MORPHOLOGICAL CHARACTERISTICS OF THE SMALL INTESTINE AND TRANSLOCATION OF THE INTESTINAL MICROFLORA IN THE POSTRESUSCITATION PERIOD

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Analysis of postresuscitation complications at autopsy frequently reveals a gastrointestinal syndrome, including petechial hemorrhages, erosions of the mucous membrane, and extensive foci of hemorrhagic necrosis of the small intestine [4, 7]. Damage to the intestinal barrier in terminal states may be indicated by the fact that infectious complications arising in such patients are caused by conventionally pathogenic enterobacteria, including *Klebsiella, Proteus, Serratia, Staphylococcus,* etc. [2, 8]. For instance, experiments on dogs recovering from clinical death caused by acute blood loss revealed bacteriemia due to Gram-negative bacilli and staphylococci [1, 6]. It is in the small intestine of resuscitated animals that the most marked structural and metabolic disturbances are found, amounting in some cases to the development of necrosis of epitheliocytes and of individual villi and the formation of erosions on the mucous membrane [5]. The above account suggests a connection between destructive changes in the wall of the small intestine and translocation of enterobacteria into various organs and tissues in terminal states. The aim of the present investigation was accordingly to analyze morphological changes in the mucous membrane of the small intestine and translocation of the intestinal microflora in the postresuscitation period.

EXPERIMENTAL METHOD

Experiments were carried out on 85 male and female rats weighing 170-220 g, recovering from clinical death due to acute blood loss for 5 min. Segments of the duodenum, jejunum, and ileum 0.8-1 cm long were removed from rats anesthetized with ether, 1 and 3 h and 1 and 3 days after resuscitation, and were kept in 10% neutral formalin. The dewaxed sections were stained with hematoxylin and eosin and structural elements of the mucous membrane subjected to morphometric examination [3]. Pieces of the corresponding parts of the small intestine were prefixed in a mixture of 1% glutaraldehyde and 4% formaldehyde in 0.05 M cacodylate buffer 1 and 3 h after resuscitation, postfixed in osmium tetroxide, and embedded in a mixture of Araldite and Epon 812. Ultrathin sections were examined in the YEM-100B electron microscope. Tissue homogenates from the liver, spleen, and mesenteric lymph nodes and blood taken from the chambers of the heart were subjected to bacteriological examination. Nonspore-bearing anaerobes were cultured in an anaerobic jar, filled with triple gas. Depending on the character of the colonies on selective media, staining by Gram's method, and morphology and biochemical properties, the strains thus isolated were identified by genus and species. The numerical results were subjected to statistical analysis by Student's t test, using a special program on the MK-61 microcalculator.

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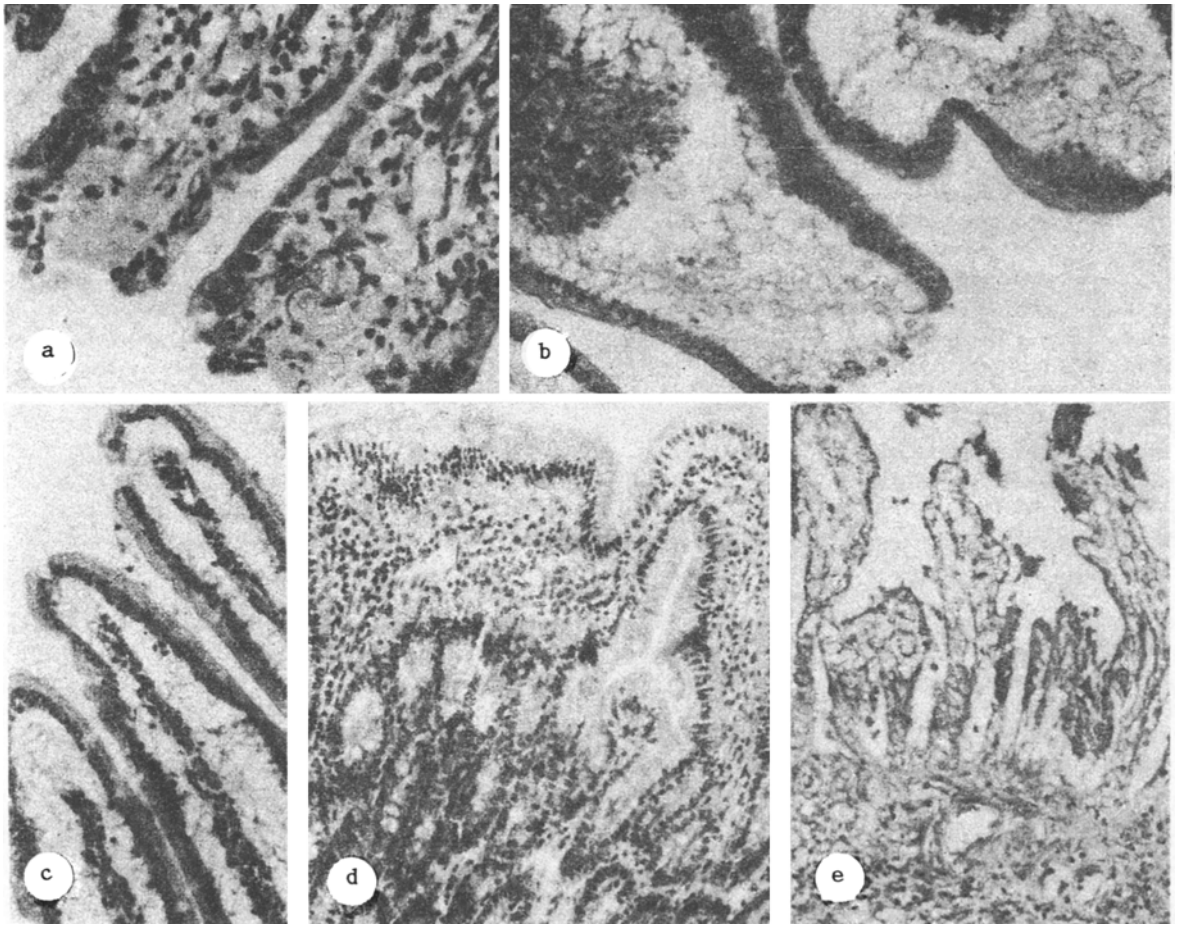

Fig. 1. Morphological changes in small intestine in postresuscitation period: a) after 1 h. Necrosis of apices of villi, desquamation of epithelium, edema of stroma. Hematoxylin and eosin. 100×; b) after 3 h. Distinct edema of lamina propria of villi, focal necrosis of epitheliocytes. Hematoxylin and eosin. 100×; c) after 3 days. Edema of lamina propria of villi. Hematoxylin and eosin. 50×; d) after 3 days. Deformation and fusion of villi. Hematoxylin and eosin. 100×; e) after 3 days. Necrosis of entire wall. Hematoxylin and eosin. 50×.

EXPERIMENTAL RESULTS

Inspection of the peritoneal cavity at autopsy, especially during the first day of the postresuscitation period, revealed atony of the small intestine, mainly of the ileum, sometimes with petechial hemorrhages on the mucous membrane, and with hemorrhagic contents; a transparent effusion was present in one rat, measuring 3-3.5 ml in volume, and bacteriologically sterile. Under the light microscope 1 h after clinical death, the most conspicuous feature was acute changes in the walls of the microcirculatory bed of the villi, consisting of spasm of the arterioles, congestion, and stasis in the venules. In the submucosa there were isolated cases of sludging in the venules and edema, with the accumulation of weakly eosinophilic fluid. The lymphatics of the villi were collapsed, their stroma cytogenous, and consisted of lymphocytes mixed with a few eosinophils. The cylindrical covering epithelium, especially on the apices of the villi, became flat and sometimes were completely desquamated, leaving the basement membrane bare (Fig. 1a). Changes also were found in the crypt region: shortening and deformation of the crypts and accumulation of large quantities of mucus. By the 3rd hour of the postresuscitation period the structural changes were still present, accompanied by edema of the villi, diapedesis of erythrocytes, and desquamation of the epitheliocytes, especially at the apices (Fig. 1b). Electron-microscopic studies at these times revealed ultrastructural changes in the epitheliocytes, more especially in cells with a brush border. Thus compared with the control (Fig. 2a), the latter showed disorganization of the microvilli, local areas of widening of the intercellular

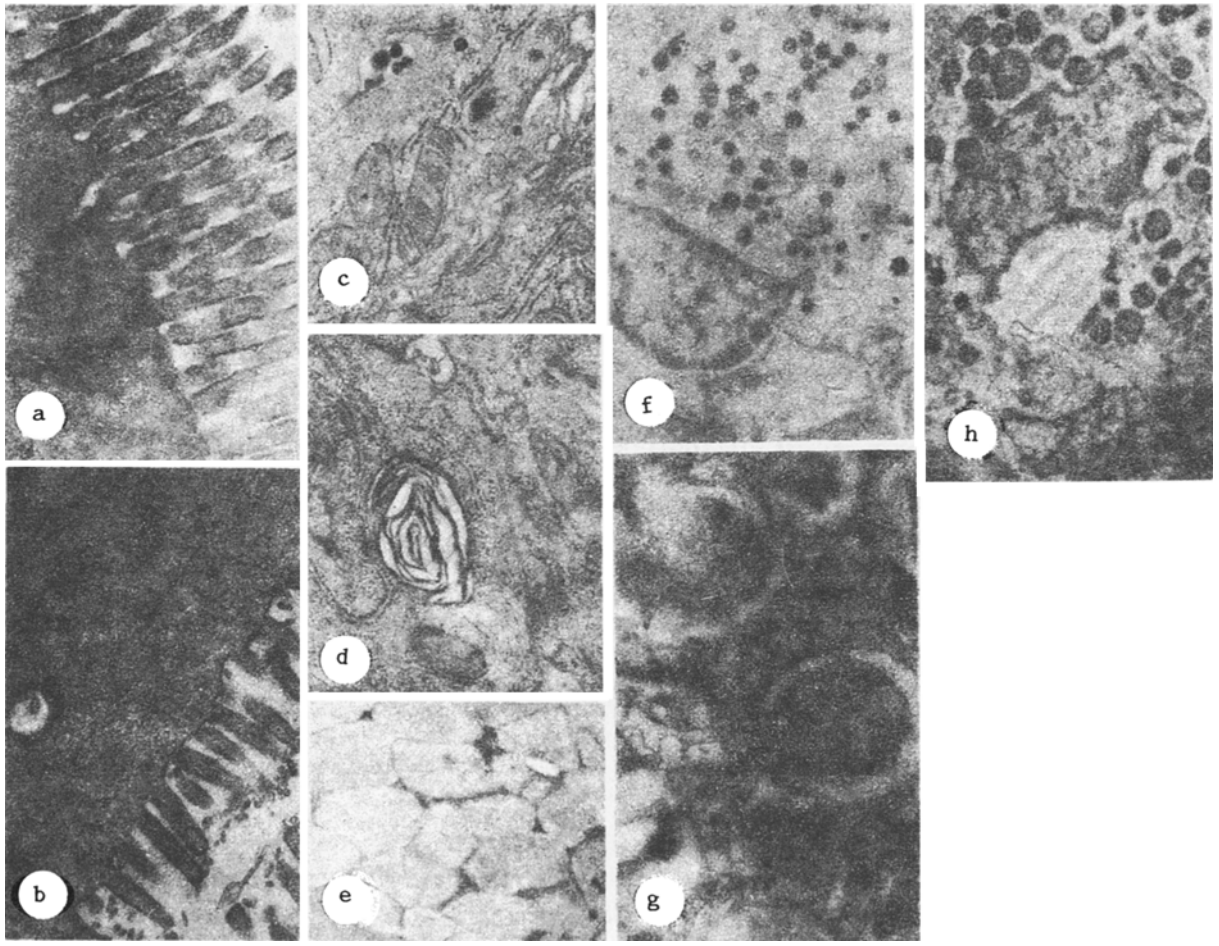

Fig. 2. Ultrastructure of various cells in epithelium and lamina propria of mucous membrane of rat ileum: a) apical part of brush-border epitheliocytes in a control rat. 22,000×; b) disorganization of microvilli of a brushborder epitheliocyte 3 h after resuscitation. 20,000×; c) mitochondria with widened intracristal spaces and local widening of intercellular spaces of brush-border epitheliocytes. 20,000×; d) local widening of intercellular spaces, myelinlike structure, slight degree of widening of rough endoplasmic reticulum of brush-border epitheliocytes. 20,000×; e) accumulation of secretory granules in goblet cells (e), type D endocrine cells (f), and Paneth's cells (9) 3 h after resuscitation; h) mast cell in lamina propria of mucous membrane 3 h after resuscitation. 12,000×.

spaces, numerous mitochondria with widened intracristal spaces in the cytoplasm, dilatation of cisterns of the rough endoplasmic reticulum, accompanied by its degranulation, and freely lying myelinlike structures (Fig. 2b-d). Processes indicating the accumulation of secretory granules in them were observed in the goblet, endocrine, and Paneth's cells (Fig. 2e-g). Many plasma cells, eosinophils, and mast cells could be seen in the lamina propria (Fig. 2h), and these cells quite often formed characteristic associations. After 24 h, besides destructive changes, regenerative processes could be seen in the mucous membrane, namely stratification of the epitheliocytes on the villi and gradual restoration of the epithelial cover (Fig. 1d). However, the edema and reduction of density of the villi were still present, together with flattening, deformation, and congestion of the venules in the submucosa. By the end of the 3rd day of the postresuscitation period, edema still persisted in individual villi (Fig. 1c). The epitheliocytes were homogenized at the apical poles, zones of translucency were seen around the nuclei, and in some places the nuclei were pycnomorphic. Desquamation of epitheliocytes at the apices of the villi was rare. Reparative processes increased in intensity. Morphometric analysis revealed significant quantitative changes in the structures of the small intestine in the postresuscitation period (Table 1). For instance, 1-3 h after resuscitation from clinical death, the villi were shortened by 1.5-2 times, and this was accompanied by cellular infiltration of the stroma and a decrease in the number of goblet cells. Toward the end of the 3rd day, no statistically significant difference

TABLE 1. Morphometric Parameters of Structural Elements of Mucous Membrane of Jejunum (J) and Ileum (I) in Postresuscitation Period (M ± m)

Group of animals	Part of intestine	Villi, μm		Crypts, μm		Cellular infiltration of stroma	Goblet cells
		height	width	depth	width		
Control	J	436,2±4,4	105,6±6,4	128,4±16,7	40,4±0,51	10340,5±3701,4	5,3±4,09
	I	266,7±32,9	134,4±47.6	100,8±19,6	32,9±5,6	10817,4±3498,6	9,82±3,50
1 h	J	175,56±17,18*	101,22±4,65	97,86±12,83	31,18±9,18	13308,4±5042,1	3,86±3,59
	I	186,2±21,7*	71,38±14,7	105,0±32,9	30,8±5,6	10615,9±3447,1	5,50±1,92
3 h	J	336,32±14,56*	102,48±7,05	95,9±29,4	32,34±1,35	10221,4±3035,5	5,54±1,02
	I	231,7±49,7	105,7±18,9	108,5±7,87	31,5±5,6	15160,6±4004,5	4,90±2,27
1 day	J	380,34±12,77*	111,06±6,17	129,22±13,85	35,42±0,75	99878,4±874,7	6,44±5,50
	I	242,3±32,9	96,6±14,7	100,8±36,4	32,2±4,9	12390,9±3781,6	14,53±4,30
3 days	J	418,46±40,69	107,94±1,79	116,54±11,64	29,56±6,83	10255,7±1180,5	6,80±4,64
	I	224,0±70,7	151,1±33,6	106,4±14,7	33,6±4,9	11044,6±3121,3	11,2±2,44

Legend. *$p < 0.05$ compared with control.

could be found in the morphometric parameters in the experimental and control groups of animals. In cases when after 3 days the state of the resuscitated animals still remained serious, gross destructive changes still remained in the wall of the small intestine. In the duodenal mucosa, for instance, incomplete restoration of the epithelial layer, desquamation on the apices of the villi, and marked edema and congestion were observed. In the ileum focal necrotic changes were found in the intestinal wall with homogeneous weakly eosinophilic imbibition (Fig. 1e).

Bacteriological investigation of homogenates of the mesenteric lymph nodes, liver, and spleen and blood cultures from 40 resuscitated rats yielded positive results with 23 animals. In six rats, moreover, translocation was observed as early as 3-9 h after recovery from clinical death, and after 3 days in 17 rats. In 15 of 23 animals translocation of 48 strains was observed into the mesenteric lymph nodes, in 10 rats 25 strains were seeded from liver and spleen tissue, and in six rats blood cultures revealed six strains. Depending on the frequency of translocation the microorganisms were distributed in the following order: *Escherichia* 25 strains, *Proteus* 11, *Lactobacillus* eight, *Bifidobacterium* seven, *Enterococcus* six, *Aerococcus* five, *Veillonella* five, Gram-positive aerobic bacteria five, *Staphylococcus* four, *Klebsiella* two, *Serratia* one strain. *Escherichia,* Gram-positive bacteria, and *Staphylococcus* were isolated from the blood of three, two, and one rats respectively. Meanwhile, associations of microorganisms, numbering 2-6 species, underwent translocation into the test tissues. Morphological changes in the intestinal wall were compared with the character of translocation of microorganisms 3 days after resuscitation. Focal necrotic changes, affecting all layers of the wall, were found in the ileum of one of the rats, and six groups of microorganisms, including *Escherichia, Proteus, Enterococcus, Aerococcus, Lactobacillus,* and *Bifidobacterium,* were seeded from the mesenteric lymph nodes and the liver of this animal.

Translocation of the aerobic and anaerobic intestinal microflora into the mesenteric lymph nodes, liver, spleen, and blood during the first hours of the postresuscitation period is thus associated with destructive changes in the wall of the small intestine. If the general condition of the resuscitated animals was still serious after 3 days, structural changes persisted in the intestinal wall, leading to penetration of the internal medium by microorganisms. In the majority of rats, however, by this time repair processes predominated in the mucous membrane of the small intestine, and for that reason positive results of bacteriological investigation of tissue homogenates and blood of these animals may perhaps be attributable not to continued translocation, but to preservation of the viability of microorganisms which had penetrated into the organs and tissues of animals surviving the terminal state.

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RESULTS OF INTRAVASCULAR IMPLANTATION OF METHOTREXATE-SATURATED EMBOLI

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Inadequately researched methods of treatment of malignant neoplasms demand the study of new therapeutic approaches to the solution of this problem. The efficacy of targeted local application of cytostatics directly into the tissue of a neoplasm has been the subject of numerous trials. Solutions of cytostatics for this purpose are injected into an artery supplying blood to the tumor tissue [6], or endolymphatic introduction of cytostatics has been used [4]. All methods of targeted administration of cytostatics into tumor tissue so far tested have been distinguished by short term action of the drug, and accordingly, in order to achieve a definite therapeutic effect, they usually have to be given repeatedly. If cytostatics are administered by these methods, just as when given in the normal way, without targeting, they give rise to marked toxic effects on hematopoiesis, the gastrointestinal tract, and other organs, making the patient's state serious [5]. It is also irrational to administer expensive therapeutic preparations by these methods.

The idea of targeted local administration of cytostatics by occluding the blood vessels of the tumor by emboli containing a therapeutic substance which would not pass through the blood vessels or lymphatics of the tumor but which would remain in it for a longer time, thus prolonging its cytostatic action, is attractive. The procedure would also inhibit growth of the tumor and would reduce its blood supply. Sufficient experience has now been obtained of the practical use of hydrogel emboli, based on polyhydroxyethylmethacrylate, for endovascular occlusion of blood vessels in a wide range of human diseases [1, 3]. Of all the emboli at present known, those from hydrogel have been found to be therapeutically optimal.

The aim of this investigation was to discover if hydrogel emboli can be saturated with methotrexate, a widely used cytostatic [5], and to study the character of histological changes in the vessel wall and surrounding tissue after endovascular implantation of the emboli.

EXPERIMENTAL METHOD

Synthesis of spherical particles based on poly-2-hydroxyethylmethacrylate has been described previously [7]. Hydrogel particles measuring 0.4-0.6 mm, washed to remove unreacted monomer, were activated in dioxan by means of *p*-nitrophenylchlorformate at 4°C for 4 h. After removal of the nitrophenol formed in this way from the hydrogel, activated hydrogel was reacted with hexamethylenediamine at room temperature for 8 h. The washed hydrogel, modified by the diamine, was added to a saturated aqueous solution of methotrexate, which was completely adsorbed on the polymer matrix.

Department of Pathomorphology, All-Union Center for Dressings, Suture, and Polymer Materials in Surgery, and Department of Vascular Surgery, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 10, pp. 439-441, October, 1991. Original article submitted October 5, 1990.