

DESQUAMATION OF THE EPITHELIUM OF THE VILLI OF THE SMALL INTESTINE—AN ELEMENT OF FUNCTIONAL MORPHOLOGY OR AN ARTEFACT ?

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It has been shown by many research workers [5, 7, 8, 9, 12, 15, 16, 19] that the solid part of the intestinal juice consists mainly of desquamated and disintegrating intestinal epithelial cells, i.e., that the secretion of the solid part of the intestinal juice is connected with the destructive phase of physiological regeneration [2, 10]. Different views exist, however, of the mechanism of death of the intestinal epithelial cells.

After fixation of the intestine in a stretched condition [14], a picture of the dislodgement of cells from the apices of the villi was observed. In some cases this process affected a single cell, and in others, groups of cells, but the breakdown in the integrity of the epithelial layer was always connected with destructive changes in the epithelial cells, and with their necrosis. Similar findings were obtained by other authors [11, 13, 17].

M. I. Razumov [6] considers that the epithelium is desquamated by means of the detachment of large sheets of cells, affecting not only the apices of the villi, but also their lateral surfaces, so that their stroma is denuded. Histological and histochemical investigation showed that the desquamating epithelium did not differ from that left behind on the villi. The process of desquamation of the epithelium is preceded by the formation of epithelial tubes, the lumen of which was described earlier as Gruenhagen's spaces. Other authors have reported similar findings [3, 4].

In view of the divergent opinions on this subject, we carried out the present investigation, based on the findings of those authors [18, 20] who consider that the formation of Gruenhagen's spaces is the result of the contraction of the muscle fibers of the stroma of the villi due to the fixing agent and the ensuing isolation from the reticular stroma of the villi of the thickened epithelium, loosely connected to it.

We believe that the detachment of the epithelial layer from the stroma is brought about by the adhesion of the villi to each other or to the undigested food mass, since the apex of the villus is attached under these

circumstances. Desquamation and detachment of the epithelium takes place during fixation and embedding to a greater degree the closer the contact between the villi.

In order to test this hypothesis, the intestines of 48 rats were irrigated *in situ*, with slight distension, at first with physiological saline for 2 min, and later with Bouin's fluid for 5 min. The lumen of the intestine was moderately distended. Histological examination of fragments taken from four different sections of the small intestine showed that, in certain villi, detachment of the epithelium from the stroma took place, although in none of the sections of the small intestine in all the rats investigated were villi with a completely denuded stroma found. With distension of the lumen of the intestine, and consequent separation of the villi from each other, in a modification of the experiment, similar results were obtained. Small areas of different sections of the intestine (in 3 dogs and 5 rats) were ligated on two sides with silk thread, and physiological saline or fixing agent was injected into the lumen of the bowel until the muscular wall was distended. As controls, neighboring areas of the intestine were used, which were not ligated nor filled with fluid. Villi deprived of their epithelium were never visible in the distended areas (Fig. 1). In the control areas a different picture was often visible, with disturbance of the integrity of the epithelial layer, as has been described in detail by other authors [3, 4, 6].

This group of experiments also includes the fixation of fragments of intestine (of 50 rats, 30 guinea pigs, and 3 *Macacus rhesus* monkeys), followed by cutting into segments and placing over a strip of cardboard without stretching. As a result of the contraction of the muscularis mucosae, the mucous membrane was everted. By the use of various fixatives (Bouin's fluid, 10% formalin solution, Carnoy's mixture) we never once observed villi with a denuded stroma.

The prevention of contact between the apices of the villi thus prevented the separation of the epithelial layer

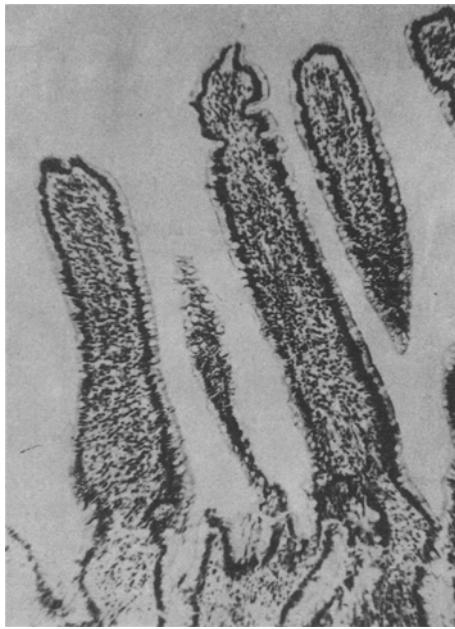


Fig. 1. Intestinal villi of a dog, the walls of the intestine being stretched with physiological saline. The epithelial layer is in close contact with the stroma and plicae are visible on the lateral surfaces. Magnification $7 \cdot 20$. Stained with hematoxylin-eosin.

from the stroma of the villi and the denudation of the stroma as a result of fixation. We consider that, because of this, two hours after rats have been taken food and the intestine is distended by the juices of the digestive glands, the patterns of detachment of the epithelial layer and denudation of the stroma of the villi is not observed in histological preparations [1].

Prevention of the contraction of the muscle fibers of the villi at the moment of fixation must lead [20] to the obtaining of normal patterns of structure of the intestine. This may be achieved in various ways. We cooled pieces of intestine (from 5 rats) for periods of from 10 minutes to 2 hours at $+4^\circ$ and fixed them in formalin at room temperature. On histological examination, the structure of the villi was found to be normal, and they were elongated in shape. The same result was observed after fixation of pieces of intestine (5 rats and one dog), at a temperature close to that of the body, in formalin cooled to $+4^\circ$.

In the intestine of rabbits, Z. M. Gadzhieva [4] did not observe the desquamation of large sheets of epithelial cells, and she considers that there are no muscle fibers in the stroma of the villi in rabbits. It seems to us that this would also explain the absence of formation of obvious epithelial tubes.

On the assumption that fixation of the intestine at a moment when the muscle fibers were contracted might

disclose the pattern of the relationship between the epithelial layer and the stroma, we perfused the intestine (of 5 rats) with warm physiological saline at a pressure of 30-35 mm Hg for 5 min, and with Bouin's fluid for 10 min. It will be seen from the photomicrograph (Fig. 2) that the epithelial layer is in close contact with the stroma; the muscle fibers are round in shape, i.e., they are contracted. After fixation of the intestine in Bouin's fluid, immediately after sacrifice of rats (5 animals) kept on the same diet, the typical pictures of desquamation of the epithelium from the stroma of the villi were seen (Fig. 3), which were described by Z. M.

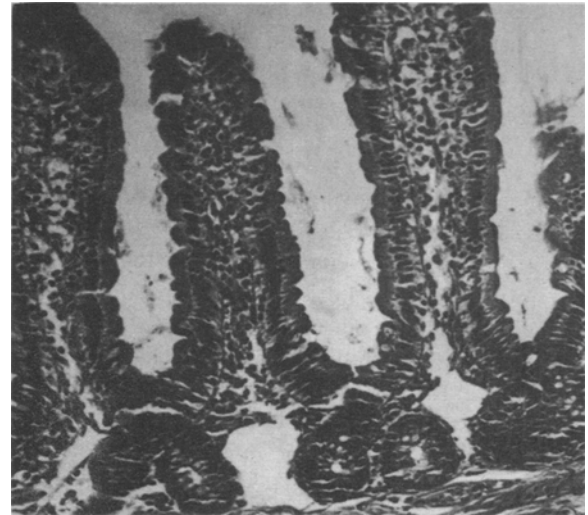


Fig. 2. Villi of a rat's intestine. Irrigation with physiological saline and fixation. No detachment of the epithelium from the stroma. Plicae of epithelium are seen. Muscle fibers round in shape. Magnification $7 \cdot 40$. Stained with hematoxylin-eosin.

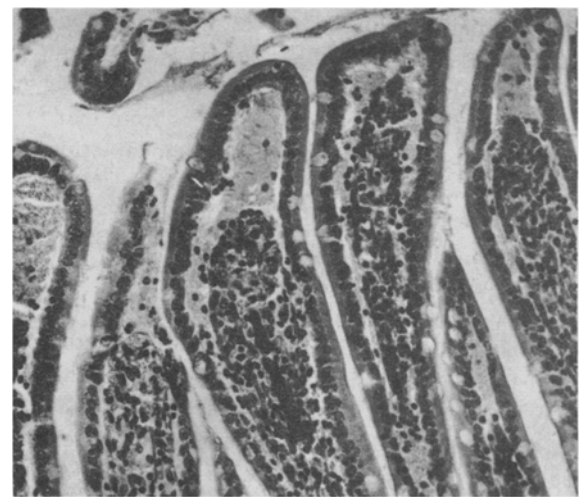


Fig. 3. Villi of a rat's intestine, fixed immediately after sacrifice. Detachment of the epithelium from the stroma is seen. Magnification $7 \cdot 40$. Stained with hematoxylin-eosin.

Gadzhieva [3, 4] as "elements of the functional morphology of the small intestine". Irrigation of the intestine in dogs for the same period of time did not completely abolish the phenomenon of detachment of the epithelium from the stroma. This may evidently be due to the more powerful musculature and to the well-developed Kerkring's valves, for in places where these valves were only feebly developed, the villi were completely covered by epithelium.

In order to verify the fact that villi with denuded stroma were not present in the normal intestine, the intestine of 5 rats were perfused with physiological saline for 5-10 min. The intestine was then separated from the mesentery, incised longitudinally, placed over cardboard with the mucous membrane outermost, and fixed in Bouin's fluid. Examination with the stereoscopic microscope (MBS-1) failed to show a single villus without epithelium although the epithelial layer could readily be separated and detached at the apices of the villi by means of a dissecting needle.

The same picture was observed during the examination of the mucous membrane of different portions of the intestine (in 3 *Macacus rhesus* monkeys) immediately after sacrifice, by means of the stereoscopic microscope.

Z. M. Gadzhieva [3] carried out histological investigations of the intestine of rats without preliminary irrigation, and found detachment of the epithelium and denudation of the stroma in certain areas of the small intestine. If this was not an artefact of fixation, we undoubtedly should be able to find desquamation of the epithelial layers in our experimental rats, even though only in a few places.

We performed experiments on five rats, in which we examined the intestine under the stereoscopic microscope during life. For this purpose, after starving the rats for 48 hours, we opened the abdomen under ether anesthesia; a small area from the proximal division of the jejunum was excised at the opposite border to the attachment of the mesentery, and its edge was sutured to the skin. The mesentery of this area of intestine was left behind in the abdomen. The mucous membrane was irrigated with physiological saline and kept in a moist and warm condition throughout the period of observation. For six hours we found no essential changes in the external appearance of the villi, and their blood supply was well maintained. No signs of detachment of the epithelium were present.

Similar experiments were conducted on three rats which had taken food, the observations lasting four hr, and in no case did we find any changes in the epithelial layer of the villi.

When the data of M. I. Razumov and Z. M. Gadzhieva is analyzed, it is easy to find a number of contradictions in their conclusions. They associate the phenomenon of desquamation of the epithelial cells with the secretion of the solid part of the intestinal juice. In their

view, living, unchanged cells are detached, whereas in the juice secreted periodically there are found cells undergoing destruction, with pyknosis or karyorrhexis of their nuclei.

The epithelial plicae on the lateral surfaces of the villi were interpreted by M. I. Razumov as an accumulation of epithelial cells, preceding the formation of epithelial tubes. Other authors [20] considered that these plicae are the result of the contraction of muscle fibers, leading to a reduction of the volume of the stroma. The same state of affairs could be seen in our own preparations, in which irrigation of the intestine with warm physiological saline caused contraction of the muscle fibers of the stroma (Fig. 2).

For the proof of a number of his postulates, M. I. Razumov had to admit that renewal of the epithelial cells takes place as a result of their formation from the reticular stroma. This assumption is contradicted by numerous morphological findings, collected in the course of the last decades.

On the basis of data in the literature and of the results of our experiments, we conclude that the phenomena described in the papers by M. I. Razumov and Z. M. Gadzhieva must be regarded not as "elements of the functional morphology of the small intestine" but rather as artefacts arising as a result of autolysis, fixation and subsequent histological treatment of areas of the intestine without taking into consideration the possible disturbance of structural relationships. It hence follows that, during the analysis of the phenomenon of detachment of the intestinal epithelium, i.e., of the destructive phase of its physiological regeneration, and of certain of its characteristic pathohistological patterns, the possibility of the production of artefacts must be taken into consideration.*

SUMMARY

As shown on rats, dogs, guinea pigs and monkeys, detachment of the epithelial layer from the stroma of intestinal villi with exposure of the stroma is actually an artefact. The latter may occur in autolysis, fixation and histological treatment of material. The normal inter-relationship of the epithelium and the stroma may be obtained by fixation in a cooled solution or by stretching the intestine, thus preventing the villi from coming in contact with each other.

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