and accounted on average for about 50% of the total number of villi in the mucous membrane of the biopsy material ($M \pm m = 55 \pm 13$). Up to one-third of villi of the biopsy material was the average proportion of "free" villi of the usual length ($M \pm m = 26 \pm 9$); a tendency was found for their number to increase significantly 3 h after feeding: 80 ± 4 compared with 5 ± 3 in a fasting state and 32 ± 14 6 h after feeding.

In the mucous membrane of whole intestinal biopsy specimens, variously shortened villi arranged singly or in groups among the mass of remaining villi could be observed; the tip of many of the shortened villi showed the same distinguishing features as the atypical ends and region of constriction of the "free" villi (Fig. 1f; Fig. 2h). Altogether these shortened villi accounted for not less than 10% of the total number of villi of the biopsy specimens. Both ordinary and shortened villi with a constriction, and more likely than not with pinching off the distal part of varied size, were found in the mucous membrane (Fig. 1e). These pictures coincided fully with the signs of constriction of "free" villi, they were observed at all levels as far as the base inclusive, and simultaneously at different levels of the villi, and they varied in degree: from a hardly perceptible boundary to separation of the distal fragment of the villus from its basal part, or of the villus as a whole from the basal layer of the mucous membrane. In the last case (Fig. 1d) their base was rounded and was con-

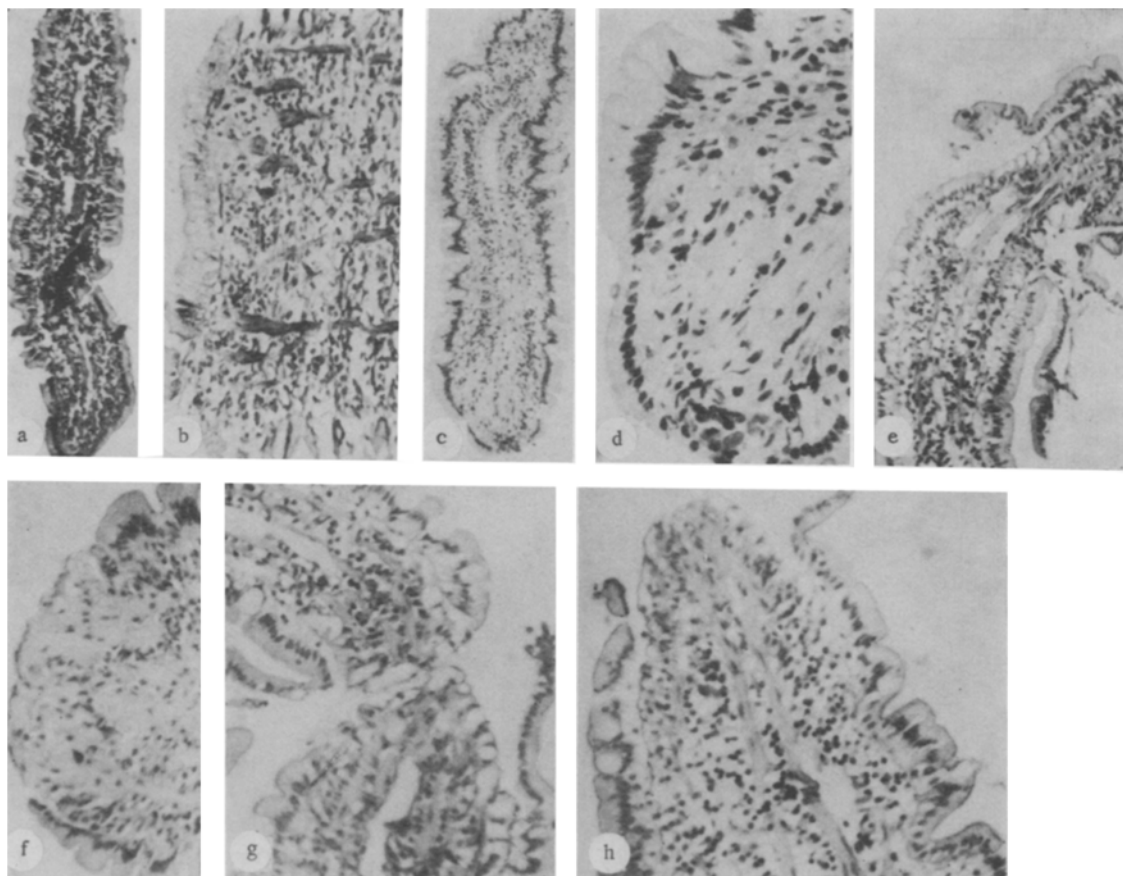


Fig. 2. Direct and indirect features of the phenomenon in microscopic sections through individual forms of villi: a, b) "free" villi from intestinal samples without and with autolysis, respectively; c, d, e, f, g) "free" villi from mucous deposits on biopsy specimens: with branching of the fold (c), with a defect of the epithelium (f), and with low epithelium (d) at the basal end, and with a varied degree of constriction (e, g); h) tip of shortened villus with defect of cover and with branching of bands of flattened epithelium. Stained with hematoxylin-eosin (a, b, e, f, g, h) and by Van Gieson's method (c, d). Magnification: $130\times$ (a, c, e); $200\times$ (b, d, f, g, h).

nected to the underlying tissue by a stalk, and as this grew thinner, the villus could separate from the mucous membrane without leaving any visible trace. The apical surface of the stump formed by pinching off of the distal fragment became similar to the apex of a shortened villus, and with the atypical ends of the "free" villi (see: Fig. 1e, f; Fig. 2f, g). On average about 6% of the villi were in the "pinching off" state, whereas up to 4% of villi in the biopsy specimens were shortened, with corresponding features.

By comparing the results described above it was possible to identify apical parts of villi which were pinched off but not yet completely detached, their larger distal fragments, whole villi with the round bodies, and the polar "free" villi, on the one hand, and the partly and completely detached, previously shortened villi with the nonpolar "free" villi of the deposits, on the other hand. In other words, the "free" villi and round bodies were nothing more than whole and shortened villi and their distal fragments, detached into the deposits on the surface of the mucous membrane after being pinched off, and which subsequently passed into the intestinal lumen.

The presence of components of the villi with signs of autolysis in the intestinal contents is objective evidence that their detachment from the mucous membrane was an intravital phenomenon. Meanwhile, the absence of any significant differences between components of the villi in the deposits and villi of the mucous membrane and the absence of signs of any degree of injury to them by the digestive juices suggest that this phenomenon may also be interpreted as an artifact of the biopsy method. To shed light on this problem experiments were carried out in which mucous deposits from detached portions of villi were separated from

biopsy material and kept for 1-9 days in an incubator at 39°C in the liquid fraction of chyme, and in physiological saline as the control. On the 1st day the detached components of the villi in the control experiments were denuded of their cover, which was found in the form of caps and sheaths in the mucous and in the physiological saline, whereas in chyme they showed no visible changes and were indistinguishable in structure from components of the villi from the intestinal samples. On the 3rd-9th day of their stay in the digestive medium the detached components of the villi, although preserving their typical shape, were much smaller than initially (Fig. 1g), and in microscopic sections of them the same picture of autolysis could be seen as in components of the villi in the intestinal samples obtained from fistulas in dogs. Consequently, the experiments in vivo and in vitro showed that the detached villi and their fragments were resistant to the intestinal medium, even after partial or total loss of their epithelial cover.

These investigations thus show that villi of typical and altered size are detached or become shortened as a result of separation of a distal fragment of varied size. This process is evidently preceded by local separation of the epithelium from the basement membrane, with removal of enterocytes, arriving from the crypts and becoming atypical under these circumstances, into a fold, the rupture of which causes exposure of the basement membrane which, in this region, assumes the position of an outer membrane. Information on stability of the basement membrane of the villi in the absence of the epithelium [5, 10, 13] can explain the structural integrity not only of the villi as they are being pinched off, but also of the villi and fragments which are detached. This phenomenon raises the question of its role in the development of pathomorphological changes in the surface of the mucous membrane, and also in the well-known pathological fact of the increased influx of epithelium into the lumen of the small intestine [4].

On the basis of the results described above, the phenomenon of detachment of intestinal villi and of their distal fragments can be regarded as an intravital, nonspecific, and uniform process which, together with extrusion of the enterocytes and detachment of surplus epithelial structures, independently of the entry and digestion of food, facilitates the systematic supply of epithelium and other endogenous materials to the intestinal medium. Since the mucous membrane of the small intestine in organotypical cultures in vitro can maintain both the structure and functional activity of the enterocytes for 24-48 h [3, 6-9, 11, 12], it is by no means improbable that structural elements detached from it can participate in the processes of luminal digestion.

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