Role of Ultrastructural Changes in the Lungs of Suckling Rabbits in the Pathogenesis of Experimental Cholera

E. A. Bardakhch'yan, Yu. M. Lomov, and N. G. Kharlanova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, No. 8, pp. 173-177, August, 1995
Original article submitted November 9, 1994

Type II alveolocytes are destroyed in suckling rabbits during adhesion of *Vibrio cholerae*, whereas in type I alveolocytes no ultrastructural disorders are detected. The number of lipid granules is increased in the lipofibroblast cytoplasm. Transendothelial micropinocytosis and endothelial edema and destruction are increased in pulmonary capillaries, and plasmatic impregnation of the stroma is observed. The development of experimental cholera is associated with progressive disorders of the regional circulation, degranulation of platelets and basophils, destruction of polymorphonuclear leukocytes and endotheliocytes, and a marked increase of vascular permeability.

Key Words: *cholera; lungs; ultrastructure*

An important role in the pathogenesis of cholera is played by cAMP, vegetative ganglia of the small intestine, and the effects of vasoactive amines and other bioactive substances (BAS) [2-4,10,11,14]. The development of cholera is associated with severe dehydration, metabolic, structural, and functional changes in various organs, and an increase of the histamine, serotonin, and prostaglandin E_2 (PGE_2) levels in the blood [6,12,14].

The pathogenetic significance of serotonin and PGE_2 in enterocyte hypersecretion has been revealed. Blocking of serotonin receptors types I and II by ketanserine and the agent ICS 250-930, respectively [13-15], completely abolished the secretion induced by cholera exotoxin [13]; injection of indomethacin, an inhibitor of the cyclooxygenase route of arachidonic acid metabolism, reduced the secretory effect of the cholerogen, though to a lesser degree [14]. Special interest therefore attaches to the nonrespiratory functions of the lungs, namely the metabolism of certain BAS characterized by pronounced vasomotor activity [8,9]. For example, a single passage of blood through the lung tissue inactivates up to 95% of serotonin and up to 90% of PGE_1, PGE_2, and $PGE_{2\alpha}$ in the vascular bed [8]. Since the enzymatic systems involved in metabolism are concentrated mainly in the vascular endothelium of the lungs, damage to this may entrain quantitative changes in the blood levels of BAS and, hence, influence the course of cholera.

Our aim, therefore, in this research was to perform an electron-microscopic examination of suckling rabbit lungs during adhesion of *V. cholerae* and the development of clinical signs of cholera.

MATERIALS AND METHODS

Experiments were carried out with 26 suckling rabbits aged 10-12 days infected intragastrically [1]. One ml of 3% sodium bicarbonate solution was infused through a polyethylene tube to animals which had fasted for 24 h in order to neutralize the stomach contents, after which 1 ml of an 18-hour culture of the virulent El-Tor 5879 strain, and then again 0.5 ml sodium bicarbonate were administered. According to the optic opacity stan-

Laboratory of Pathomorphology, Rostov Research Antiplague Institute (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0008-0818$12.50

dard, the infective dose was 10^5 bacterial cells. The animals were sacrificed by ether overdosage after 4 h, that is, during adhesion of *V. cholerae* (n=8), 1 day (n=9), and 2 days (n=3) postinoculation in the presence of clinical signs of cholera. In the control (3 animals per batch), suckling rabbits were administered 1.5 ml sodium bicarbonate and 1 ml isotonic NaCl solution. Fragments of the lung were embedded in Epon after glutarosmium fixation. The total picture of changes in pulmonary tissue was studied on semithin slices stained with toluidine blue. Later, slices of the same blocks made with an LKB 8800 ultramicrotome were contrast-stained with uranyl acetate and lead citrate and examined under a JEM-100B electron microscope.

RESULTS

Normal saline administered to control suckling rabbits causes no changes of any kind in the lungs, although examination of semithin slices usually shows cells whose cytoplasm contains lipid inclusions. Electron-microscopic examination shows a regular spherical shape of the lipid inclusions (granules) surrounded by a membrane and a light rim round a more osmiophilic core. In the lungs such cells are called lipofibroblasts and serve to accumulate phospholipid precursors whose active synthesis is carried out in type II alveolocytes producing surfactant [7].

During adhesion the number of lipid granules appreciably increases, and in some lipofibroblasts they become transformed into lamellar bodies resembling those in type II alveolocytes. Analysis of surfactant-producing cells showed that a negligible proportion of them is irreversibly changed and undergoes destruction, with intracellular organelles, lamellar corpuscles, and surfactant seen in the alveolar lumens. In type I alveolocytes no ultrastructural changes are seen, just edema being observed in some of them.

The worst damage is observed in pulmonary capillaries. Intensive transepithelial micropinocytosis, with which liquid transport is commonly associated, takes place in virtually all endotheliocytes. Pinocytosis usually gives way to partial edema of the cytoplasm which, in turn, may spread to encompass the whole cell (Fig. 1, *a*). An uncommonly high lability of the endotheliocyte plasmalemma is worthy of note, which manifests itself in the formation of outgrowths, focal swellings, and microvilli acquiring an unusual shape. At sites of endothelial thinning, the endothelium is frequently detached from the basal membrane and protrudes far into the capillary lumen. Defects in the endothelial lining are possible, due to which the permeability of the aerohematic barrier sharply increases. Plasmatic impregnation of the stroma is observed. Less frequently individual red cells and fibrin threads are observed in the alveolar lumen. Fibrin precipitation is observed in the vascular lumen as well (Fig. 1, *b*).

A characteristic sign of injury to endotheliocytes is desquamation of apparently intact endotheliocytes (Fig. 1, *c*) and formation of myelin figures followed by microclasmatosis of the corresponding parts of the cytoplasm (Fig. 1, *d*); cells with edema are particularly liable to clasmatosis (Fig. 1, *a*). In parallel with this, intravascular disorders are recorded which, along with fibrin precipitation, are characterized by circulatory disorders (plethora, formation of "coin rolls") and destruction of polymorphonuclear leukocytes.

These ultrastructural lesions of the alveolar epithelium and microcirculatory bed are still present when clinical signs of cholera manifest themselves on days 1-2 postinfection. Sites of atelectases are sometimes seen, although dystelectases are much more frequent. While type I alveolocytes look relatively intact, in surfactant-producing cells the lamellar corpuscles lose their concentric structure and become fragmented, gaps are formed instead of them, and in some alveolar spaces altered corpuscles are seen which are evidently incapable of unfolding their layers. Lipid granules, as a rule, completely disappear from lipofibroblasts and these cells become fibroblasts capable only of collagen production. One to two days after infection the phagocytic activity of alveolar macrophages increases, this being manifested in the formation of plasmalemma outgrowths and the formation of numerous secondary lysosomes in their cytoplasm.

The most pronounced changes in the lungs during manifest cholera occur inside the microcirculatory vessels and involve the vessels proper. Mixed clots preventing normal blood flow are observed in the capillary lumen (Fig. 2, *a*). Some polymorphonuclear leukocytes are destroyed, and the adjacent red cells include osmiophobic sites of different shape. Such cavities usually contain plasma and less frequently myelin figures. However, our findings do not provide an answer as to whether this phenomenon is a result of microphagocytosis, when the myelin figure finds its way into red cells from the surrounding plasma or whether, on the contrary, the myelin figures are forced out from red cells as a result of exocytosis. Degranulation of platelets and basophils, due to which appreciable amounts of serotonin and hista-

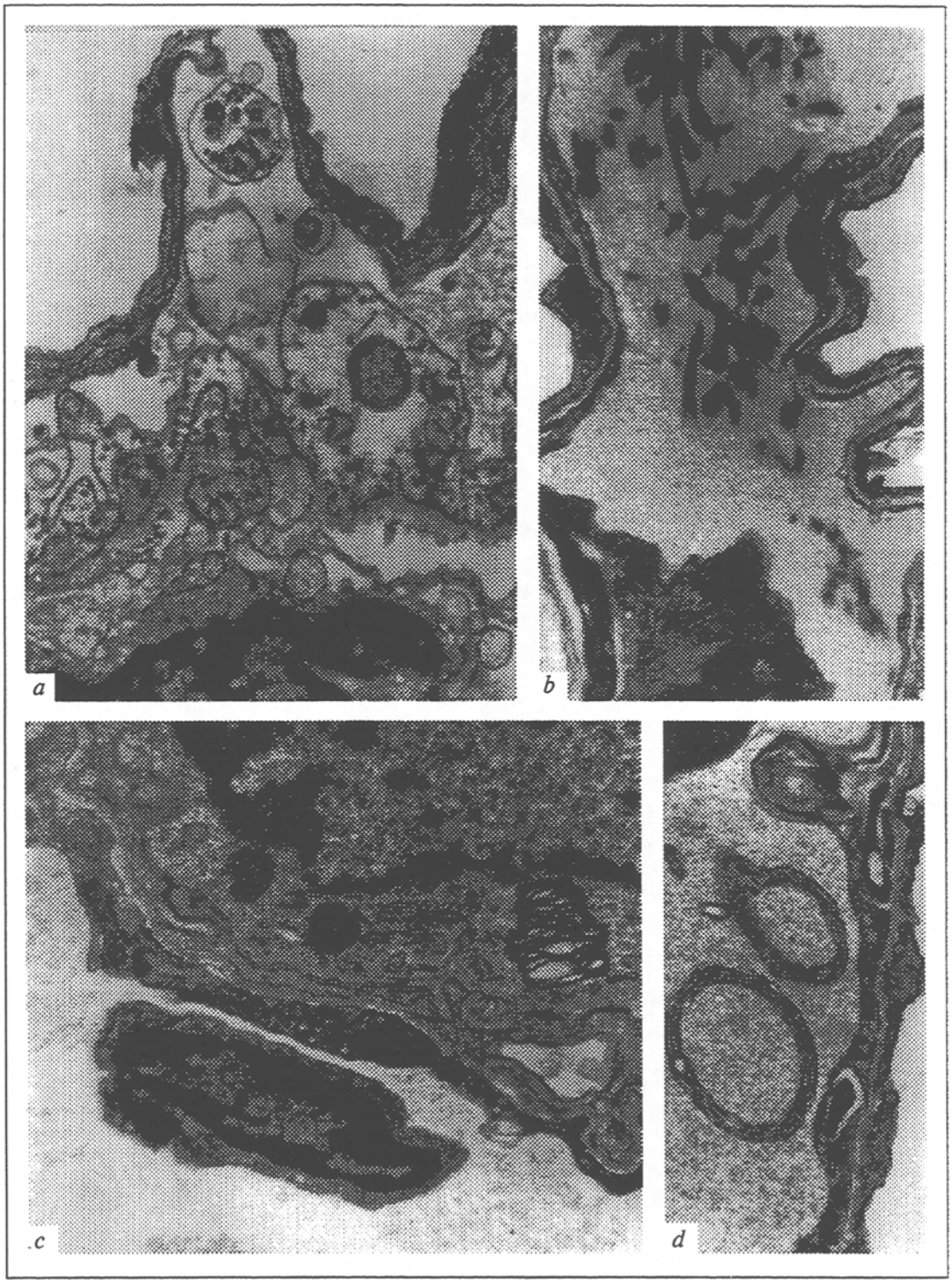

Fig. 1. Ultrastructural changes in the lungs during adhesion of *V. cholerae*. *a*) endotheliocyte edema and clasmatosis of cytoplasm sites. ×8000; *b*) fibrin in the capillary lumen. ×8000; *c*) desquamated endothelial cell in the capillary lumen. ×8000; *d*) formation of myelin figure in the endothelium and clasmatosis of thinned sites of endotheliocyte cytoplasm. ×15,000.

mine are released into the blood, is worthy of special note (Fig. 2, *b, c*).

Changes at the level of the vascular wall, besides those described for the adhesion period, may be supplemented by episodes of mitochondrial microphagocytosis (Fig. 2, *d*), whose biological significance was discussed previously when we were experimenting with intravenous endotoxin infusions

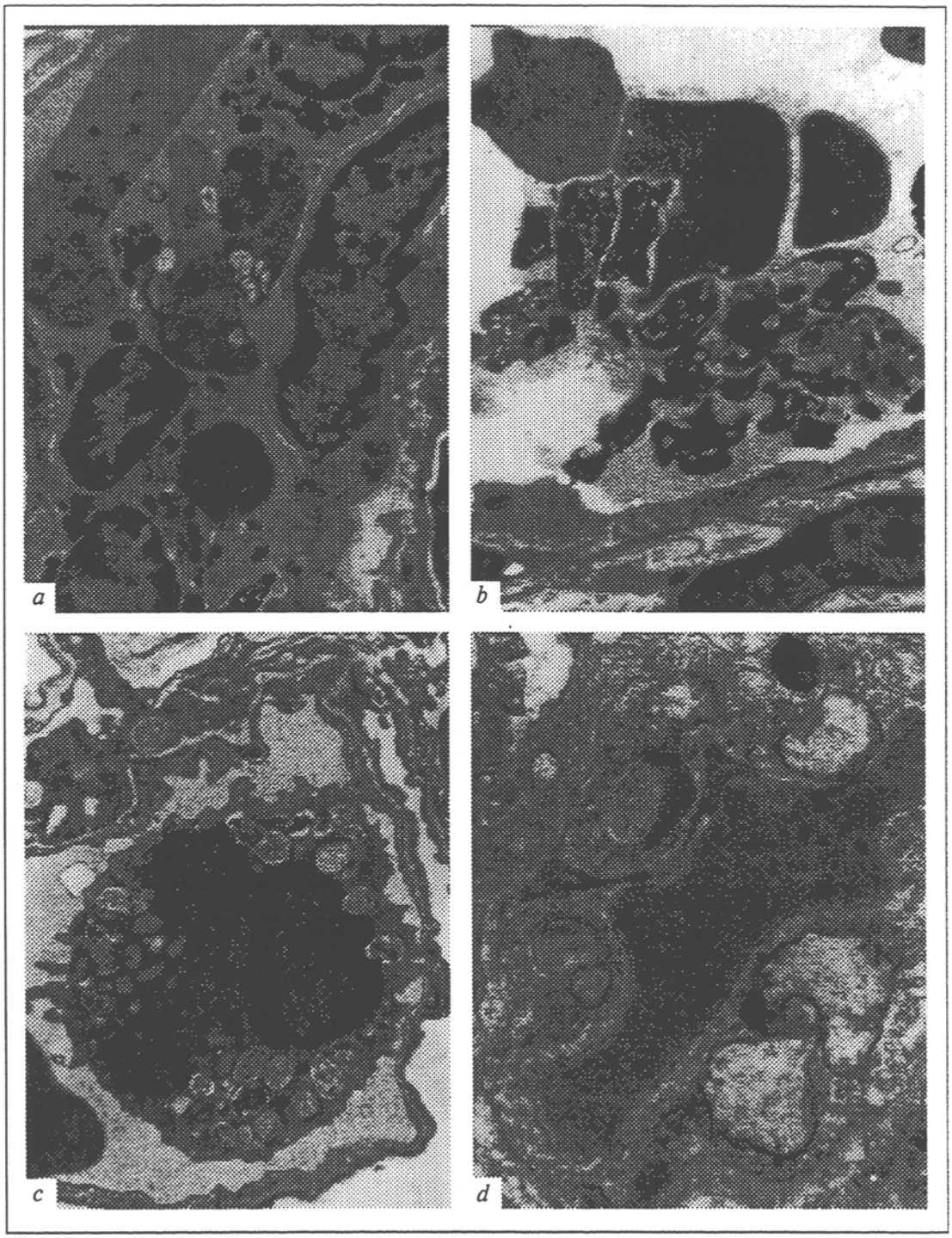

Fig. 2. Ultrastructural changes in the lungs during manifest cholera. *a*) mixed clot consisting of destroyed polymorphonuclear leukocytes and red cells with osmiophobic zones. ×5000; *b*) mixed clot consisting of red cells and degranulated platelets. ×3000; *c*) degranulated basophil. ×6000; *d*) mitochondrial macrophagocytosis in an endothelial cell. ×10,000.

[5]. Finally, extravascular disorders are characterized by plasma release and, less often, by diapedetic microhemorrhages.

Hence, phasic changes in the content of lipid granules in lipofibroblasts are detected in the lungs: during adhesion of *V. cholerae* their number increases, while during manifest cholera it decreases. Hemocirculatory changes in the course of cholera development are unidirectional and exhibited in progressive disorders of the local circulation, platelet and basophil degranulation, destruction of polymorphonuclear leukocytes and endotheliocytes, and increased

permeability of the aerohematic barrier. Damage to endotheliocyte membranes and the concomitant deficiency and failure of the enzymatic systems are conducive to the accumulation of histamine, serotonin, and prostaglandins, including PGE_2 ("cholera" prostaglandin), causing hypersecretion of the small intestine enterocytes.

REFERENCES

1. V. P. Avrorov, I. D. Bardykh, and V. V. Lobanov, *Problemy Osobo Opasnykh Infektsii,* № 3, 70-71 (1977).
2. E. A. Bardakhch'yan, Yu. M. Lomov, and I. A. Gusatinskii, *Byull. Eksp. Biol. Med.,* **113**, № 5, 549-552 (1992).
3. E. A. Bardakhch'yan, Yu. M. Lomov, and I. A. Gusatinskii, *Arkh. Pat.,* **55**, № 1, 29-34 (1993).
4. E. A. Bardakhch'yan, Yu. M. Lomov, and I. A. Gusatinskii, *Morfologiya,* **104**, № 5-6, 39-47 (1993).
5. P. E. Povilaitite, E. A. Bardakhch'yan, and G. A. Vilkov, *Tsitol. Genet.,* **20**, № 5, 323-326 (1986).
6. V. I. Pokrovskii, V. V. Maleev, and A. K. Adamov, *Clinical Picture, Pathogenesis, and Treatment of Cholera* [in Russian], Saratov (1988).
7. L. K. Romanova, *Regulation of Repair Processes* [in Russian], Moscow (1984).
8. I. R. Tupeev and O. A. Gomazkov, *Pat. Fiziol.,* № 1, 78-82 (1984).
9. N. G. Kharlanova and E. A. Bardakhch'yan, *Anest. Reanimatol.,* № 4, 37-41 (1988).
10. V. A. Shakhlamov, *Arkh. Pat.,* **42**, № 5, 75-83 (1980).
11. V. A. Shakhlamov, *Vestn. Akad. Med. Nauk SSSR,* № 11, 10-16 (1983).
12. V. A. Shakhlamov, T. G. Barkhina, M. N. Lyapin, and R. S. Trager, *Byull. Eksp. Biol. Med.,* **84**, № 12, 731-734 (1977).
13. E. Beubler and G. Burg, *Gastroenterology,* **96**, № 5, 42 (1989).
14. E. Beubler, G. Kollar, A. Saria, *et al., Ibid.,* № 2, 368-376.
15. K.-H. Buchheit, *Naunyn Schiedebergs Arch. Pharmacol.,* **339**, № 7, 704-705 (1984).