INTERACTION OF *Yersinia pseudotuberculosis* WITH THE SMALL INTESTINAL EPITHELIUM IN EXPERIMENTAL INFECTION

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KEY WORDS: pseudotuberculosis; *Yersinia*; small intestine; epitheliocytes; enteritis.

The pathogenesis of pseudotuberculosis, especially of the early stages of its development, has received little study. According to the clinical-anatomical classification of *Yersinia* infections [1], the abdominal form is found in 50% of cases. According to most authorities, the morbid anatomical changes in the form of erosive-ulcerative enteritis, are located mainly in the terminal part of the ileum, which is considered to be the main portal of entry of the agent [1-3, 7-10, 12]. In experiments with peroral infection of mice and guinea pigs, with intraduodenal infection of rabbits, and on a model of a ligated loop of rabbit intestine [4-6, 11, 12] it has been shown that invasion of the mucosa of the small intestine by the microorganisms, followed by generalization of the infection, plays an important role in the pathogenesis of *Yersinia* infections.

The aim of this investigation was to study the character of interaction of *Yersinia pseudotuberculosis* with the mucosa of the small intestine.

EXPERIMENTAL METHOD

Experiments were carried out on 20 rabbits weighing 2.5-3.0 kg. Strain 1179 of *Y. pseudotuberculosis* serovar I, in the S-form, isolated from a patient, was used for infection. The feces and blood of the animals were first investigated to exclude spontaneous infection. The rabbits were starved for 2 days and then infected by feeding with milk mixed with a microbial suspension in a dose of 10^8 bacterial cells. Material was taken 30 min, 1, 5, 12, and 18 h, and 2, 3, 5, 7, and 10 days after infection. Pieces of duodenum, jejunum, and the terminal ileum were excised. Pieces measuring 1 × 1 mm were fixed in 2% glutaraldehyde in Millonig's buffer, postfixed in 2% OsO_4, dehydrated in alcohols, and embedded in Araldite. Semithin sections 1 μ thick were cut on an LKB ultramicrotome, stained with azure II-fuchsin, and examined in an "Orthoplan" light microscope.

EXPERIMENTAL RESULTS

At autopsy on rabbits 12 h after infection accumulation of yellowish fluid was observed in the lumen of the duodenum and jejunum, with congestion of vessels in the intestinal wall and mesentery, a pale exudate in the peritoneal cavity, and scattered punctate hemorrhages beneath the serous membrane of the terminal part of the ileum and appendix. Signs of enteritis were distinctly seen before the 5th-7th day after infection. The loops of small intestine were filled with seromucous exudate, the mucosa was friable and hyperemic, and was covered with a mucinous bran-like deposit.

Microscopic investigation revealed lesions of uniform type in the epithelium of all parts of the small intestine. Differences were observed in the time course of development of the pathological changes in its proximal and distal parts. In the duodenum and jejunum 30 min after infection small groups of microorganisms, typically rod-shaped and brightly stained with azure, were found in the lumen close to the surface of the villi. The cytoplasm of the enterocytes was vacuolated. Single pale swollen cells, with microorganisms in their cytoplasm, stood out distinctly in the epithelium. Penetration of yersinias into the epithelium was observed

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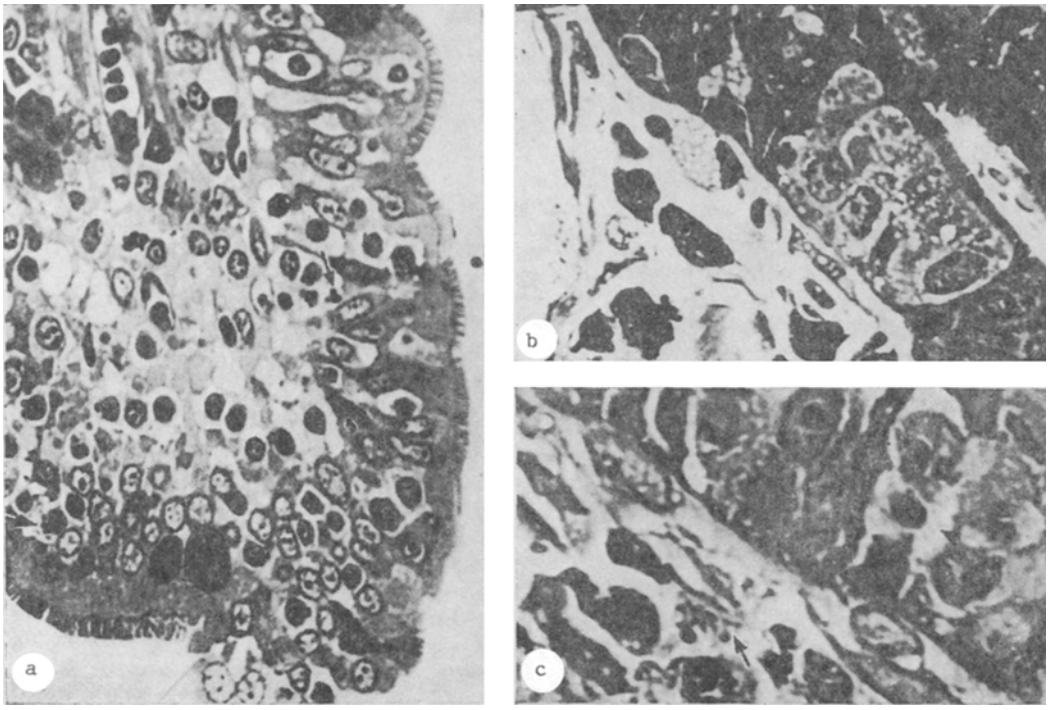

Fig. 1. Histopathological picture of mucosa of small intestine after oral infection with *Y. pseudotuberculosis*. a) Fragmentation of brush border and apical destruction of epithelium of villus of jejunum: few yersinias seen in translucent cytoplasm of enterocytes (arrow), 1 h after infection. 640×; b) invasion by yersinias and their intraepithelial multiplication in duodenal enterocytes: a group of enterocytes with translucent cytoplasm, edema of the lamina propria of the mucosa, 5 h after infection. 1000×; c) yersinias in enterocytes with translucent cytoplasm (triangle): microcolonies of yersinias in lamina propria of jejunal mucosa (arrow), 2 days after infection. 1600×. Here and in Fig. 2, semithin sections, stained with azure II-fuchsine.

1 h after infection (Fig. 1a). Marked invasion of the bacteria and their intraepithelial multiplication were observed after 5 h; some epitheliocytes were converted into "balloons," filled with large numbers of yersinias (Fig. 1b). Regions of disruption of the brush border were present, apical destruction of villi and widening of the interepithelial spaces, with the presence of intraepithelial lymphocytes, were observed. The nuclei of individual enterocytes were "foamy" in appearance. The goblet cells were distended with deeply stained secretion. Desquamation of the epithelium was observed in some places. After 12-18 h breaking up of epithelial cell complexes was found, with desquamation of the epithelium and erosion formation. On the 2nd day of infection microcolonies of yersinias were detected on the surface of the brush border, in the cytoplasm of the epitheliocytes, and inside large vacuoles in the thickness of the epithelium (Fig. 1c). Destruction of extensive areas of the epithelial layer and erosion and ulceration of the mucosa were observed 3-5 days after infection (Fig. 2a). As a result of disintegration of the desquamated epitheliocytes many yersinias were liberated into the lumen of the intestine, and some were undergoing degeneration (Fig. 2b). Regeneration of the epithelium of the intestinal villi began after 7-10 days. However, some enterocytes containing yersinias in their cytoplasm could still be distinguished. These cells had no brush border, and microcolonies of yersinias were nesting in these microdefects of the mucosa. A few palely stained rods could be seen in the intestinal lumen between the villi and sometimes in the crypts of Lieberkühn.

Yersinias were not found in the lumen of the ileum until 5 h after infection. Chains of microorganisms were palely stained. Small groups of yersinias with the typical morphology were located in microdefects of the epithelium, formed in places where globlet cells had degenerated. After 12-18 h mild invasions of enterocytes by yersinias was observed, with involvement of two or three neighboring cells. The epithelium of some cells had no brush border, and dystrophy and evaginations of the cytoplasm in the form of vesicles into the intestinal lumen were present. After 2-3 days numerous yersinias were observed along the

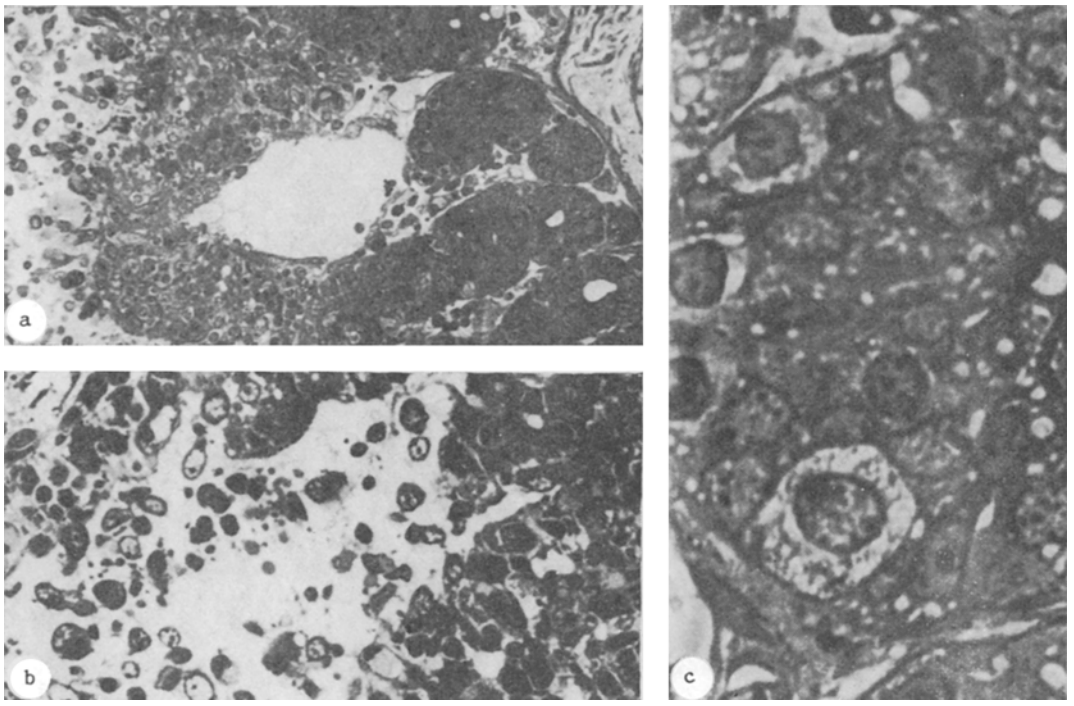

Fig. 2. Histopathological picture of mucosa of small intestine after oral infection with *Y. pseudotuberbulosis*: a) erosive enteritis, desquamation of epitheliocytes, marked dilatation of lacteal in center of jejunal villus, 3 days after infection, 400×; b) destruction and desquamation of epitheliocytes of jejunal villus, numerous yersinias in intestinal lumen, 5 days after infection. 640×; c) multiplication of yersinias in translucent cytoplasm of swollen enterocytes of ileal crypt, 3 days after infection. 1600×.

villi, mainly degenerative forms, and multiplication of the microorganisms in enterocytes was discovered (Fig. 2c). On the 5th day of infection destruction of the epithelium and disintegration of its cells were observed, with seeding of the intestinal canal and the formation of erosions and ulcers of the mucosa. On the 7th-10th day partial repair of the epithelium was found, but intraepithelial concentrations of yersinias in single cells lining the villi and crypts still remained.

In all parts of the small intestine microorganisms quickly penetrated from the epithelium into the lamina propria, passing through the basement membrane, where they were found 30-60 min after infection.

The results are evidence that after alimentary infection, invasion of the mucosa and multiplication of *Y. psuedotuberculosis* begin in the proximal parts of the small intestine — the duodenum and Jejunum. Penetration of yersinias into the epithelium of the ileum begins later, with evacuation of infected contents along the intestinal tract. More intensive penetration of microorganisms into the epithelium of the mucosa was observed in the duodenum and jejunum. Invasive activity of the yersinias in the ileum was reduced, probably because of the loss of pathogenicity under the influence of digestive juices and the protective forces of the host, manifested by degeneration of microorganisms present in the intestinal lumen. Yersinias multiplying in the cytoplasm of the enterocytes caused severe dystrophy of the affected cells with no marked tendency to spread along the epithelial layer, and they penetrated into deeper layers of the mucosa of the small intestine. As a result of the cytotoxic action of *Y. pseudotuberculosis*, multiplying in the epithelium, an erosive-ulcerative enteritis developed. Destruction of the epithelium was followed by a second wave of endogenous seeding of the intestinal canal, which could lead to a chronic carrier state and to allergic sensitization of the host.

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RELATIVE HYPERTROPHY OF THE RIGHT VENTRICLE IN SILVER FOXES SELECTED FOR DOMESTICATION

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To analyze the genetic mechanisms of domestication of animals, for the last 25 years silver foxes have been bred in the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, for domestication. Previous investigations have shown that domestication of animals is accompanied by the appearance of some special physiological and morphological features [4, 5]. In particular, the study of the internal organs showed that the weight of the right ventricle is increased to some degree in animals capable of domestication compared with those which are incapable. The investigation described below was devoted to a study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 88 silver-black foxes aged 2-4 years, obtained at the Experimental Game Farm of the Institute. The animals investigated included 23 male and 20 female foxes bred for domesticated behavior (capable of domestication) and 23 male and 22 female foxes obtained from a State Farm Population, not subjected to corresponding selection (incapable of domestication). The groups were selected in accordance with the analog principle of age composition.

After electrocution the animals were weighed and the heart was removed and fixed with 4% depolymerized paraform in 0.1 M phosphate buffer, pH 8.0 [10]. This fixation did not appreciably change the weight of the tissues. The walls of the right ventricle (RV) and the wall of the left ventricle with ventricular septum (LV) were isolated from the fixed hearts and weighed separately.

For stereologic study at light and electron-microscopic levels of the relative and absolute total volumes and surface areas of the myocardial structural components [1, 11, 12, 14] groups of six male foxes capable of domestication and six incapable, of identical age composition and all killed on the same day, were chosen. The technique of alkaline dissociation of the myocardium [3, 7] was used on these same animals to determine the total number and concentration of the muscle cells and of their nuclei in RV.

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