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MICROSCOPIC STRUCTURE OF ALBINO RATS' SMALL INTESTINE

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ABSTRACT

Introduction: The small intestine of albino rats is a transitive canal between the stomach and the cecum that is closely located from each other, reaches a length of one meter, which in comparison ratio to body weight significantly exceeds the corresponding segment in humans.

The aim: The paper is aimed at thorough histological study of the wall and structure of albino rats' small intestine mucosa.

Materials and methods: 30 mature albino male rats were involved into the study. The specimens of albino rats' small intestine, fixed in 10% neutral buffered formalin solution, have been studied. The study was carried out using conventional histological methods for obtaining serial paraffin sections stained with hematoxylin-eosin. Epoxy plastination of individual tissue samples of the small intestine was performed. Subsequently, polished thin sections were made, stained with 1% methylene blue and 1% borax solution. The obtained specimens were studied on the "Konus" light microscope equipped with Sigeta DCM-900 9.0MP digital microphoto attachment with the Biorex 3 software adapted for studies of such type.

Results and conclusions: For the first time in the practice of histological study of the epithelial covering of the mucous membrane of the small intestine, attention is drawn to the specific pattern of its organization on the intestinal villi. It has been found that epithelial covering consists of alternating cluster epithelial aggregations separated by fissured depressions. Since no mentioning about them has been found in the publication, these cluster aggregations of enterocytes can be called epithelial buds of the intestinal villi. Consequently, it can be concluded that with the exception of some specific morphological features, the small intestine of albino rats is homologous to human one by its histological structure, which means that it can be used as a model for various experimental studies.

KEY WORDS: small intestine, epithelial buds, enterocytes, albino rats

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INTRODUCTION

The small intestine of albino rats is a transitive canal between the stomach and the cecum that are closely located from each other, reaches a length of one meter, which in comparison ratio to body weight significantly exceeds the corresponding part in human [1, 2, 3, 4, 5, 6, 7]. However, publications report that they are considered to be completely homologous by the histological structure of their wall, which proves the validity of the experimental modeling of the pathological processes of the small intestine on albino rats [8, 9, 10]. And yet, with a more demanding approach to the data available in the literature, a number of questions arise that cannot be neglected when trying to increase the reliability of the findings of experimental studies and, thus, verify the extrapolation of their validity to human one.

THE AIM

The paper is aimed at thorough histological study of the wall and structure of albino rats' small intestine mucosa.

MATERIALS AND METHODS

30 mature albino male rats weighted 200,0±20,0 g were involved into the study. The specimens of albino rats' small intestine, fixed in 10% neutral buffered formalin solution, have been studied. Euthanasia of rats was made under thiopentone anesthesia overdose (75 mg / kg animal body weight intramuscularly in the upper third of the hip of the hind paw) [8, 11].

Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) at the Ukrainian Medical Stomatological Academy in compliance to the regulations on keeping experimental animals adopted by the European Parliament and Council Directive (2010/63 / EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the V National Congress on Bioethics (Kiev, 2013), (Minutes No. 155 as of 26.04.2017 of meeting the Commission on Biomedical Ethics at Ukrainian Medical Stomatological Academy) [12, 13, 14].

The study was carried out using conventional histological methods for obtaining serial paraffin sections of 4 μm thick (Microm HM 325), stained with hematoxylin-eosin. Additionally, methods of epoxy plastination of individual tissue samples of the small intestine were used. Subsequently, polished thin sections were made, stained with 1% methylene blue and 1% borax solution [15, 16, 17]. The obtained specimens were studied on the "Konus" light

microscope equipped with Sigeta DCM-900 9.0MP digital microphoto attachment with the Biorex 3 (serial number 5604) software adapted for these studies. Morphometric studies were performed using the system for visual analysis of histological specimens.

RESULTS

Considering the great length of above region of the gastrointestinal tract of albino rats, it is possible to restrict studying of the autopsy material to only three of its selected portions, namely, 1) the duodenum; 2) small intestine between the Peyer's patches and 3) in the zone of Peyer's patches localization.

The findings of the study has established that the wall of the albino rats' duodenum consists (if not considering the finest outer covering in the form of adventitia or serous membrane) of only two membranes, namely, mucous and muscular. In this regard, the first one is about three times thicker than the second one (Fig. 1). If the muscular layer is a simple uniform layer (about 10 layers) of the fusiform smooth myocytes, then the mucous membrane is rich in content of various structures, but among them entirely muscle plate is located. Consequently, its lamina propria is directly associated with the muscular layer, i.e., in the wall of the duodenum of albino rats the submucosa does not exist at all, in contrast to human one. Therefore, all the glandular structures of the mucous membrane, such as the crypts of Lieberkühn and the Brunner's glands, are entirely localized in its lamina propria. It has long been known that the first of them are tubular invaginations of the epithelium, originating between the intestinal villi and extend down into the lamina propria (in albino rats this corresponds to the border with the muscular layer). These formations are notable for the Paneth cells contained in their walls, which produce the anti-microbial enzyme lysozyme (muramidase), which is commonly assigned to the humoral factors of the innate (nonspecific) immunity. Interestingly, scarce intraepithelial lymphocytes reside among the Paneth cells at the basal portion (Fig. 2). Notably, in the duodenum of albino rats, crypts of Lieberkühn also serve as the excretory ducts for the mucous Brunner's glands, entering them, the acini of which are in the form of compact glomeruli between the basal portions of the crypts and the muscular layer.

In contrast to the proliferated (invaginated) crypts, the mucous membrane of the duodenum forms numerous villous projections, which, among other similar formations of the small intestine, are characterized by a variety of shapes and large sizes. Figure 3 shows that such formations are bushy-branched, so that a common base and a number of short branches can be identified in each villus. They are based on connective tissue papillae, which are derivatives of the lamina propria of the mucous membrane, covered with simple columnar epithelium. Compacted bundle of collagen fibers is located in their axial position, surrounded by a fine-fiber network of reticular fibers, containing predominantly lengthwise amorphous substance, within which blood microvessels of the capillary type are found. Publications report that no lymph vessels of the appropriate caliber are found in the duodenal villi. Noteworthy, this interstitial space, occupying the place between the axial connective tissue induration, and the covering epithelium is abundantly infiltrated by the lymphocytic elements.

It is known that the epithelial covering of the mucous membrane of the small intestine is monostratal. However, its description, available from the publications, does not entirely coincide with the observed pattern during the study of histological specimens, on which the covering epithelium of the intestinal villi is not uniformly organized. Magnified microimages clearly show that it has an uneven, tuberous relief on its external surface, due to the fact that the components of its epitheliocytes are distributed in separate cluster aggregations, which are separated by fissured depressions (Fig. 4). Since no mentioning about them has been found in the publication, these cluster aggregations of enterocytes can be called epithelial buds. Each epithelial cluster consists of a certain number of high (columnar) cells, located very close to each other, the borders between which, even in higher magnifications of the light microscope, can hardly be identified. They are better identified by their nuclei, which, being in a row, form a kind of twisted chain that repeats the form of convex surfaces of epithelial buds and depressions between them. Due to the fact that the intestinal epitheliocytes are closely interconnected, their brush borders form a continuous common coating on the convex surfaces of the epithelial buds, interrupting only in the zones of fissured depressions between them. It is well known that the brush borders of enterocytes are formed by numerous microvilli in the form of finest projections of the apical plasmolemma. Such type of increasing the area of the plasma membrane is characteristic of cells carrying out transmembrane transport of substances. This suggests that in the duodenum, the process of absorbing the products of hydrolysis of high molecular nutrients is performed in the zone of free surfaces of epithelial buds, whereas in the zones of fissured depressions such process cannot occur because of the absence, according to the findings of the research, of brush borders. Therefore, the question arises about the role of these depressions in the digestive process of the duodenum. However, it is not known whether such a structural organization of the intestinal villi epithelium is peculiar in other laboratory animals, and, moreover, that it is particularly significant in humans. To date, no solution to such issues has been found.

Essential formations of the mucous membrane of the mesenteric part of the small intestine of albino rats are represented by innumerable intestinal crypts, residing in the lamina propria and bordering its basal portions with the muscular layer. Their shape and cytological composition are similar to the aboveglands of Lieberkühn, which, as in the duodenum, open with narrow orifices between the villi. In contrast to latter, the mucous (Brunner's) glands are not found in the rest of mesenteric portion of the small intestine. Apparently, their absence is compensated by the diffuse distribution of mucous cells among the epithelial

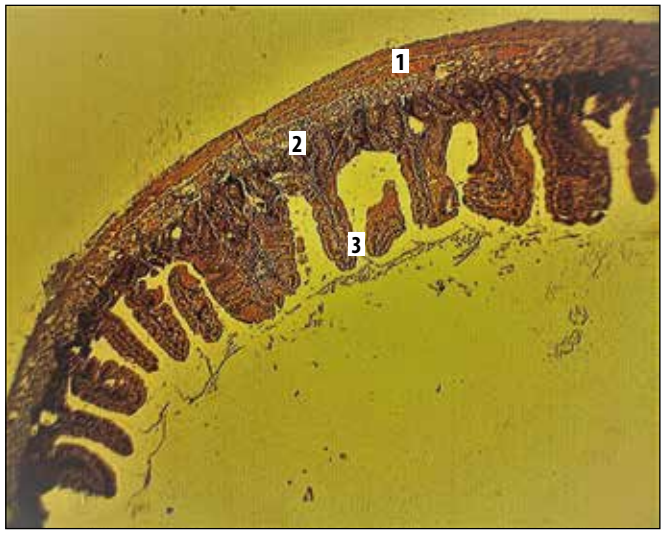

Figure 1. The wall of duodenum of albino rat. Paraffin section; H&E stain; 4× magnification.
1 – muscular layer; 2 – mucous membrane; 3 – intestinal villi.

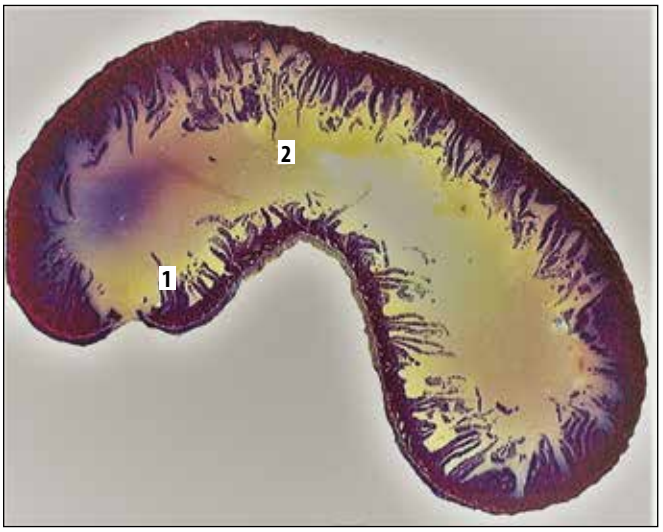

Figure 3. Villous projections of the albino rat's duodenal mucosa. Epoxy slice in tangential section; methylene blue stain; 2× magnification.
1 – villi of the mucosa; 2 – inner lumen.

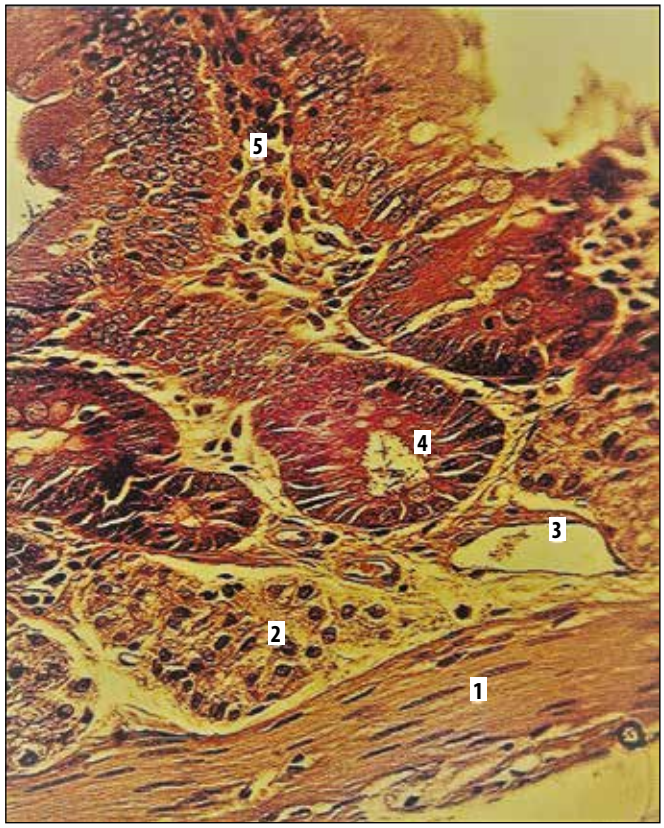

Figure 2. Microscopic structure of the wall of albino rat's duodenum. Paraffin section; H&E stain; 40 × magnification.
1 – muscular layer; 2 – acini of the mucous (Brunner's) glands; 3 – blood microvessels; 4 – basal portions of the crypts of Lieberkühn; 5 – lymphocytic infiltration of the connective tissue.

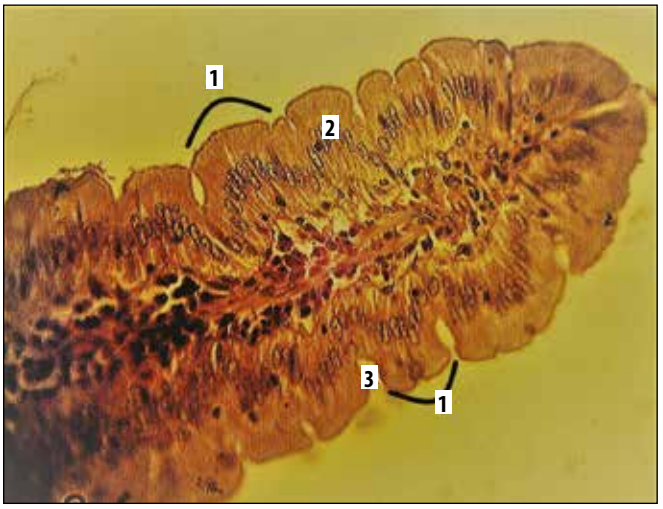

Figure 4. The structure of the villus of the duodenal mucosa. Paraffin section; H&E stain; 40× magnification.
1 – epithelial buds; 2 – nuclei of prismatic, columnar epithelial cells; 3 – fissured depressions between epithelial buds.

cells of the intestinal villi, which are variable in shape, but of the same type in their internal structure. It should be noted that they differ from similar formations of the duodenum by the presence of centric lymphatic microvessel in their connective tissue, around which connective tissue is diffusely infiltrated by the cells of the lymphocyte series, from the mass of which migration of individual lymphocytes to the covering epithelium is noted (Fig. 5). Moreover, among these cells, sporadic transverse and oblique profiles of blood microvessels are found. Furthermore, the intestinal villus of the covering epithelium is organized in the form of cluster aggregations that can be called epithelial buds, similar to duodenum ones, though sporadic mucous cells are found among their prismatic columnar epithelium (in contrast to the duodenum).

In addition, solitary lymphoid nodules are found in the intermediate zone of the small intestine between the Peyer's patches in an irregular order. Noteworthy, these lymphoepithelial formations, occupying the entire thick-

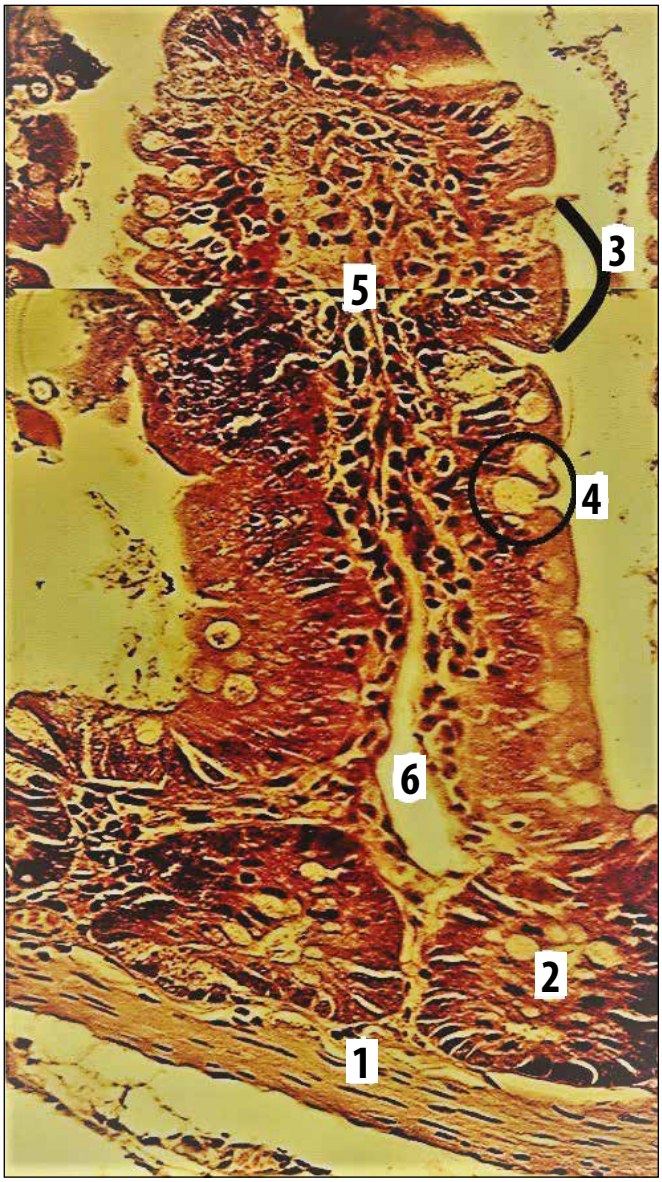

Figure 5. The villus of albino rat's small intestine. Paraffin section; H&E stain; 40× magnification.
1 – muscular layer; 2 – the basal portions of the intestinal crypts; 3 – epithelial buds; 4 – mucous cells; 5 – lymphocytic infiltration of the connective tissue base of intestinal villus; 6 – lymphatic vessel.

ness of the mucous membrane, are surrounded by typical intestinal villi, being circumferentially connected with the basal portions of crypts. At the same time, the bases of these nodules are directly adjacent to the muscular layer, whereas their apical surface is covered with similar columnar epithelium, forming the small villi, which are not mentioned in the publications. Since the objective of our prospective investigations is the experimental study of the nature of structural changes in the lymphoid aggregations of the mucous membrane of the albino rats' gastrointestinal tract in the effect of antibiotics to its microflora, a more detailed description of their single and grouped nodules in its small intestine will be presented in the subsequent publications.

DISCUSSION

The small intestine is the longest part of the digestive tract, intended to perform the major functions in the process of the consumption of nutrients by the body, which are mainly products of enzymatic hydrolysis of proteins, fats and polysaccharides. The final phase of this process is the absorption of these nutrients into the internal environment of the body. The conveyor nature of digestion in the small intestine is generally expressed in the well-known anatomical distinguishing of such parts as the duodenum, jejunum and ileum, the length of which in a human is approximately (separately) is 30 cm - 2 m - 3 m, respectively. Evidently, the length of the rat small intestine is much smaller. However, the data reported in the literature are questionable. Thus, according to them, the length of the rat duodenum is comparable to that of a human one, and the rest of its small intestine is approximately equal to 1 meter. Consequently, the entire rat small intestine is only by 4-5 times shorter than that of a human one. Obviously, in a limited volume of the rat abdomen of such a length, the digestive tube can fit if it is sufficiently thin [18, 19]. Unfortunately, no data on its thickness have been found in the literature to date. It is known, the transverse size of the human small intestine varies in length from 2 to 3 cm [2, 4].

The mucous membrane of the human small intestine, preserving its general principle of the structure, has not only its own distinctive features, but also differs in certain morphological features in its various parts. A common anatomical feature for it is the presence of numerous transverse-ring folds throughout it, due to the loose submucosa and smooth muscle tone. But their number and degree of manifestation gradually decreases towards the distal part of the ileum. In the duodenum, the folding of the mucous membrane is somewhat different in that from the side of the medial wall of its descending part where a permanent longitudinal fold is located, which inferiorly becomes higher and ends with a large papilla. It opens with one common hole of the bile duct and pancreatic duct. A little superiorly, the second papilla of a smaller size is located, on which the accessory pancreatic duct opens.

The mucous membrane of the small intestine, in the straightened state, is distinguished by a dull, velvety appearance, due to the numerous tiny (about 1 mm long) finger- or foliate processes called the intestinal villi [20, 21, 22]. The mucous membrane of the small intestine has a greatly developed local representation of the immune system in the form of intestinal lymphoepithelial clusters called lymphoid nodules. Mostly they are in the form of single nodules and their group clusters [9, 10, 23].

CONCLUSIONS

1. The wall of the small intestine of albino rats, in its entire length (about one meter) from the pyloric part of the stomach to the place of the entering to the cecum, consists of two, most pronounced, coaxial membranes, namely, mucous and muscular, which are not separated by a well-marked submucosa.

2. The essential basic structures of the entire intestinal tract of albino rats are simple tubular (Lieberkühn) glands, i.e. intestinal crypts, which in the duodenum, along with their particular secretory properties, serve as excretory ducts for the acini of the mucous (Lieberkühn) glands located between the muscular layer and the lamina propria of the mucosa.
3. The orifices of intestinal crypts are distributed regularly along the mucous membrane surface, being hidden in depth between the intestinal villi, the size of which steadily decreases in the caudal direction while maintaining, as is known from the publications, the typical principle of structural organization.
4. For the first time in the practice of histological study of the epithelial covering of the mucous membrane of the small intestine, attention is drawn to the specific pattern of its organization on the intestinal villi. It has been found that epithelial covering consists of alternating cluster epithelial aggregations separated by fissured depressions. Since no mentioning about them has been found in the publication, these cluster aggregations of enterocytes can be called epithelial buds of the intestinal villi.

Consequently, it can be concluded that with the exception of some specific morphological features, the small intestine of albino rats is homologous to human one by its histological structure, which means that it can be used as a model for various experimental studies.

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Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

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