MORPHOLOGIC AND FUNCTIONAL CHARACTERISTICS OF THE CECUM IN EXPERIMENTAL INFLUENZA

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A considerable number of studies of the alimentary tract in acute respiratory virus infections has now been undertaken and conclusive proof has been obtained that the intestine and, in particular, the epithelial cells of its mucous membrane, may be damaged by respiratory viruses [7-9]. However, it is not only destruction of epitheliocytes by the virus, but also its immunosuppressive activity that may cause activation of the conventionally pathogenic bacterial flora of the intestine and development of an inflammatory process, which is particularly important in relation to the vermiform appendix [5]. This state of affairs prompted the investigation described below, devoted to morphological and functional changes in the cecum in experimental influenza and, in particular, in its distal part, regarded as the likely morphological and functional equivalent of the human appendix [3, 11].

EXPERIMENTAL METHOD

Under ether anesthesia, influenza virus A (H_3N_2/Aichi/68) was injected intranasally into BALB/c mice in a sublethal dose of between 3 and 30 ID_{50}/0.1 ml. The animals were withdrawn from the experiment 3 days and 1 week after infection. Mice receiving 0.1 ml of physiological saline served as the control. Fragments of the distal part of the cecum were fixed in a 10% solution of neutral formalin and in a 1% solution of acetic acid in cold ethanol, and embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin, toluidine blue, by the methods of Brachet and Grimelius, and with Schiff's reagent. To assess the state of the mast cell population morphometric methods were used [2]. Immunoglobulin-containing cells were identified by the direct immunoperoxidase method [1]. The results were subjected to statistical analysis by variance methods, and the significance of compared mean values was assessed by Student's test. Pieces of the distal parts of the cecum for electron-microscopy were fixed in a mixture of 1% glutaraldehyde solution and 4% paraformaldehyde in 0.05 M cacodylate buffer. Postfixation was carried out in a 1% solution of OSO_4, followed by contrasting with lead citrate; the specimens were examined in the IEM-100B electron microscope (Japan).

EXPERIMENTAL RESULTS

Histologic investigation revealed characteristic changes of a cytopathic and vasospastic character in the tissues of the brain, trachea, bronchi, and the parenchyma of the lungs. Concentrations of oxyphilic exudate contaminated with cells of macrophage type were found in the lung tissue. Some of these cells had an oxyphilic or pale cytoplasm of foam type, in which tiny fuchsinophilic inclusions were found. In certain parts of the mucous membrane of medium-sized and small bronchi, papillary outgrowths of epithelium with marked dystrophic changes of the cells were identified (Fig. 1a).

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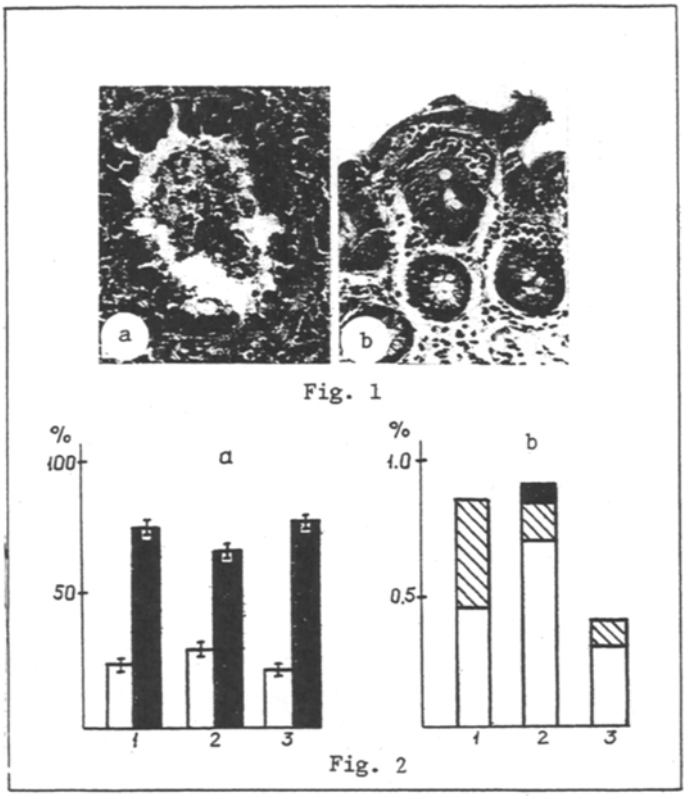

Fig. 1

Fig. 2

Fig. 1. Proliferative changes in epithelium of different organs in experimental influenza: a) papillary outgrowths in bronchial epithelium and peribronchial lymphoid infiltration (3 days after infection). Hematoxylin-eosin, 120×; b) formation of papillary outgrowths, dystrophically changed epitheliocytes, edema of lamina propria of mucous membrane (3 days after infection; hematoxylin-eosin. 100×).

Fig. 2. Morphometric criteria of state of mast cell population of cecum in experimental influenza. 1) Control animals, 2) 3 days after infection, 3) 7 days after infection; a) relative frequency of dark (black columns) and pale (white columns) cells; b) index of degranulation and relative frequency of its forms; unshaded part of columns – mild, obliquely shaded – moderate, and black – severe form of degranulation.

Groups of epitheliocytes with marked dystrophic changes and vacuolation of the cytoplasm, and with the formation of papillary outgrowths of epithelium resembling those in lung tissue were found on microscopic examination of the cecum (Fig. 1b). At sites of papillary outgrowths the dystrophically changed epitheliocytes were enlarged, round in shape, their cytoplasm was somewhat condensed and oxyphilic, and their nuclei were enlarged and somewhat translucent. Signs of marked edema and congestion of the vessels were seen in the lamina propria of the mucous membrane. Diapedetic hemorrhages could be seen in certain areas. The number of intraepithelial lymphocytes was doubled at this time ($p < 0.05$). A small increase in the number of goblet cells also was observed (Table 1). The total density of the infiltrating cells of the lamina propria of the mucous membrane was higher than in the control; 52.0 ± 22.3% of infiltrating cells of the lamina propria of the mucous membrane consisted of plasma cells, compared with 45.0 ± 2.2% in the control. An increase in the number of immunoglobulin-containing cells also was noted, up to 67.0 ± 21.0% of the number of plasma cells (34.0 ± 21.2% in the control). The number of endocrine cells was reduced by half and most of them contained a few secretory granules. Mast cells were located mainly around the vessels of the submucosa. Just as in the control, the majority of them were dark cells. The degranulation index was virtually unchanged, although at this time the ratio between the different types of degranulation was changed,

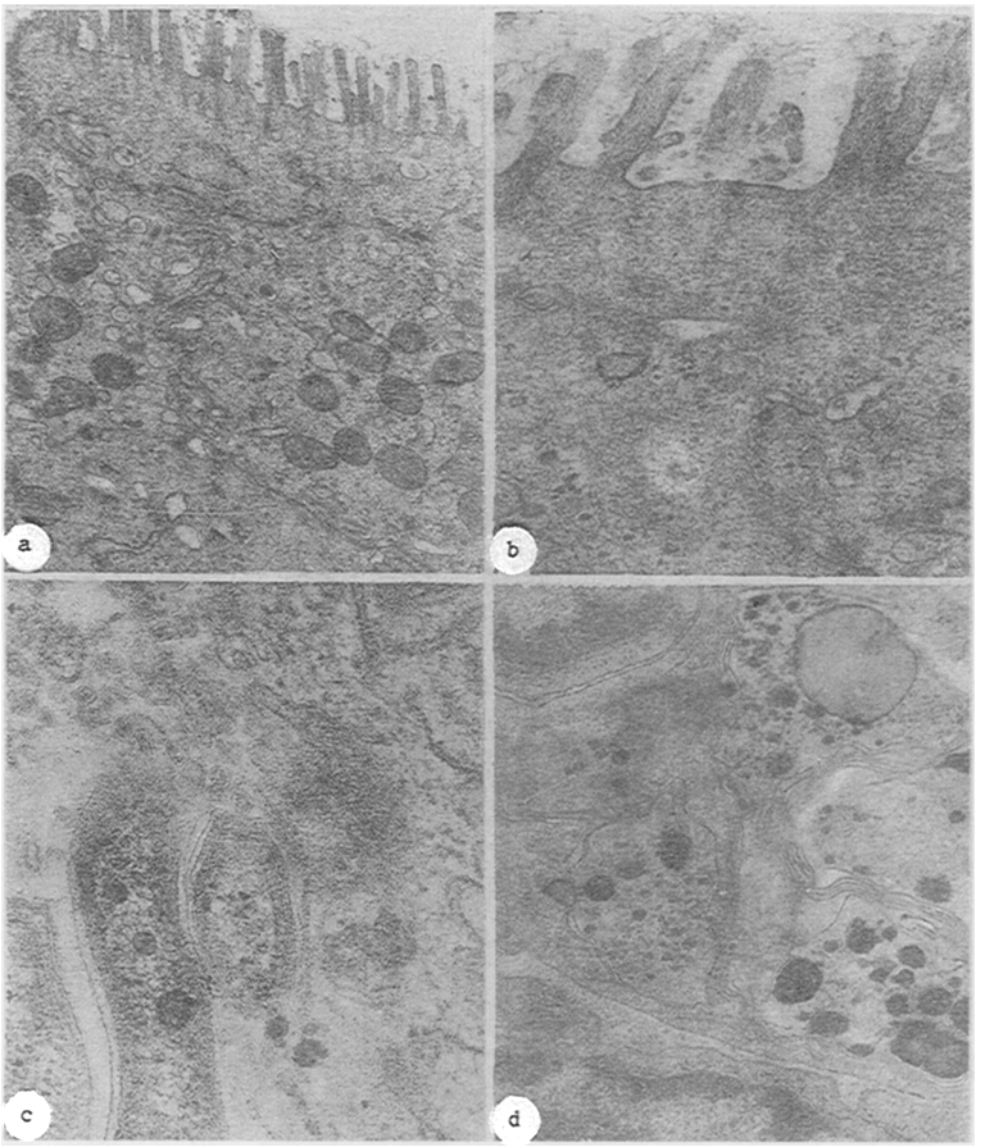

Fig. 3. Intracellular virus particles in experimental influenza 3 days and 1 week after infection: a, b) in cytoplasm, in free state and in microvesicles of brushborder epitheliocytes. Magnification: 13,000 (a), 21,000 (b); c) in modified mitochondria and in free state in cytoplasm of brush-border epitheliocytes. Magnification 28,000×; d) in cytophagosomes of a macrophage. 18,000×.

with an increase in the number of cells with severe and mild forms of degranulation and a marked decrease in the number of cells with the moderate form of degranulation (Fig. 2). Signs of marked edema, and dystrophic changes in the ganglia cells, amounting to karyolysis and plasmolysis were found in the nervous plexuses of the intestine, mainly the intermuscular plexuses.

A combination of ultrastructural changes of different types were observed at the same period of infection. In one group of brush-border epitheliocytes lying side by side their surface was enlarged due to active high microvilli and numerous interdigitations of the lateral plasmalemma. In one group of epitheliocytes either local and considerable widening of the intercellular spaces, or moderate widening but present over the whole of the lateral surface of the plasmalemma, were observed. Disorganization of the microvilli was often found in such cells, leading collectively to reduction of the cell surface of the epitheliocytes. Besides changes of destructive character (widening of the perinuclear space, translucency of the cytoplasm of individual cells) and partial degranulation of the rough endoplasmic reticulum (RER), processes of a compensatory-adaptive character also were observed, evidence of repair of the

TABLE 1. Morphometric Parameters of Cellular and Tissue Elements of the Cecal Mucosa in Experimental Influenza A (M ± m)

Parameter	Control	Time of infection	
		3 days	1 week
TDCI	13142±424	18730±656	18115±557*
PC	6022±778	9694±1250	11116±505*
IGCC	2022±770	6537±536	7236±438*
EC	28±5	14±3	17±4
MC	50,5±3,4	33,2±7,1	40,1±3,2
IEL	2,64±0,5	4,73±0,3	3,06±0,2
GC	12,9±3,2	17,1±0,2	13,4±0,7

Legend. TDCI) Total density of cellular infiltration; PC) plasma cells; IGCC) immunoglobulin-containing cells; EC) endocrine cells; MC) mast cells; IEL) intraepithelial lymphocytes; GC) goblet cells. Asterisk indicates data for which $p < 0.05$ compared with control.

intracellular structures and intensification of the detoxicating function of the epitheliocytes (hypertrophy and hyperplasia of the Golgi's lamellar complex, dilatation of the cisterns of the smooth endoplasmic reticulum with their hyperplasia, and an increase in the number of active mitochondria). The marginal arrangement of the larger masses of chromatin and displacement of the nucleoli toward the periphery of the nucleoplasm of the epitheliocytes, the intraepithelial lymphocytes, lymphocytes of the lamina propria of the mucous membrane, and of macrophages must be particularly emphasized. Similar data on margination of chromatin were described previously at the light-optical level in viral infection [6]. An important factor in this pathology is the increase in number of goblet cells and of Paneth's cells. The lamina propria of the mucous membrane was rich in cellular association, incorporating lymphocytes, plasma cells, eosinophils, and mast cells; macrophages were seen occasionally. The plasma cells were rich in organelles, and their perinuclear space and the cisterns of their RER were greatly widened.

A distinguishing feature of the ultrastructure of the epitheliocytes was the fact that they contained virus particles, in the form either of small dark osmiophilic inclusions (mainly located in the supra- and/or paranuclear zones), both in the free state and in microvesicles, and sometimes in mitochondria and in the nucleoplasm as round, oval, rod-shaped, or irregularly shaped formations (Fig. 3a, b). Close to these particles degranulation of RER, widening of the perinuclear space and of the cisterns of RER, and translucency of the mitochondrial matrix were observed. Local translucency of the hyaloplasm also was observed in these areas. Larger virus particles were found both in the cytoplasm of the brush-border epitheliocytes and also in macrophages of the lamina propria of the mucous membrane 2 weeks after injection (Fig. 3c, d).

After 1 week the microscopic picture resembled on the whole that observed at the previous time of the investigation but the pathological changes were rather less severe. The number of intraepithelial lymphocytes was reduced and was the same as their number in the control, but significantly different from the value obtained at the previous time. Meanwhile, against the background of a very small reduction of the total density of the infiltrating cells of the lamina propria of the mucous membrane the number of plasma cells and of immunoglobulin-containing cells continued to increase, reaching 1116 ± 505 (61.4 ± 21.8%) and 7236 ± 438 (39.9 ± 21.9%) respectively. At this time of infection a tendency was noted for the number of endocrine cells and of mast cells to decrease (Table 1). Among the mast cells, the entire 100% consisted of cells with moderately severe and mild forms of degranulation.

Thus, after intranasal injection of influenza virus we obtained clinical and morphologic confirmation of the presence of this infection. We found both a cytopathic and a cytoproliferative effect of influenza virus. Some workers [8, 9] observed similar manifestations of this process in many respiratory virus infections under both natural and experimental conditions. The presence of virus particles both in the free state and in the composition of subcellular structures, not only of epitheliocytes, but also of lymphoid cells of intestinal tissue, is evidence of the pantropism of influence A virus. Largely similar changes have been observed in infection by oncoviruses [10] and human immunodeficiency viruses [4], and this was regarded as an immunosuppressor effect.

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