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# Effect of Lidocaine on Cholera Vibrio-Induced Changes in the Jejunum, Renal Medulla, and Lungs of Suckling Rabbits

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Lidocaine injected into suckling rabbits infected with a virulent strain of *Vibrio cholerae* abolishes the development of hydropic and ballooning degeneration the jejunal enterocytes. Secretory granules and lipid inclusions accumulate in jejunal enterochromaffin cells and interstitial cells of renal medulla, respectively, and are not released into the vascular bed. In pulmonary tissue ultrastructural changes are mild, and capillary epithelium is undamaged, indicating that lidocaine stimulates pulmonary enzymes which inactivate biologically active substances implicated in the pathogenesis of cholera.

**Key Words:** *cholera; lidocaine; electron microscopy*

Activation of phospholipase $A_2$ ($PLA_2$) by cholera toxin stimulates the formation of platelet-activating factor, a potent mediator of inflammation and destruction, and probably a key factor of the cholera toxin-induced secretion [12]. Phospholipase $A_2$ is a key component of the pulmonary antisurfactant system, activation of which in cholera leads to intense destruction of the surfactant [3]. In association with $Ca^{2+}$ $PLA_2$ cleaves arachidonic acid from membrane phospholipids, which is used in the synthesis of prostanoids (prostaglandins and thromboxanes) and eikosanoids (leukotrienes and fatty acid peroxides and hydroperoxides).

Particular attention has been focused on the role of prostaglandin $E_2$ ($PGE_2$) in the intestinal dehydration syndrome. In fact, choleragenic intoxication is accompanied by 3- to 5-fold rise of serotonin and $PGE_2$ [10]. It has been showed that cholera toxin causes dehydration by inducing the release of serotonin which stimulates $PGE_2$ production [10]. Biochemical findings correlate with ultrastructural changes in enterochromaffin (EC) cells of the small intestine in rats caused by choleragen [8] and, as we have shown, in interstitial cells of rabbits infected with *Vibrio cholerae*. Moreover, the development of experimental cholera strongly depends on the state of pulmonary vascular endothelium where serotonin and most of PG are inactivated [3]. Finally, lidocaine, a potent anesthetic, inhibits $PLA_2$ [11].

In this study we examined the effect of lidocaine on ultrastructural changes in cells producing serotonin and $PGE_2$ and on pulmonary tissue in experimental cholera.

## MATERIALS AND METHODS

A total of 34 suckling rabbits were used, 25 of which were infected intragastrally with a 18-h culture of *Vibrio cholerae* strain El Tor 5879 [2]. Thirteen rab-

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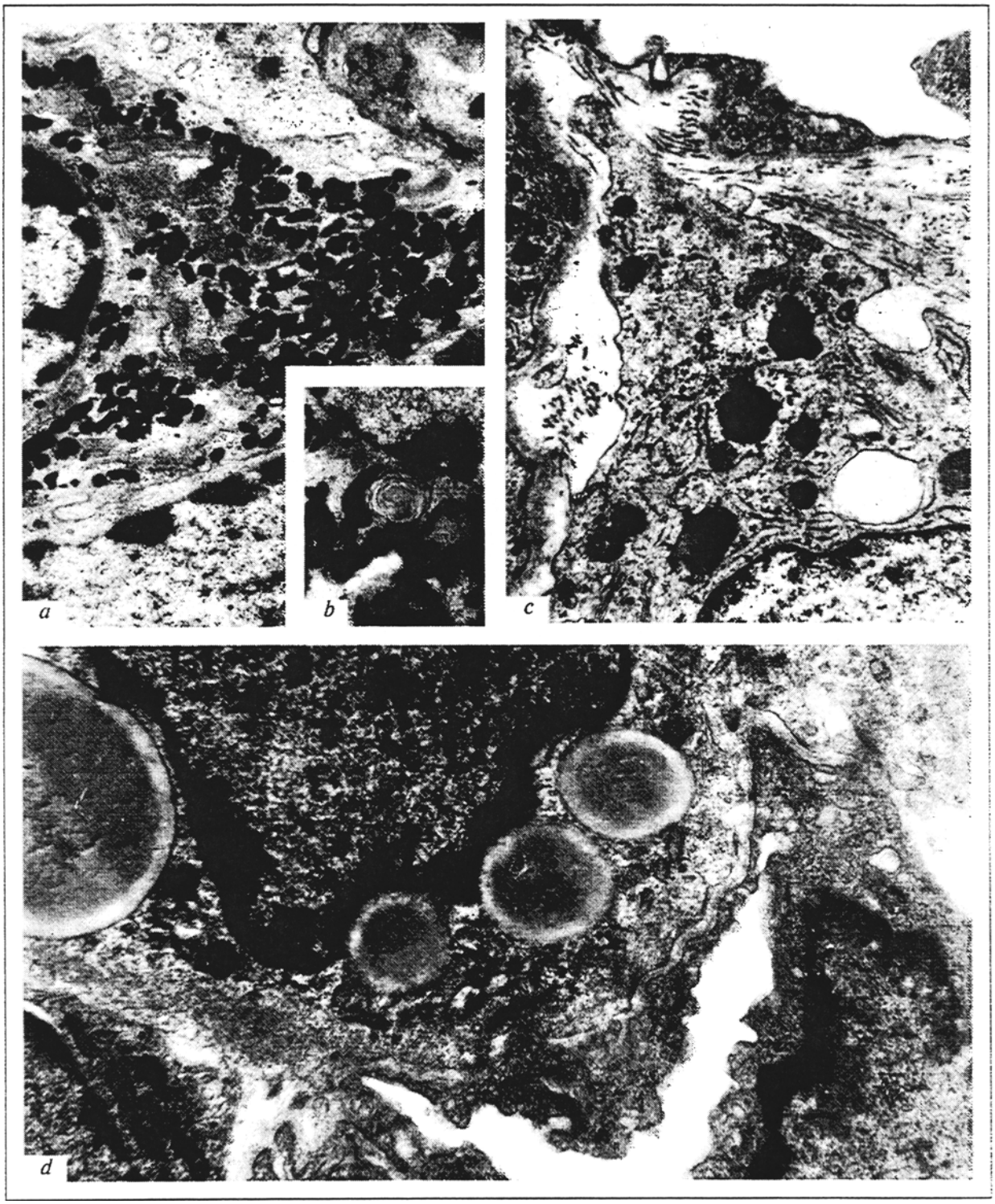


**Fig. 1.** Ultrastructure of cells from the jejunum, renal medulla, and lungs of lidocaine-treated suckling control rabbits. *a*) Fragment of a jejunal enterochromaffin (EC) cell with numerous secretory granules (×10,000); *b*) myelinlike structure at the nucleus of an EC cell (×8000); *c*) fragment of interstitial cell with solitary lipid inclusions and dilated granular endoplasmic reticulum, with the plasma membrane forming a deep invagination comparable in size to the vacuole near the nucleus (×8000); *d*) pulmonary fibroblasts with numerous lipid inclusions (×6000).

bits (group 1) received no treatment and 12 (group 2) were injected intramuscularly with lidocaine in a dose of 0.4 mg/100 g body weight 4 h postinfection and twice at a 3-h interval on the next day. Three out of nine control rabbits received sodium bicarbonate and isotonic NaCl solutions, three rabbits were treated with lidocaine as described above, and three remained intact. Material for electron-microscopic

examination (jejunum, inner zone of renal medulla, and lungs) was taken 24 h postinfection in group 1 and 2 h after the last lidocaine injection in group 2, and processed as described previously [3]. Changes in the lungs and enterocytes and *Cholerae vibrio* adhesion patterns were examined in toluidine blue-stained semithin sections which were also used to analyze interstitial cells by counting lipid granules per 50 cells. The results were analyzed by standard statistical methods. Then semithin sections cut from the same blocks were contrasted with uranyl acetate and lead citrate and examined in a JEM-100B electron microscope.

## RESULTS

Lidocaine did not damage jejunal enterocytes. The number of secretory granules in EC cells was the same or slightly higher than in intact rabbits or those given NaCl (Fig. 1, *a*). Myelinlike structures were seen in some cells in close proximity to the nucleus (Fig. 1, *b*). Enterochromaffin cells are open-type cells, i.e., their apical pole is oriented towards intestinal lumen [9]; however, in suckling rabbits they are "closed," i.e., do not contact with intestinal lumen. Although at the electron-microscopic level the EC cell population is heterogeneous (EC, $EC_1$, $EC_2$), these cells are considered as a single cell type with granules containing serotonin, melatonin, motilin, substance P, and enkephalin [7].

In interstitial cells, lidocaine caused a slight, statistically insignificant decrease in the number of lipid inclusions (Table 1). Dilated cisternae of the granular endoplasmic reticulum, solitary large vacuoles, and small mitochondria with poorly distinguishable cristae were seen (Fig. 1, *c*). The plasma membrane defects similar to those occurring in intact rabbits were observed.

Lungs were particularly resistant to lidocaine. Cells of the alveolar lining and endothelial cells of blood capillaries remained virtually unchanged. Fibroblasts with large lipid granules (lipofibroblasts) surrounded by a narrow electron-transparent zone were seen in the interstitium (Fig. 1, *d*).

It should be noted that while lipid inclusions of interstitial cells from renal medulla contained predominantly PG precursors, the lipidlike material in pulmonary lipofibroblasts served for the accumulation of phospholipid precursors used by type pneumocyte to produce surfactant. It is thought that lipofibroblasts of any localization only produce collagen once their interaction with other cells had been disrupted [4].

Examination of the material from lidocaine-treated rabbits with cholera revealed no ultrastructural changes detected previously in animals with experimental cholera [2,3]. In occasional epithelial cells deep invaginations of the apical plasma membrane crossed terminal network and formed broad ramified channels beneath it (Fig. 2, *a*). Structural alterations in the cell plasma membrane structure are probably provoked by high intracellular levels of lysolecithin, cholesterol, dihydrocholesterol, and hydroxyacetic and stearic acids. As a result, the viscosity and orderliness of the lipid layer in the erythrocyte membranes decrease, which increases the fluidity and permeability of the membrane [1].

We think that secretory granules in EC cells play an important role (Fig. 2, *b*). In fact, they occupied almost all cytoplasm. The granules varied in shape from oval and round to more complex irregular figures. In lidocaine-treated infected and control rabbits, all granules were electron dense. The organelles of EC cells were usually unchanged, except for the presence of myelinlike structures in the perinuclear zone (Fig. 2, *c*), their content in infected rabbits being higher than in controls.

Interstitial cells contained granules of approximately equal densities (Fig. 2, *d*) and in nearly the same numbers as in the control group (Table 1). The organelles were slightly changed, but these changes were much less pronounced than those in interstitial cells of animals with experimental cholera infection or colibacteriosis [6].

It is noteworthy that endothelial cells of pulmonary capillaries remained intact (Fig. 2, *e*). Type I pneumocytes were also undamaged, the number of lamellar bodies (cytophospholiposomes) in type II pneumocytes slightly decreased, and the number of lipid granules in lipofibroblasts dropped. Of particular importance is the finding that since the endothelial

**TABLE 1.** Effect of Lidocaine on the Number of Lipid Granules in Interstitial Cells of Renal Medulla from Sucking Rabbits Infected with Cholera Vibrio (*M*±*m*)

| Intact controls (*n*=3) | Controls treated with | | Test rabbits | |
|---|---|---|---|---|
| | soda and NaCl solutions (*n*=3) | lidocaine (*n*=3) | infected (*n*=13) | infected and treated with lidocaine (*n*=12) |
| 6.35±0.28 | 6.61±0.42 | 5.80±0.31 | 1.48±0.36* | 5.25±0.24 |

Note. *$p<0.001$ in comparison with sodaium bicarbonate and NaCl-treated controls.

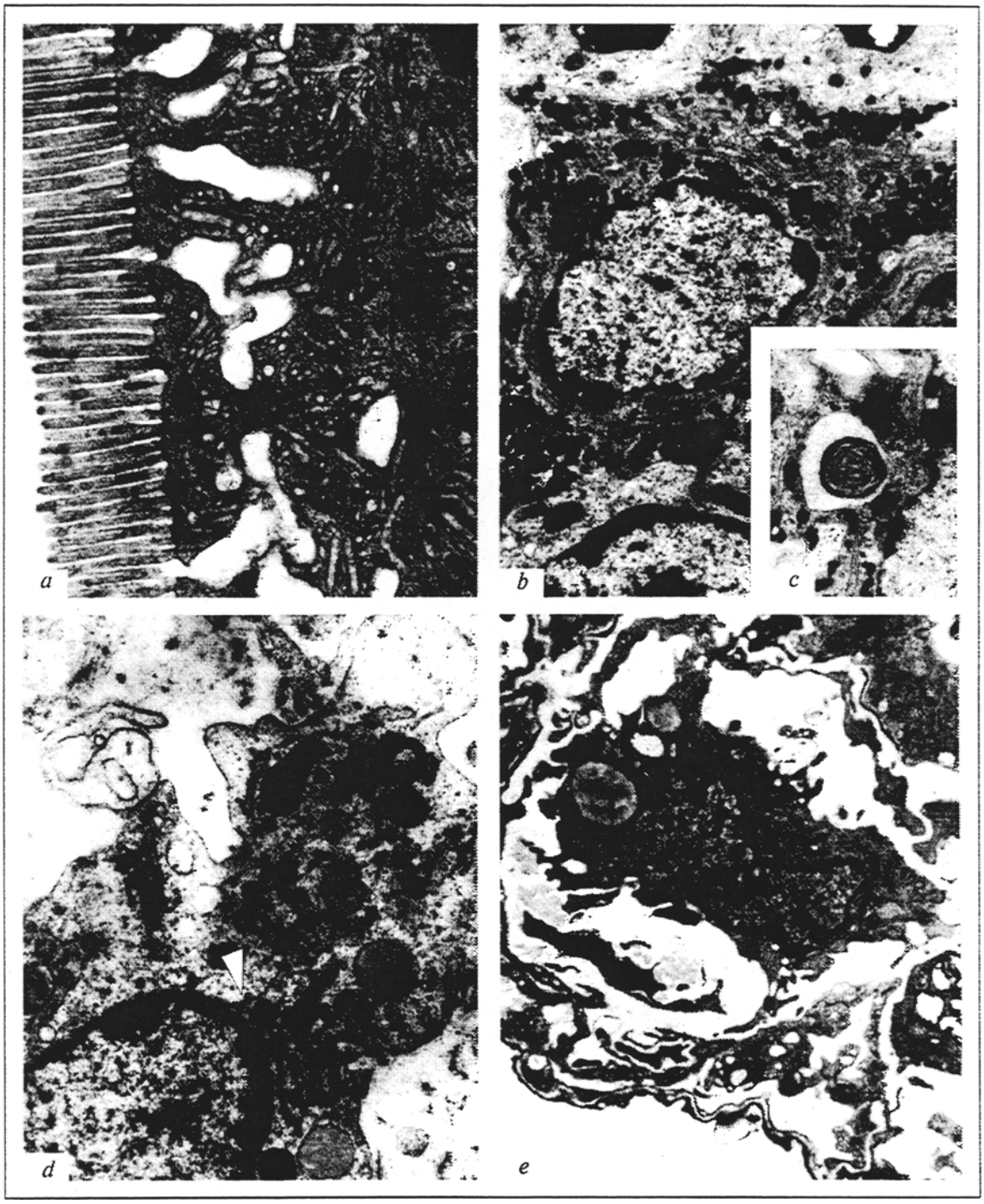


**Fig. 2.** Ultrastructural appearances of cells from the jejunum, renal medulla, and lungs of infected suckling rabbits treated with lidocaine. *a*) Deep invaginations of the apical plasma membrane in an enterocyte (×10,000); *b*) cytoplasm of enterochromaffin (EC) cell with many secretory granules and unchanged organelles (×8000); *c*) myelinlike structure, surrounded by a light zone, located near the nucleus (×6000); *d*) fragment of interstitial cell cytoplasm with lipid inclusions, dilated granular endoplasmic reticulum, and perinuclear space forming local swelling (arrow) (×8000); *e*) pulmonary lipofibroblast with solitary lipid inclusions (×4000).

membrane structures remained intact, there were no alterations appear in membrane-bound enzymes which inactivate 95% serotonin and 90% $PGE_1$, $PGE_2$, and $PGE_{2\alpha}$ upon a single passage of blood through the lungs [5].

Thus, lidocaine promotes the accumulation of secretory granules in EC cells without the release of their contents in the extracellular space so that local (paracrine) action of diarrhea-inducing secretion products is ruled out. On the other hand, the sero-

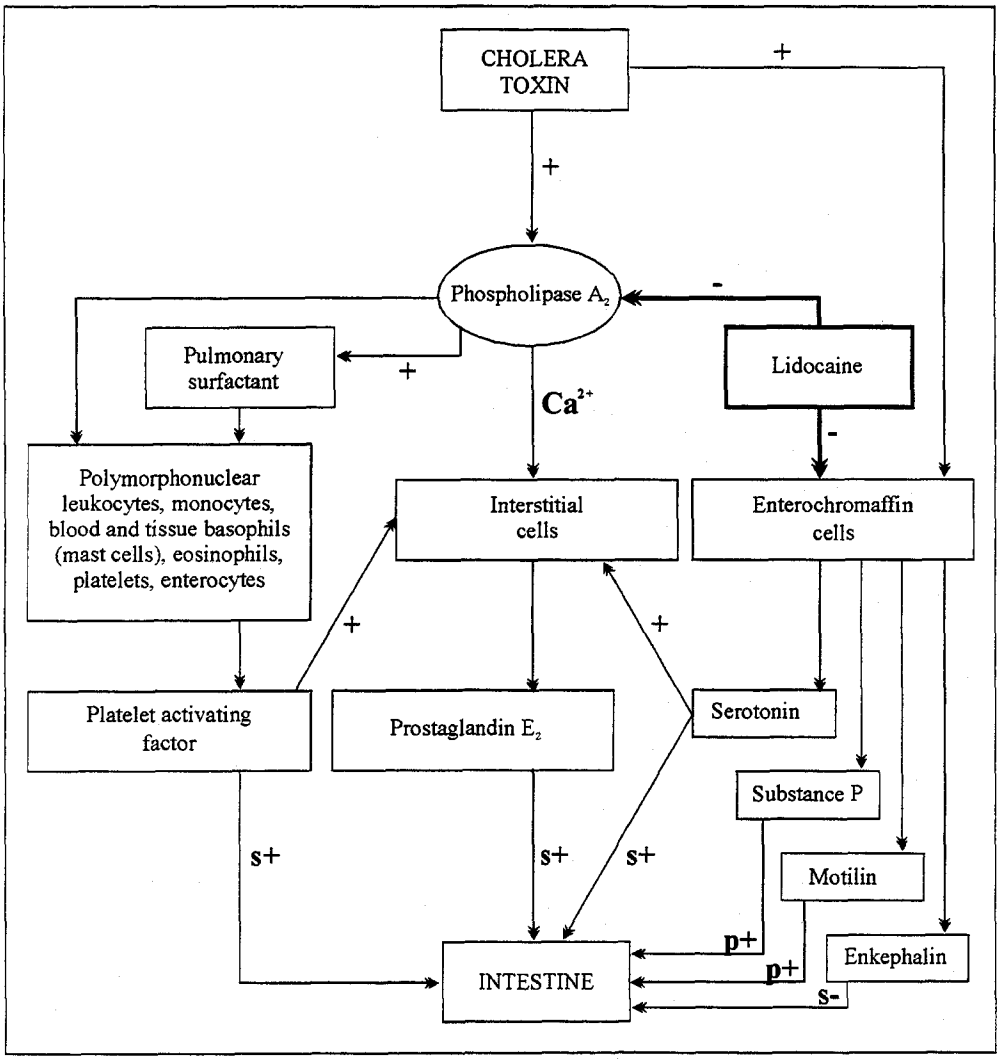


**Fig. 3.** A scheme showing the effect of lidocaine on some elements involved in the development of experimental cholera. *s*) secretion; *p*) peristalsis; +) enhancement (activation); -) inhibition (blockade).

tonin-mediated mechanism of $PGE_2$ production is blocked. Furthermore, lidocaine inhibits $PLA_2$ activity, thus excluding the possibility of arachidonates (including $PGE_2$) being formed from membrane phospholipids (Fig. 3).

Our results show that lidocaine administered to suckling rabbits infected with a virulent strain of cholera vibrio arrested the development of hydropic and ballooning degeneration in jejunal enterocytes. It also promoted preservation of vascular endothelium in the lungs, providing normal functioning of enzyme systems inactivating serotonin and $PGE_2$, which mediate enterocyte hypersecretion in cholera. Moreover, microcirculation and capillaries in the jejunum and renal medulla of lidocaine-treated animals remained unchanged. After administration of lidocaine, secretory granules accumulated in EC cells and lipid inclusions in interstitial cells without releasing their contents into the bloodstream. Thus, our findings open prospects for the use of drugs inactivating $PLA_2$ or blocking serotonin release in the treatment of cholera.

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