MORPHOLOGICAL CHANGES CAUSED BY DERMONECROTIC FACTOR OF *Vibrio cholerae* AND CERTAIN OTHER INTESTINAL PATHOGENS

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Considerable interest is currently being shown in the dermonecrotic factor (DNF) of *Vibrio cholerae,* which is also found in certain other microorganisms. This substance causes necrosis of the skin and also has a general toxic action, participating in the complex mechanism of pathogenesis of cholera infection [6]. Correlation has been found between the necrotic and hemolytic activity of vibrios of the El-Tor group [8]. Subsequent investigations [9] showed that the hemolytic and dermonecrotic activity of *V. cholerae* have no such correlation. It has been shown [4, 5] that DNF in typical cholerogenic strains of vibrios is not constantly present, but in hemolytic noncholerogenic and NAG mutants, it is more constantly present and in higher titers. Adamov [1] classes it as an endotoxin, but Uraleva and co-workers [7] as an imperfect cholerogen. Morphological changes in the skin of rabbits have been discovered but only in isolated studies [3]. Initially (after 6 h) edema of the subcutaneous cellular tissue, stasis, hemorrhages, concentrations of polymorphs and fibrin, and fragmentation of collagen fibers developed at the site of injection. After 24 h, many altered polymorphs were present against the background of edema, with an abundance of mononuclears in the dermis. Information on morphological changes after injection of supernatants of cultures of cholera vibrios caused by DNF is very scanty. Inconstancy of the zones of cutaneous necrosis has been noted [4, 6]. They varied in diameter from 3-7 mm to 30 mm. Usually pustules appeared after 24-48 h at the site of injection. They were based on separation of the epidermis by purulent exudate with the development of diffuse coagulation necrosis in the latter.

The aim of these investigations was to study changes caused by DNF of *Vibrio cholerae* and certain other intestinal pathogens and to discover the particular features of their morphopathogenesis.

EXPERIMENTAL METHOD

Living cholera vibrios of the cholera of El-Tor group were injected into the preliminarily depilated skin of Chinchilla rabbits weighing 2-2.5 kg. The vibrios included 16 virulent strains, 22 weakly virulent strains, 18 avirulent strains, and nontoxic strains (37D, S64, S80, S20) and were generously provided by O. I. Pomukhina, N. M. Ostroumova, and G. M. Grizhebovskii. The other pathogens used were *Shigella flexneri* strain 59536, *Salmonella typhi* strain 97723, *E. coli* 0.4; and vibrios of the non-01 group (17 strains). These microorganisms were mainly injected live in a dose of $2 \cdot 10^9$ bacterial cells in 0.1 ml, although in some experiments with strains 550 and 569B, other doses were used ($12.5 \cdot 10^8$, $6.25 \cdot 10^8$, $5 \cdot 10^8$, $2.5 \cdot 10^8$, but heat-killed (56°C, 30 min) cultures of vibrios also were used, whole and in a dilution of 1:2. Sometimes the experiments were repeated up to 5 times (strains 569B, 550). In other experiments cell-free filtrates of cultures of *V. cholerae* (strains 550, B-53-2-38), grown by the method in [5] using cellophane film, were also injected intradermally in a dose of 0.1 ml. The results were read macroscopically and pieces of skin and internal organs were taken from the killed rabbits for histological investigation 24-48 h after injection of

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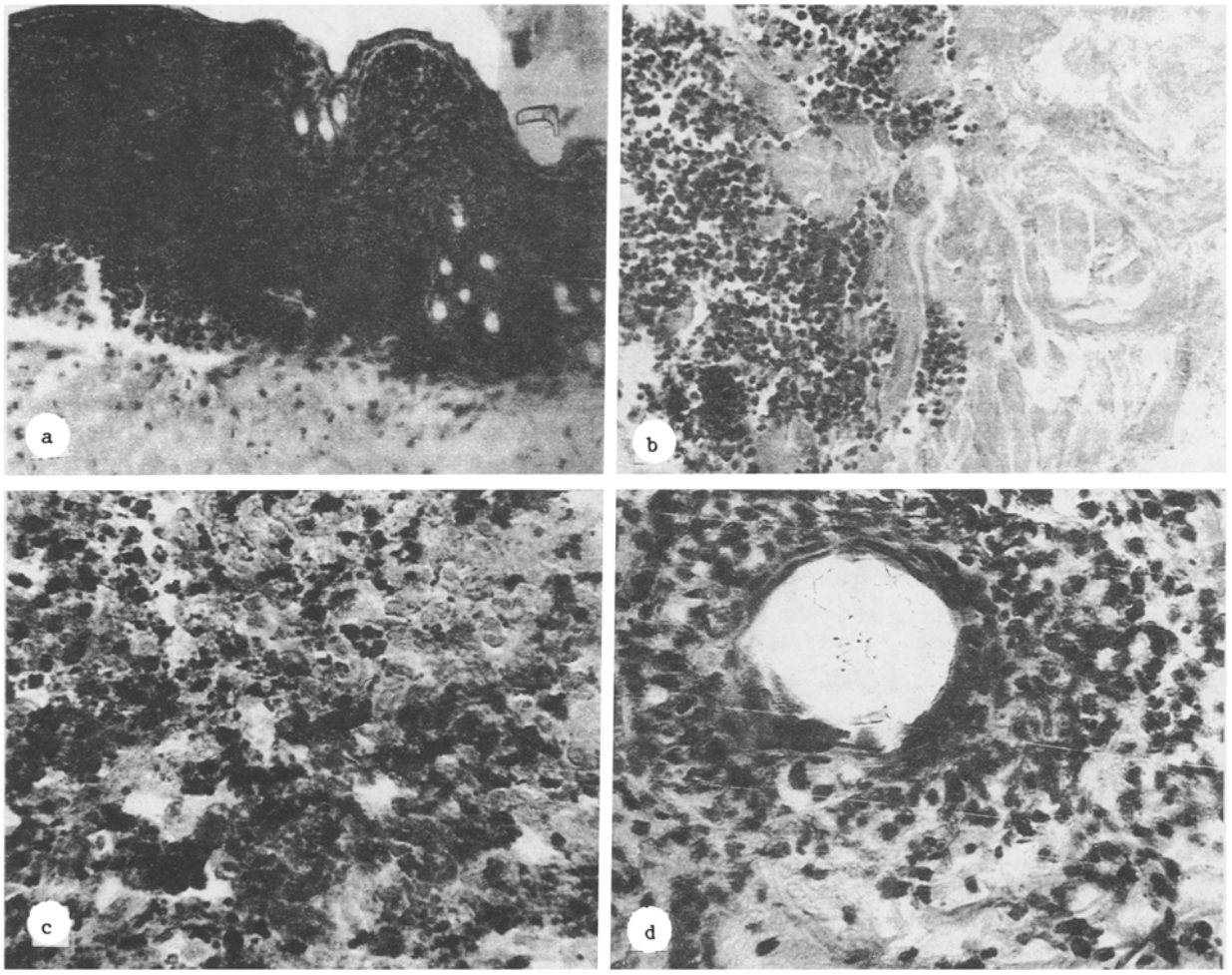

Fig. 1. Changes in rabbit skin 48 h after intradermal injection of $2 \cdot 10^9$ living bacterial cells of *V. cholerae* strain 569B. a) Focal subepithelial concentration of polymorphs with marked pycnosis of nuclei, b) suppurative inflammation and focal coagulation necrosis in dermis, c) seropurulent inflammation in dermis of skin in region of injection of cholera vibrios, d) seropurulent vasculitis in subcutaneous cellular tissue on boundary with adjacent muscles. Hematoxylin and eosin. Magnification: a, b) 90; c, d) 160.

the preparations. The material was fixed in 10% neutral formalin solution. Paraffin sections were stained with hematoxylin and eosin. Fibrin was detected by the methods of Weigert and Shueninov, acid muc polysaccharides with toluidine blue, and elastic fibers by Weigert's method.

EXPERIMENTAL RESULTS

The morphological investigation showed that 24-48 h after intradermal injection of living cholera vibrios of the 01 and non-01 groups, an exudative inflammation developed in the rabbits with congestion and edema of varied degrees of severity.

Connective-tissue fibers of the skin were considerably swollen or even underwent necrosis over a wide area. In some cases clusters of leukocytes of different sizes formed beneath the endothelium, and after 48 h their nuclei underwent pycnosis (Fig. 1a). A unique type of scab was formed, and usually occupied part of the papule (in the region of injection). In addition, the whole of the subcutaneous cellular tissue in the focus of injection of the microorganisms (5-10-15 mm in diameter) was either sparsely or densely infiltrated with polymorphs, among which there were a moderate number of lymphocytes and macrophages (Fig. 1c). In some cases necrosis and seropurulent inflammation were observed in the dermis and in muscles adjacent to the subcutaneous cellular tissue. In this same region, especially if considerable necrosis was present, a marked degree of vasculitis developed (Fig. 1d), with large and small, recent hemorrhages. In some cases the necrotic changes spread only on the surface

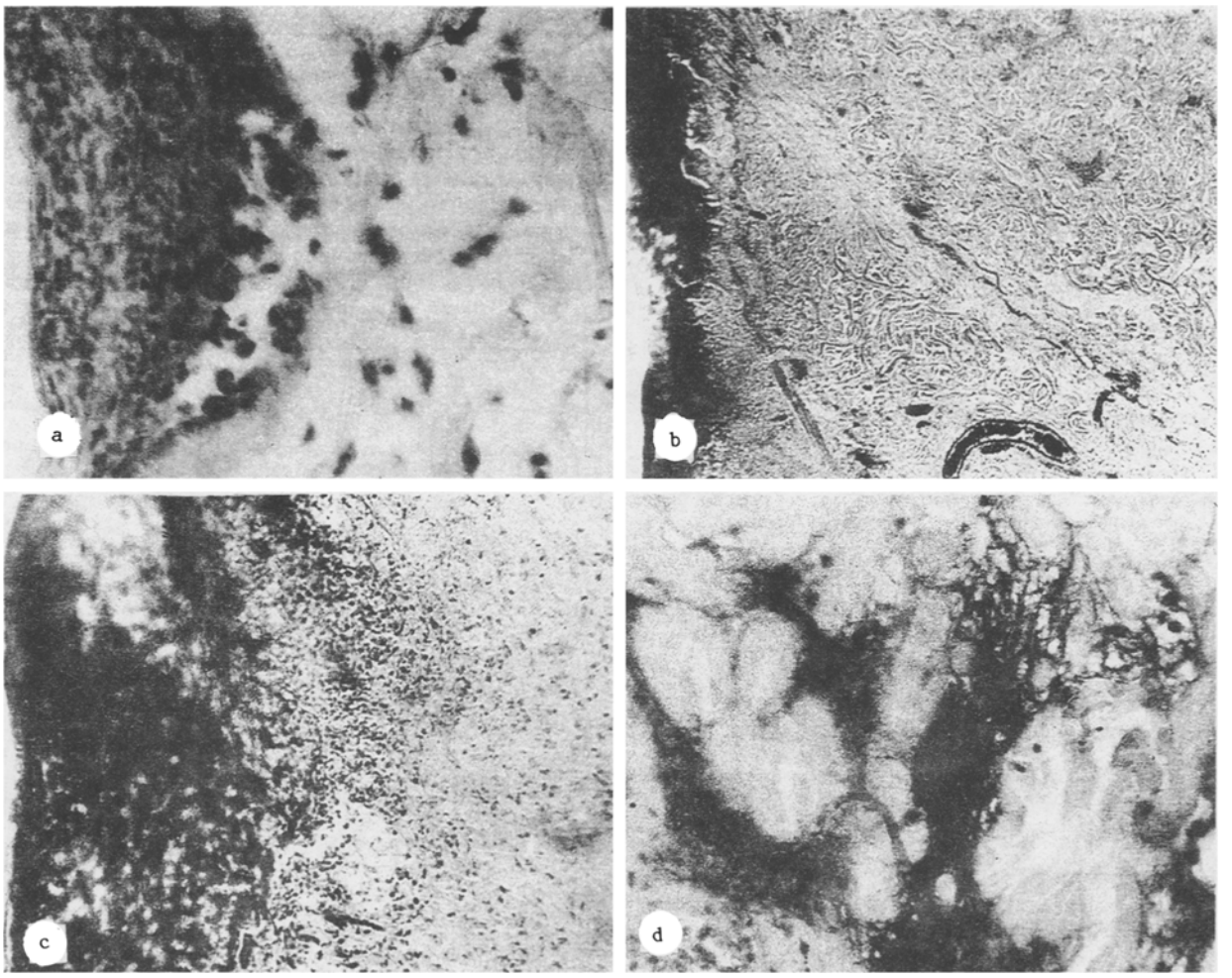

Fig. 2. Changes in rabbit skin 48 h after intradermal injection of 0.1 ml of supernatant of *V. cholerae*. a) Focal intraepithelial concentration of polymorphs with marked pycnosis of nuclei, b) necrosis of cutaneous epithelium, c) necrosis of epithelium and of stratum papillare of skin with evidence of demarcation inflammation, d) concentration of fibrin in dermis. a, b, c) Stained with hematoxylin and eosin, d) stained for fibrin by Weigert's method. Magnification: a, c) 90, b) 35, d) 160.

layer of the stratified squamous epithelium, in others, they spread throughout the thickness of the epidermal layer, and sometimes involved also the connective-tissue part of the stratum papillare of the epidermis of the skin.

After intradermal injection of filtrates of agar cultures of different cholera vibrios into rabbits virtually the same changes were found as those described above after injection of living microorganisms, although there were also certain morphological differences. For instance, in the region of injection of filtrates of cultures necrotic changes in the subcutaneous cellular tissue were absent. The presence of a focal scab was found, where the surface layers of the stratified epithelium of the skin were involved in the necrotic process (Fig. 2a-c). The degree and frequency of discovery of the changes described above depended on the concentration of the preparations injected. Meanwhile, in the zone of seropurulent inflammation, which was usually located in the dermis, focal concentrations of fibrin were found (Fig. 2d). These changes were observed when the filtrate of cultures of cholera vibrios were prepared from living microorganisms. If one such preparation was obtained from previously killed vibrios, only diffuse or focal lymphohistiocytic infiltration developed in the subcutaneous cellular tissue.

After intradermal injection of whole cholera toxin no necrotic lesions were observed in the skin or subcutaneous cellular tissue. There was likewise no scab. Instead, there was marked congestion and profuse edema of the subcutaneous cellular tissue. Intradermal injection of cholera vibrios of the non-01 group caused the development of the same changes in the skin as injection of virulent cholera vibrios.

Morphological examination of the skin after injection of atoxic and other (shigellas, salmonellas, *E. coli*) strains of enteropathogenic microorganisms extensive, diffuse areas of polymorphocellular infiltration were found in the dermis and subcutaneous cellular tissue, and sometimes confluent seropurulent inflammation could actually be observed in the subcutaneous cellular tissue.

Incidentally, the development of superficial areas of focal necrosis of the skin after intradermal injection of living microorganisms or filtrates of them was due to the fact that the blood supply to the skin rises from the deep part of the dermis. From it arterioles rise to the surface, and having passed through the dermis, form a subpapillary plexus beneath the epidermis [3]. The genesis of superficial necrosis of the skin as described above also was confirmed by morphological changes in the blood vessels: marked acute vasculitis in the dermis, and on the boundary of the subcutaneous cellular tissue and adjacent muscles.

After intradermal injection of living cholera vibrios or their supernatants, concentrations of polymorphs are formed in the skin both within and beneath the epithelium, most frequently in the form of foci of various sizes, and they die relatively quickly. Sometimes necrosis affects the whole thickness of the epithelial layer of the skin at once, but sometimes it also involves the deeper layers and spreads to the stratum papillare of the epidermis. In such cases, a demarcation barrier develops on the boundary with unchanged tissues. In the deeper layers of the dermis, in the region of injection of the supernatant, seropurulent inflammation with a varied degree of severity appears, and involvement of blood vessels in the pathological process can be clearly seen. Considerable destructive changes arise in the blood vessels, accompanied by exudation of fibrin into the surrounding tissues.

In the experiments with living cholera vibrios the changes mentioned above were well defined quite frequently in the focus of inflammation, located usually in the dermis; large and small foci of coagulation necrosis also were formed, which was not observed when supernatants of cholera vibrios were used. Incidentally, focal seropurulent inflammation in the region of injection of the preparations showed no tendency to form an abscess or to become encapsulated. Considerable congestion and edema, against the background of mild, mainly lymphohistiocytic, infiltration were found only in the skin, 24 h after injection of the cholerogen-toxin.

Analysis of the experimental results showed no direct correlation between the hemolytic activity of cholera vibrios and the action of their DNF.

LITERATURE CITED

1. A. K. Adamov, The Immunology of Cholera [in Russian], Saratov (1981), p. 319.
2. O. V. Alekseev, The Skin: Structure, Function, General Pathology and Therapy [in Russian], A. M. Chernukh and E. P. Frolov (eds.) Moscow (1982), pp. 59-76.
3. L. S. Nazarova, T. V. Bugorkova, and A. K. Adamov, Collected Transactions of the Plague Research Centers of the USSR [in Russian], Saratov (1985), pp. 65-70.
4. O. I. Pomukhina, Abstracts of Proceedings of a Scientific and Practical Conference on Current Problems in Cholera [in Russian], Rostov-on-Don (1984), pp. 156-158.
5. O. I. Pomukhina and V. S. Uraleva, Collected Transactions of the Plague Research Centers of the USSR [in Russian], Saratov (1987), pp. 67-73.
6. O. I. Pomukhina, V. S. Uraleva, L. A. Kozyreva, et al., Abstracts of Proceedings of an All-Union Conference [in Russian], Rostov-on-Don (1988), pp. 101-103.
7. V. S. Uraleva, L. I. Kolesnikova, and O. P. Feitsalova, Collected Transactions of the Plague Research Centers of the USSR [in Russian], Saratov (1983), pp. 88-92.
8. N. Kovacs, Zbl. Bakt. I. Abt. Orig., 123, 456 (1932).
9. Y. Watanabe and W. F. Verwey, J. Infect. Dis., **116**, No. 3, 363 (1966).