EXPERIMENTAL INTESTINAL INFECTION CAUSED BY ENTEROPATHOGENIC STRAIN Escherichia coli O124:K72, AN AGENT OF DYSENTERY-LIKE DISEASES IN ADULTS AND CHILDREN

Yu. E. Polotskii and N. R. Vasser

UDC 616.34-022.6(0124:K72)-092.9

Fasting guineas pigs, infected enterally with an enteropathogenic strain of Escherichia coli O124 (several billions of bacterial cells), isolated from an adult patient during a water-borne epidemic of O124 enterocolitis, developed enterocolitis with death of some of the animals. Considerable proliferation of the bacteria was observed in the jejunum, ileum, and cecum. Histologically, the infectious process connected with intraepithelial parasitization of the microorganisms was similar to experimental dysentery.

* * *

The similarity between disease produced in adults and children by enteropathogenic strain Escherichia coli (ESEC) O124:K72 and dysentery has recently been established [6, 7]. In previous experiments with intranasal infection of mice [8-10] and conjunctival infection of guinea pigs [11], the writers demonstrated the similarity between the biological properties of ESEC O124 and those of shigellas. Like shigellas, ESEC O124 proliferated in epithelial cells, destroyed them, and produced suppurative and ulcerative inflammation. To make a further comparison between ESEC O124 and shigellas, it was important to obtain an experimental intestinal infection by enteral inoculation. In a preliminary communication describing these experiments [12], the writers stated that by the use of Formal's dysentery model [13, 14] – enteral infection of guinea pigs with ESEC O124 – the animals developed enterocolitis with considerable proliferation of the microorganisms, accompanied by death of some of the animals.

The object of the present investigation was to study intestinal lesions produced experimentally by ESEC O124:K72 and to compare them with lesions of the experimental enterocolitis produced by shigellas and described by Formal and co-workers [13-17, 20], which has been studied in detail by Bibinova and co-workers [1].

EXPERIMENTAL METHOD

Strain ESEC O124:K72 No.5446/5559 was isolated from an adult patient during a water-borne epidemic of O124 enterocolitis described previously [6]. Altogether 39 guinea pigs weighing 300-400 g were used, and 10 of them received trypan blue instead of ESEC O124 (control). In Formal's method [13, 14], guinea pigs were deprived of food for 4 days, and then after neutralization of the gastric juice with a suspension of chalk (125 mg in 5 ml water 3 h before infection), they were given an 18 h broth culture of ESEC O124, in a volume of 10 ml through a gastric tube, in doses of between $0.1 \cdot 10^9$ and $4 \cdot 10^9$ bacterial cells. After 1 h, 1 ml of tincture of opium was injected intraperitoneally to inhibit intestinal peristalsis. The animals were killed with chloroform, and at autopsy the number of ESEC O124 cells was counted per ml (or per gram) of contents of all parts of the gastro-intestinal tract, and segments of the intestine were fixed with 10% formalin. Frozen sections were stained with Sudan – α-naphthol by Goldman's method for leukocytes, and paraffin sections were stained with hematoxylin – eosin, with thionine, with azure, and by the Hotchkiss and Brachet methods.

Department of Pathological Anatomy, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. Laboratory of Bacterial Intestinal Infections, Pasteur Institute of Epidemiology and Microbiology, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Ioffe.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 2, pp. 20-23, February, 1970. Original article submitted July 31, 1969.

EXPERIMENTAL RESULTS

Infection with ESEC O124 in doses of $0.1 \cdot 10^9$–$0.65 \cdot 10^9$ bacterial cells did not produce the disease in 8 guinea pigs, and virtually no O124 bacteria were found in seedings taken after 24 h.

Twenty-one guinea pigs infected with ESEC-O124 in doses of $1.3 \cdot 10^9$–$4.4 \cdot 10^9$ cells developed enterocolitis. Some of the animals had a severe form of the disease, and six guinea pigs died or were sacrificed in a terminal state after 24–72 h; the rest recovered. The jejunum, ileum, and cecum of the animals which died or were sacrified were filled with liquid exudate, and cultures taken from this showed marked proliferation of ESEC O124 (from $0.2 \cdot 10^9$ to $3.6 \cdot 10^9$ bacterial cells/ml). In the other animals, whose condition improved, the number of these organisms in the intestine gradually fell, and by 72 h after infection, some guinea pigs were completely cleared of ESEC O124.

The histological investigation showed that all guinea pigs infected with billions of ESEC O124 cells developed foci of inflammation in the mucous membrane of the jejunum, ileum, and cecum (after 9 h in the jejunum, next in the ileum, and after 24 h in the cecum), and these were particularly severe in the cecum 48 h after infection in seriously ill or dying animals. In the affected areas of the mucous membrane small defects appeared in the epithelium, and the crypts, especially in the region of the Peyer's patches, were filled with purulent exudate, their epithelium was destroyed, the underlying tissues were infiltrated with leukocytes, abcesses formed (Fig. 1a), and the surface of the mucous membrane was covered with purulent exudate (Fig. 1b). Large ulcers developed in the cecum of some animals (Fig. 1c). In the animals which recovered, the inflammation subsided, especially after 48 h, and by 72 h after infection, only a few diffusely scattered leukocytes in the mucous membrane and small isolated areas of undifferentiated regenerating epithelium could be observed. Meanwhile, in one such guinea pig two large ulcers of the cecum, with their floor filled with granulation tissue, were found.

A study of the fate of the inoculated bacteria showed that during the first few hours after infection solitary bacilli were present in some epithelial cells of the jejunum. By 24 h after infection, near foci of injury to the epithelium and crypt cells, but sometimes in intact epithelium also, isolated epithelial cells containing bacteria in their cytoplasm were found. After 48 h, in seriously ill and dying animals, usually small collections or even complete microcolonies of bacteria could be seen at the edges of zones of epithelial destruction in the intact epithelial cells (and also in desquamated epithelial cells among the leukocytes of the purulent exudate) (Fig. 2). Sometimes bacilli also were found in the epithelium of those parts of the mucous membrane where no inflammatory changes could yet be detected. The ESEC O124 cells evidently died rapidly in the cytoplasm of polymorphonuclear leukocytes, for only occasionally were a few half-destroyed bacilli found in these cells. Sometimes bacteria were found in the cytoplasm of macrophages in the mucuous membrane itself, close to zones of injury to the epithelium, but the bacilli in these cells appeared to be unchanged or even to have divided. Free microorganisms in the tunica propria were found principally in the animals which died, and in these cases marked postmortem changes were present.

In sections taken from animals which recovered, a few Gram-negative bacilli were found only in the lumen. Sometimes the number of Gram-positive bacilli and cocci (autoflora of the large intestine) in the lumen was increased in these guinea pigs, even in the jejunum, which, under normal conditions, usually does not contain microorganisms.

In experimental intestinal infection with ESEC O124, an infectious disease developed with focal lesions of the intestinal epithelium, evidently as a result of intraepithelial proliferation of the microorganisms. Numerous experimental investigations and studies of dysentery in monkeys have shown that the virulence of shigellas is determined by their intraepithelial parasitization [2–5, 18, 19, 21]. Bibinova and co-workers [1] found that in experimental enterocolitis of guinea pigs caused by shigellas, lesions of the intestinal epithelium developed as a result of intraepithelial proliferation of shigellas and not because of passage of the microorganisms through the epithelial cells and their subsequent proliferation in the tunica propria of the mucous membrane, as American workers previously considered [15–17, 20]. The experimental intestinal infection described above is very similar to the experimental "shigella" enterocolitis studied in detail by Bibinova and her collaborators. At the same time, in experiments with ESEC O124, many times larger doses of microorganisms were needed in order to reproduce enterocolitis, yet the mortality among the animals was much lower. The impression was gained that ESEC O124 cells are less virulent than shigellas, and only in the case of massive infection did they produce a more violent infection, although one with a rapid course, in the development of which an important role was played by intraepithelial

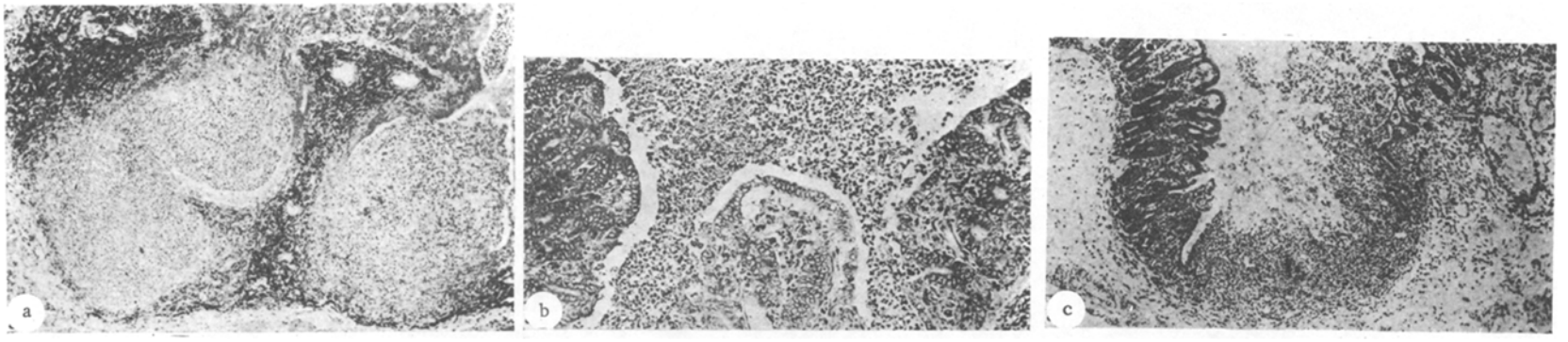

Fig. 1. Well-defined inflammatory changes in cecal mucosa of guinea pigs 48 h after enteral infection with ESEC O124 in a dose of 1.3×10^9–4.4×10^9 bacterial cells. a) Crypts in the region of a Peyer's patch distended with purulent exudate, their epithelial lining destroyed in the basal portions, purulent exudate spreading into the subjacent tissue forming abscesses (thionine, 70×); b) mucous membrane covered with purulent exudate, filling crypts (thionine, 120×); c) ulcer with complete destruction of mucous membrane and dense infiltration with leucocytes at its base, marked edema of the submucosa (thionine, 100×).

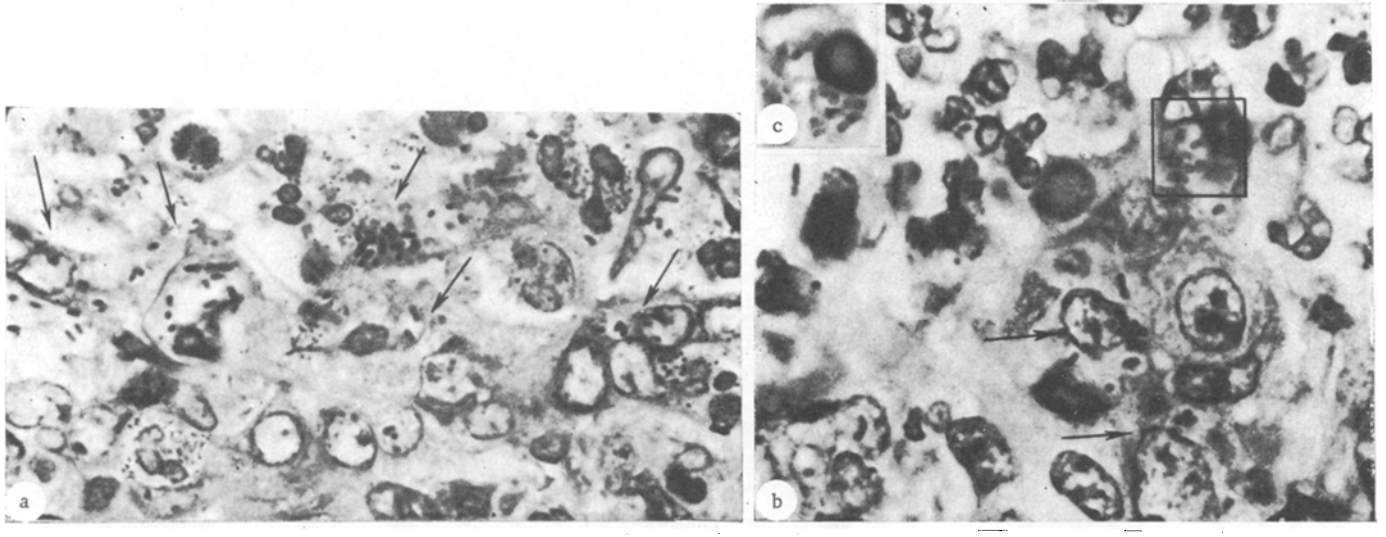

Fig. 2. Proliferation of bacteria in cytoplasm of epithelial cells of cecum close to sites of destruction of epithelium 48 h after enteral infection with ESEC O124 in a dose of 1.3×10^9–4.4×10^9 bacterial cells. a) Clusters of dividing bacilli in cytoplasm of epithelial cells of eroded area of mucous membrane, infiltrated with leucocytes (azure II–eosin, 2240×); b) above: purulent exudate in lumen of eroded crypt. Groups of dividing bacilli in cytoplasm of epithelial cells lining crypt in the center. In frame: epithelial cell filled with bacilli and with a pycnotic nucleus (thionine, 2650×); c) microcolony of ESEC O124 bacterial cells in an epithelial cell with pycnotic nucleus, not in focus in Fig. 2b (in the area enclosed by the frame) (thionine, 3300×).

parasitism of the microorganisms, and with a subsidiary role of toxic action. This was demonstrated by the more localized character of the focal lesions of the intestinal epithelium, the more violent and intensive leukocytic response, the lower resistance of the ESEC O124 cells to the action of leukocytes, the absence of lesions in the colon, and the more marked vascular disturbances. It should be noted that when these diseases arise in man, O124 enterocolitis develops after more massive infection, and runs a more violent but shorter course than dysentery, probably on account of the lower virulence of ESEC O124 cells than of shigellas [6, et al.]

LITERATURE CITED

1. L. S. Bibinova, T. N. Khavkin, N. S. Nikul'nikova, et al., Trudy Leningrad Nauch.-Issled. Inst. Épidemiol. Mikrobiol. im. Pastera, 33, 329 (1968); Zh. Gig. Épidemiol. (Prague), 12, No. 3, 314 (1968).
2. M. V. Voino-Yasenetskii, Zh. Mikrobiol., No. 11, 56 (1963).
3. M. V. Voino-Yasenetskii, Arkh. Pat., No. 10, 3 (1966).
4. M. V. Voino-Yasenetskii, in: Symposia of the 9th International Congress on Microbiology [in Russian], Moscow (1966), p. 146.
5. M. V. Voino-Yasenetskii and T. N. Khavkin, Zh. Mikrobiol., No. 4, 98 (1964).
6. É. M. Novgorodskaya et al., Trudy Leningrad, Nauchn.-Issled. Inst. Épidemiol. Mikrobiol. im. Pastera, 27, 372 (1964); Zh. Mikrobiol., No. 8, 21 (1966).
7. É. M. Novgorodskaya, Trudy Leningrad. Nauch.-Issled. Inst. Épidemiol. Mikrobiol. im. Pastera, 33, 7 (1968).
8. Yu. E. Polotskii and V. A. Arbuzova, in: Authors' Abstracts and Short Communications at a Review Conference of the Pasteur Institute with the Participation of Representatives of the Northwestern Regions [in Russian], Leningrad (1965), p. 8.
9. Yu. E. Polotskii and V. A. Arbuzova, Trudy Leningrad. Nauchn. Obshch. Patologoanat., No. 7, 145 (1966).
10. Yu. E. Polotskii and V. A. Arbuzova, Zh. Mikrobiol., No. 1, 61 (1967).
11. Yu. E. Polotskii and N. R. Vasser, in: Authors' Abstracts and Short Communications at a Reveiw Conference of the Pasteur Institute with the Participation of Representatives of the Northwestern Regions [in Russian], Leningrad (1966), p. 9.
12. Yu. E. Polotskii and N. R. Vasser, in: Authors' Abstracts and Short Communications at a Review Conference of the Pasteur Institute with the Participation of Representatives of the Northwestern Regions [in Russian], Leningrad (1966), p. 14.
13. S. B. Formal, G. J. Dammin, E. H. La Brec, et al., J. Bact., 78, 604 (1958).
14. S. B. Formal, G. D. Abrams, H. Schneider, et al., J. Bact., 85, 119 (1963).
15. S. B. Formal, E. H. La Brec, H. Schneider, et al., in: Proceedings of the Symposium on the Characterization and Uses of Human Diploid Cell Strains, Opatija (1963), p. 642.
16. S. B. Formal, E. H. La Brec, and H. Schneider, Fed. Proc., 24, No. 1, Part 1, 29 (1965).
17. E. H. La Brec, H. Schneider, T. J. Magnani, et al., J. Bact., 88, 1503 (1964).
18. H. Ogawa, S. Honjo, M. Takasaka, et al., Jap. J. Med. Sci. Biol., 19, 23 (1966).
19. H. Ogawa, A. Nakamura, R. Nakaya, et al., Jap. J. Med. Sci. Biol., 20, 315 (1967).
20. A. Takeuchi, H. Sprinz, E. H. La Brec, et al., Am. J. Path., 47, 1011 (1965).
21. A. Takeuchi, S. B. Formal, and H. Sprinz, Am. J. Path., 52, 503 (1968).