

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE DUODENUM IN THE EARLY STAGES OF EXPERIMENTAL COLIBACILLOSIS

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Despite definite progress in the study of the widely spread disease colibacillosis, many aspects of the development of this infectious process and the role of certain factors of immunity still require further investigation [5, 7]. Among the many factors influencing the local immune response of the intestine, attention has been drawn to a possible role of mast cells and cells of the APUD system in the complex mechanism of autoregulation of immunity. We know that almost half of the cells of this latter system are located in the gastrointestinal tract and, in particular, in the duodenum [4]. It is the proximal part of the small intestine, for which enterotoxigenic strains of *Escherichia coli* are considered mainly to multiply, that is regarded as the most sensitive part to the action of enterotoxins [6, 8]. We have studied morphological and physiological changes in the wall of the duodenum in the early stages of experimental colibacillosis.

EXPERIMENTAL METHOD

Experimental colibacillosis was produced by the method described previously [1]. Altogether 45 mice were used in the experiments. The comparison group consisted of five mice receiving physiological saline perorally instead of a culture of *E. coli*. Pieces of tissue from the duodenum were taken 1, 3, 6, 8, 14, 18, and 24 h after infection, and some of the material was fixed in cold 1% acetic acid solution in ethanol, some in 10% neutral formalin solution, and it was embedded in paraffin wax. To prepare semithin sections the material was fixed in a 2.5% solution of glutaraldehyde, embedded in Epon-Araldite, and stained with azure A and fast green. Paraffin sections were stained with hematoxylin and eosin, by Brachet's and Grimelius' methods, and with toluidine blue. To detect immunoglobulin-containing cells, enzyme immunoassay was used [2]. The relative percentage of interepithelial lymphocytes (IEL), the overall density of cellular infiltration (ODCI), and the number of plasma cells (PC), immunoglobulin-containing cells (IGCC), and endocrine cells (EC) per unit area were determined morphometrically. The mast cell (MC) population was characterized by a combined morphometric approach [3]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Histologic investigation of the duodenum 1 h after infection revealed signs of inflammatory edema, and the lymphatics were grossly dilated and the blood vessels filled with erythrocytes (Fig. 1a). Among the epithelial cells of the villi only single cells with degenerative changes could be seen. Already in this early period inflammatory infiltration of the lamina propria of the mucous membrane had developed (Table 1). Among cells in the focus of infiltration most were lymphocytes and plasma cells (Fig. 1b), but sometimes single macrophages and stab cells were found, and of the plasma cells 21% contained immunoglobulins. The number of IEL was increased somewhat (25 ± 4 compared with 21 ± 2 in the control; $p < 0.05$). The number of EC detected by the presence of the histochemical reaction product in argentaffin granules, was much smaller than

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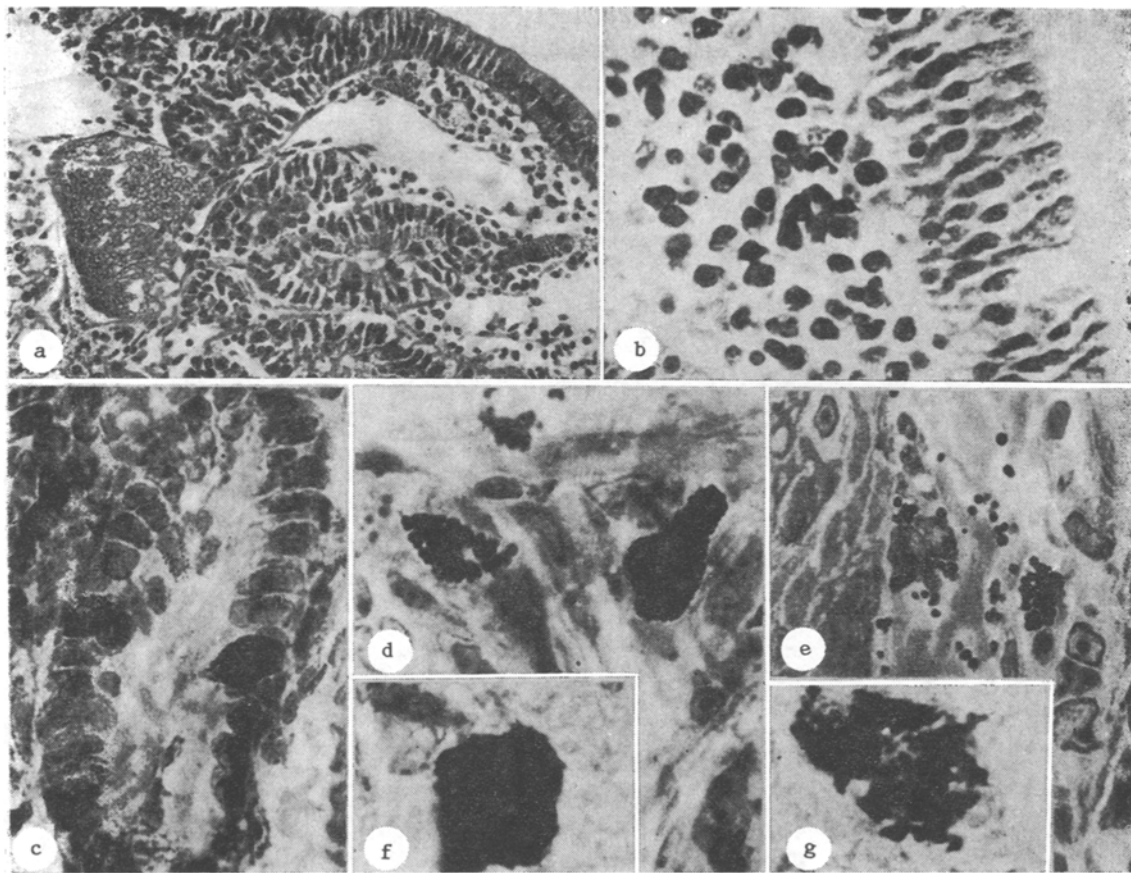


Fig. 1. Morphological and physiological changes in duodenal wall 1 h after infection: a) dilatation of lymphatics and blood vessels. Hematoxylin-eosin, 102 \times ; b) plasma-cell infiltration of stroma of villi. Brachet's method, 315 \times ; c) endocrine cells in duodenal crypt. Grimelius' method, 300 \times ; d-g) mast cells in duodenal submucosa in control (d, e) and their degranulation in experiment (f, g); d, f) azure A and fast green, semithin section. Magnification: d) 788 \times , f) 630 \times ; e, g) toluidineblue. Magnification: e, g) 630 \times .

TABLE 1. Morphometric Parameters of Duodenal Mucosa in Early Periods of Experimental Colibacillosis, Expressed Per mm² of Lamina Propria

Parameters	Times after infection, h				
	control	1	6	14	24
ODCI	12 645 \pm 407	17 528 \pm 1070***	14 689 \pm 419***	17 235 \pm 874***	18 083 \pm 613**
PC	5 110 \pm 660	7 747 \pm 237***	6 388 \pm 232*	6 122 \pm 303	6 602 \pm 368
IGCC	1 333 \pm 513	1 759 \pm 180	1 999 \pm 291	4 550 \pm 246***	4 516 \pm 369**
EC	243 \pm 15	88 \pm 6***	77 \pm 15**	90 \pm 23**	78 \pm 13**
MC	28 \pm 5	37 \pm 4	33 \pm 7	—	25 \pm 5

Legend. * p < 0.05 compared with previous time; ** p < 0.05 compared with control; —) not tested.

in the control. Besides cells packed with granules, many degranulated or even completely empty cells also were seen (Table 1; Fig. 1c). Characteristic changes took place in the MC population, in which pale cells began to be more numerous than dark, and the degranulation index was sharply increased on account of moderately and severely degranulated forms (Fig. 1d-g; Fig. 2).

Edema and stasis of erythrocytes still remained in the duodenum 3-6 h later. In some parts of the lamina propria, diapedetic hemorrhages were observed. Sometimes paving of leukocytes was found in the blood vessels. Cells of nerve plexuses showed degenerative changes, vacuolation of the cell cytoplasm, and in some cases, karyolysis and plasmolysis of

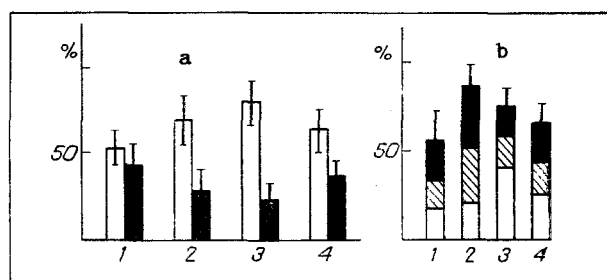


Fig. 2. Morphometric criteria of state of MC population in duodenum in early period of experimental colibacillosis: 1) control animals; 2) 1 h, 3) 6 h, 4) 24 h after infection; a) relative frequency of dark (black columns) and pale (unshaded columns) cells; b) index of degranulation and relative frequency of its forms: unshaded part of columns — weak, obliquely shaded — moderate, black — strong forms of degranulation.

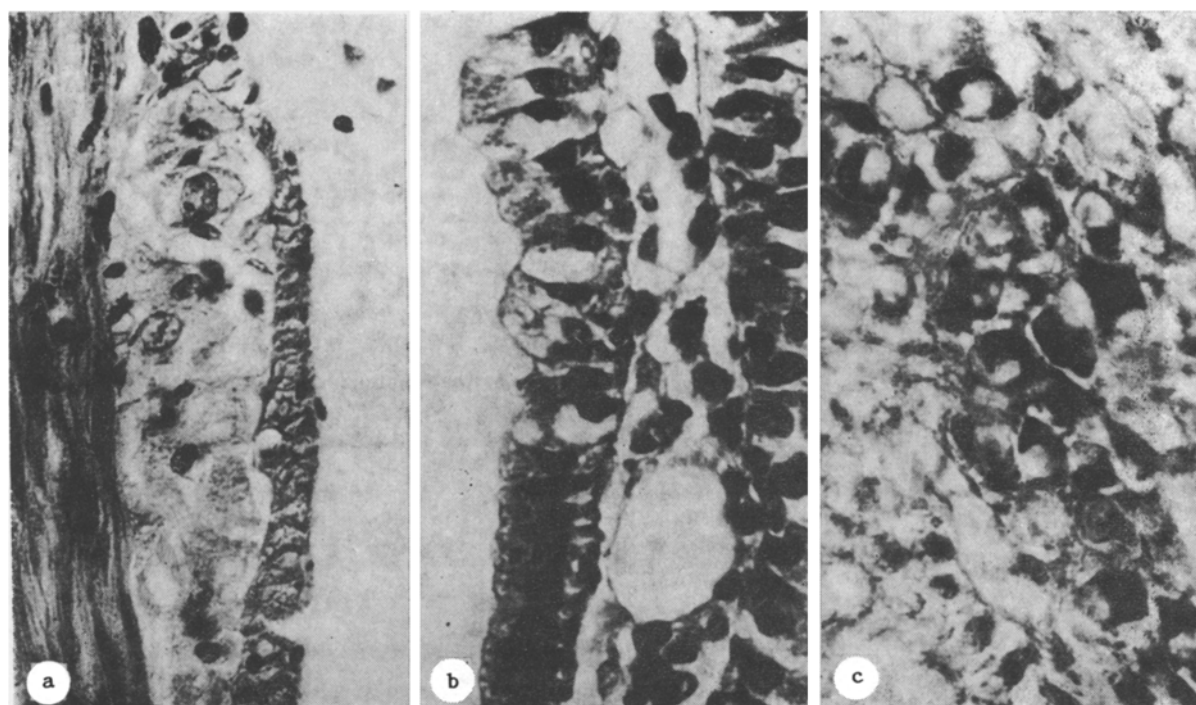


Fig. 3. Morphological characteristics of duodenal wall 14 h after infection: a) degeneration of ganglion cells of intermuscular nerve plexus. Hematoxylin-eosin, 256 \times ; b) vacuolar degeneration of epithelial cells of villi. Hematoxylin-eosin, 315 \times ; c) immunoglobulin-containing cells of lamina propria of duodenal mucosa. Immunoperoxidase method: 400 \times .

individual ganglion cells. During this period the inflammatory edema intensified in the region of the basement membrane, where microcavities formed in certain area. At the apices and on the lateral surfaces of individual villi freely lying lymphocytes, plasma cells, and desquamated epithelial cells could be identified. The ODCI was reduced a little, mainly on account of a decrease in the number of PC. Meanwhile, the number of IGCC among the latter increased to 33%. Degranulated forms predominated in the MC population, as before, with a relative decrease in the proportion of strongly degranulated and an increase in the proportion of weakly degranulated forms among them (Fig. 2).

The above-mentioned vascular changes were still present 8-14 h after infection. Edema of the villi of the mucosa was particularly marked in their upper part. Degenerative changes were increased in cells of the submucosal and intermuscular nerve plexuses (Fig. 3a). Vacuolation of cytoplasm of the epitheliocytes was observed not only on the apices of the villi, but also in the region of the crypt. Characteristic alternation of damaged and undamaged cells was observed in these cases (Fig. 3b). During this period ODCI of the stroma of the villi increased again, but the number of PC continued to fall. Meanwhile the number of IGCC among them increased appreciably (Fig. 3c).

Inflammatory edema became well marked in all membranes of the duodenum 18-24 h after infection. Infiltration by lymphocytes and plasma cells increased, but the number of IGCC and EC decreased. Toward the end of the first day the number of IEL also increased (31 ± 3 ; $p < 0.05$).

Thus only 1 h after infection it was possible to observe the development of an infectious process, expressed as disturbance of the circulation, increased cellular infiltration of the stroma of the villi, and intensive differentiation of lymphoid cells, characterized by a shift of histogenesis toward plasma cells, with biochemical induction of antibody formation. Direct participants in this process are the MC and EC of the duodenum, among which degranulated forms begin to predominate, evidence of local hormone release. The data described above suggest that a reaction of delayed-type hypersensitivity of local character may play a role in the acute period of experimental colibacillosis, a possible explanation of which may be massive antigenic stimulation of the duodenum and the close connection of this organ with mast and endocrine cells.

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