

MECHANISM OF DETACHMENT OF THE EPITHELIAL LAYER OF THE VILLI OF THE SMALL INTESTINE

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During the preparation of histological sections of the small intestine the epithelial layer frequently becomes detached, sometimes leaving the bare stroma of the villi. Various interpretations of this phenomenon are given in the literature. Some workers consider that detachment of the epithelium takes place after death and is due to treatment of the material for investigation [2, 4, 10, 12], while others regard these changes as pathological [1, 11], and a third group claims that detachment of the epithelium and exposure of the bare villi is an active physiological process associated with secretion in the small intestine [5, 6, 9].

The supporters of the first point of view maintain that detachment is based on the weak attachment of the epithelium to the underlying stroma. They postulate that detachment of the epithelium may take place either as a result of mechanical injury to the mucous membrane [3] or on account of contraction of the villi during fixation [2, 12]. They suggest that a factor contributing to detachment of the epithelium from the stroma is adhesion of the villi with each other and with the chyme [2].

To prevent detachment of the epithelium from the underlying stroma, several methods of fixation of the material are suggested. Some authors consider that detachment will not occur if the intestine is fixed, not immediately after death of the animal, but after a short interval [11]. Others are of the opinion that detachment may be avoided if the intestine is stretched before fixation, either mechanically (by introducing physiological saline into its lumen [2, 4]), or by injecting a solution of kava-kava into the blood stream to paralyze the smooth muscle [12]. In our opinion, experiments involving distension of the lumen of the small intestine with physiological saline, on the basis of which N. P. Bochkov concluded that detachment of the intestinal epithelium is an artefact, are inconclusive because in none of them was the author able to prevent detachment from taking place.

The various methods of treatment of the material suggested for prevention of detachment of the epithelial layer are themselves not without effect on the state of the intestine. Moreover, the investigators cited above did not take into account the various phases of digestive activity, which differ in their morphological expression in different parts of the intestine at one and the same time [8].

The object of this research was to study the influence of the method of fixing the material on the phenomenon of detachment of the epithelial layer of the small intestine. It was directed towards verifying the hypothesis that the epithelium is not detached if the intestine is fixed some time after the animal's death [11] and also towards verifying the results described by N. P. Bochkov [2].

EXPERIMENTAL METHOD

In the first series of experiments an attempt was made to discover the relationship between detachment and the time elapsing between the animal's death and immersion of the intestine in the fixing solution. The intestines from 24 albino mice and 13 albino rats were immersed in the fixing solution at the following intervals after sacrifice: 1st group of mice - 15 min, 2nd group - 30 min, 3rd group - 1 h, 4th group - 1.5 h, 5th group - 3 h, 6th group - 4 h; 1st group of rats - 30 min, 2nd group - 1 h after sacrifice.

In the second series of experiments the processes of detachment of the epithelial layer were studied during stretching of the intestinal mucous membrane (4 rats). The intestine was stretched by injecting physiological saline into the



Fig. 1. Small intestine after distension with physiological saline. The epithelium is firmly attached to the underlying stroma and forms a continuous lining over the villi. Hematoxylin-eosin. Medium power.

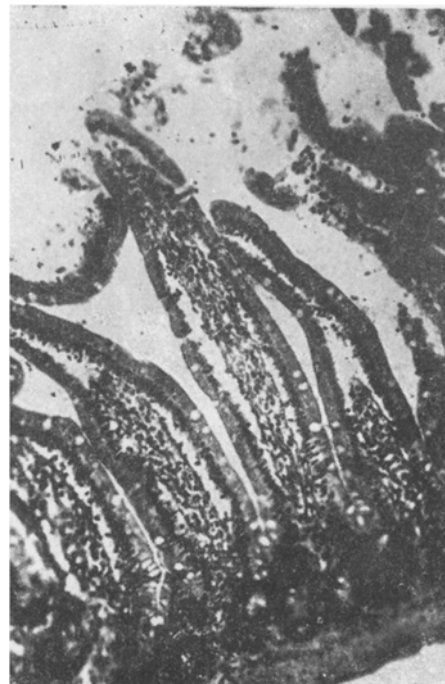


Fig. 2. Small intestine after distension with physiological saline. Detachment and desquamation of epithelium from underlying stroma. Hematoxylin-eosin. Low power.



Fig. 3. Contents of small intestine washed out during distension of bowel. Sheets of epithelium. Hematoxylin-eosin. Low power.

lumen of the proximal portion, from which it escaped through an incision made in the distal portion of the small intestine. The fluid escaping through this incision was collected in a flask and centrifuged. The sediment was fixed and embedded in celloidin. The intestine was immersed in the fixing solution in a stretched state.

In the animals of the third series of experiments (20 albino rats), which acted as controls, the intestine was immersed in the fixing solution immediately after sacrifice.

All the animals were killed with ethyl chloride. The intestine was fixed in toto by immersion in 10% formalin solution and examined histologically over its whole length. Investigations with the binocular stereoscopic microscope (MBS-1), on the basis of which N. P. Bochkov determined the state of attachment of the epithelium to the underlying stroma in a series of experiments on the perfused intestine, were shown by our observations to be inadequate for such conclusions to be drawn. The material was embedded in celloidin. Longitudinal sections through the whole length of the intestine were cut from celloidin blocks and stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

Investigation of the intestine of the control animals showed that, with the same conditions of treatment and fixation of the material, differences in the degree of attachment of the epithelium to the underlying stroma were present.

In some areas of the intestine the epithelium formed an unbroken lining on the surface of the mucous membrane and was firmly attached to the underlying stroma, while in other areas it was detached from the stroma. In places the detached epithelium lay loose in the lumen. These changes did not affect individual villi or groups of villi, but large segments of the bowel. No relationship could be determined between the anatomical features of the segment under study and the state of the epithelial layer. Detachment of the epithelium was observed in both proximal and distal portions of the intestine.

The study of the intestine of the animals in the first series of experiments produced nothing different from what was observed in the intestine of the control animals. When the intestine was fixed at various intervals after sacrifice, the degree of attachment of the epithelium to the underlying stroma also varied. As in the control animals, the epithelium in some parts was firmly attached and formed a continuous lining on the surface of the mucous membrane. In other areas the epithelial layer was detached from the stroma. Desquamation of epithelial cells into the lumen of the intestine was also observed. As the interval between sacrifice and fixation of the material grew longer, the degree of detachment of the epithelium from the stroma became more marked. Desquamation of epithelial cells into the lumen of the intestine was observed over more of its length. Further, with an increase in the time elapsing between sacrifice and fixation, signs of autolysis became more conspicuous. These affected increasingly wide areas of the mucous membrane and, in the specimens fixed after longer intervals, the submucosa and even the muscular layer. The areas of normal mucous membrane became correspondingly smaller.

Hence, our observations did not confirm the view that fixation of the intestine not immediately, but a certain length of time after sacrifice may prevent detachment of the epithelium from the underlying stroma [11]. In this respect our results agree with the observations of N. G. Gundobin, who found that stripping of the villi became more marked as the interval between death and fixation of the material grew longer.

The study of the intestine of the animals in the second series of experiments showed that the attachment of the epithelium to the underlying stroma retained the character described above when the intestine was stretched. In some areas of the small intestine the epithelium formed a continuous lining over the mucous membrane (Fig. 1), and in other segments of the bowel it was detached, and in places lying loose in the lumen (Fig. 2).

Investigations of the intestinal contents washed out with physiological saline during distension of the lumen showed that, besides food residues, cell detritus, and basophilic granular masses, single epithelial cells and sheets of epithelium, sometimes reproducing the shape of the villi, were present (Fig. 3).

Detachment and desquamation of the epithelium from the stroma were observed in all the animals investigated. The discovery of sheets of epithelium, reproducing the shape of the villi, in the intestinal contents demonstrates that the process of distension of the intestine with physiological saline itself leads to the detachment of hitherto unseparated sheets of epithelial cells.

The phenomena of detachment, desquamation, and discovery of sheets of epithelium in the intestinal contents demonstrate that the attachment of the epithelium to the underlying stroma in certain areas of the intestine is weak. In other areas, despite the severe mechanical trauma during distension of the intestine with physiological saline, the epithelium is firmly attached to the reticular stroma. The variation in the degree of attachment of the epithelium to the underlying stroma along the length of the small intestine was visible in the control series and also in the animals of the first and second series of experiments. In our investigations the intestine was immersed in the fixing solution instantaneously and as a whole piece. Consequently, the conditions of fixation were identical for all parts of the organ. We therefore regard this variation in the degree of attachment of the epithelium to the underlying stroma as an expression of variation in the functional state of the mucous membrane. This is confirmed to some degree by the irregular distribution of chyme along the length of the intestine, for it was never found along the whole length, but filled only small areas of the intestine.

It appears that the detachment of the epithelium from the underlying stroma is based on physiological processes associated with the secretory activity of the intestine [9]. The conclusion that detachment of the epithelium of the villi is a physiological process does not rule out the possibility that these changes may be pathological. Detachment of the epithelium also takes place in enteritis [1, 10]. However, the detachment of the epithelium in this condition also involves the crypts of Lieberkühn [7]. Our findings support the view that irrigation of the intestine with physiological saline may cause artefacts [3].

The conclusions reached from these investigations do not conflict with the views put forward by most authors to explain the phenomenon of detachment of the epithelium of the villi. The fallacy of the view ascribing this

phenomenon to artefact is evidently accounted for by the incorrect technical approach to investigation of the intestine. Disregard of the phased nature of digestion, also observed in the intestine when not containing food, was responsible for the false conclusions derived more from a stereotyped interpretation of general theoretical notions of the continuity and integrity of the epithelium than from factual data.

Our previous investigations [8] showed that the relief and the condition of the mucous membrane may be correlated with the state of the intestinal tone. Wolf-Heidegger [12] used a substance paralyzing smooth muscle in his experiments, so that his conclusions cannot be accepted completely.

In all the experiments without exception variation was observed in the degree of attachment of the epithelium of the villi of the small intestine to the underlying stroma. Additional factors (increasing the length of the interval between sacrifice and fixation of the intestine, irrigation of the intestine with physiological saline) did not prevent but, on the contrary, aggravated this phenomenon.

SUMMARY

Examination of mucosa of the small intestine of albino rats and mice, fixed at various periods after death (from 15 minutes to 4 hours), as well as of mucosa and contents of the intestine, distended with physiological saline, demonstrated contrary to the existing opinion that these methods did not eliminate epithelial detachment from the underlying stroma. Investigations carried out showed that the relation of the epithelium to the underlying stroma differed in various areas of the intestine. The view that epithelial desquamation is a physiological phenomenon connected with the secretory function of the stomach is maintained.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. Some or all of this periodical literature may well be available in English translation. A complete list of the cover-to-cover English translations appears at the back of this issue.
