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Inhibition of Water Absorption in the Intestine by *Staphylococcus aureus* Delta-Toxin

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Water absorption in intestinal segments was monitored by measuring the concentration of polyethylene glycol, a nonabsorbable reference marker, in a balanced salt solution continuously perfused through the lumen. *Staphylococcus aureus* delta-toxin inhibited water absorption in rabbit jejunum and ileum perfused in vivo and in guinea pig ileum perfused in vitro. Cholera toxin also interfered with water absorption in guinea pig ileum maintained in vitro, but *S. aureus* enterotoxin B had no demonstrable effect on this tissue.

The ability for overgrowth of *Staphylococcus aureus* in the intestine, especially during broad-spectrum antibiotic therapy, is widely recognized and may be manifest by responses ranging from mild diarrhea to severe enterocolitis. Although it has been suggested that staphylococcal enterotoxins may play a role in the pathogenesis of staphylococcal enteritis (1), there is little direct evidence to support this concept.

In recent years much information has accumulated about bacterial products capable of causing diarrhea by altering water and electrolyte transport in the intestine. With cholera enterotoxin and *Escherichia coli* heat-labile enterotoxin, the mechanism involves stimulation of mucosal adenyl cyclase which leads to increased intracellular levels of cyclic adenosine 3',5'-monophosphate (9). This in turn alters ion transport and, secondarily, water movement. The staphylococcal enterotoxins, however, do not seem to act in this manner. Although there can be no doubt that these products are responsible for emesis in cases of staphylococcal food poisoning, this condition is clinically distinct from staphylococcal enteritis.

Most strains of *S. aureus* are capable of producing the delta-toxin. This material does not appear to be antigenic, but it does act on membranes of a wide variety of mammalian and nonmammalian cells (2). Because of these properties, the possibility that delta-toxin might affect water absorption in the intestine was entertained. Subsequent studies revealed that water absorption in rabbit and guinea pig ileum could be inhibited by delta-toxin but not by *S. aureus* enterotoxin B.

MATERIALS AND METHODS

Toxins. Delta-toxin was prepared and assayed by using *S. aureus* strain PG114 as previously described (8). In this study only the soluble form of delta-toxin, with a specific activity of approximately 100 50% hemolytic doses/mg, was used.

Cholera enterotoxin (lot 172) was prepared under contract for the National Institute of Allergy and Infectious Diseases by Richard A. Finkelstein, University of Texas Southwestern Medical School, Dallas (4).

S. aureus enterotoxin B (lot CB-40) was generously supplied by Merlin S. Bergdoll, Food Research Institute, University of Wisconsin, Madison.

Ringer solution. A calcium-free Ringer solution, used for all in vitro procedures, had a composition of (per liter): 7.14 g of NaCl, 0.365 g of KCl, 0.167 g of KH_2PO_4, 0.311 g of $MgSO_4 \cdot 7H_2O$, and 0.416 g of $NaHCO_3$. The solution, prepared as a 10× concentrate, was diluted just before use and equilibrated with a 5% CO_2 + 95% O_2 gas mixture. When necessary, the pH was adjusted to 7.3 with addition of 7.5% $NaHCO_3$.

Perfusion of rabbit intestinal segments in vivo. New Zealand white rabbits, weighing 2 to 3 kg and having free access to food and water, were initially anesthetized with sodium pentobarbital given intravenously. Anesthesia was maintained with small doses of pentobarbital administered intravenously and supplemented by ether given by inhalation. After making a midline incision, the desired region of the small intestine was located and divided into two adjacent 15-cm segments while leaving the blood supply intact. Segments were cannulated, flushed with Earle solution, and perfused according to the method of McGonagle et al. (10). The perfusing solution consisted of 40 ml of Earle solution with polyethylene glycol 4000 (PEG) (Gallard-Schlesinger Chemical Manufacturing Corp., Long Island, N.Y.), 2 mg/ml, as a nonabsorbable marker. One segment was perfused with solution containing delta-toxin, whereas the other functioned as control. With successive experiments, proximal and distal segments alternated as controls. Aliquots of perfusing solution were removed periodically, and the PEG concentration was measured by the method of Hyden (6). During the period of perfusion, the rectal temperature was monitored with a thermistor probe and

normal body temperature was maintainerd by providing heat with a heating pad placed beneath the animal.

Perfusion of guinea pig ileal segments maintained in vitro. Guinea pigs, weighing 500 to 900 g and allowed food and water ad libitum, were anesthetized with ether. The ileum was delivered through a midline incision and cut near the ileocecal valve and again about 20 cm proximally. Luminal contents were removed from the segment by flushing with 50 ml of warm Ringer solution in a syringe with blunted needle. After severing mesentery and blood supply, the segment was attached to a perfusion apparatus somewhat modified from that designed by Darlington and Quastel (3) (Fig. 1). Short pieces of latex tubing slipped over the glass cannulae before insertion into the segment afforded leakproof seals upon securing intestine to the apparatus with surgical silk thread. Sutures were spaced such that 15-cm lengths of ileum were perfused, and care was taken that the direction of perfusion was the same as the normal intestinal flow. Perfusion was carried out with 40 ml of calcium-free Ringer solution containing glucose (2 mg/ml), PEG (2.5 mg/ml), and silicone antifoam (Y-4988, Union Carbide) (10 μg/ml). Circulation and aeration were maintained by a gas lift provided by a 95% O_2 + 5% CO_2 mixture. The apparatus with attached segment was immersed in Ringer solution held at 37 C and aerated with the same gas mixture.

After mounting the first segment, an adjacent ileal segment was removed from the anesthetized animal and likewise mounted in a companion apparatus. The average elapsed time from severing the blood supply to commencement of perfusion was about 4 min. The desired toxin was added to the perfusing fluid of one segment, whereas the other served as control. Controls were alternated among proximal and distal segments with successive experiments.

At zero time and intervals thereafter, aliquots of perfusing solution were centrifuged to remove mucus and the PEG concentration in the supernatant fluid was measured. On occasion when more antifoam was required, an equal quantity was added to both segments simultaneously.

RESULTS

Water absorption in rabbit intestine. The lumen of jejunal segments with blood supply intact was perfused with Earle solution containing PEG and delta-toxin (1 to 2 mg/ml). An adjacent segment perfused without toxin served as a control. Perfusion was continued for 6 h, at which time the animals were sacrificed. Water absorption, as revealed by concentration of PEG in the perfusing solution, was linear with time in control segments and was not significantly different among proximal and distal segments (Fig. 2). Proximal segments responded to delta-toxin differently than did distal segments. Water absorption in proximal segments was reduced by delta-toxin at a concentration of

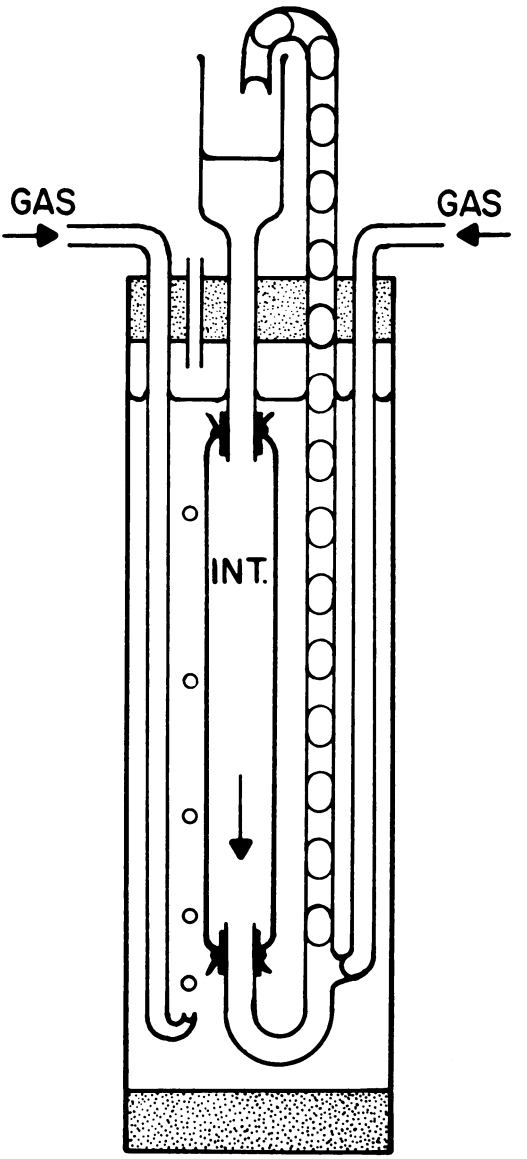

FIG. 1. *Apparatus for in vitro perfusion of intestinal segments as modified from Darlington and Quastel (3). The gas lift on the right (5% CO_2 + 95% O_2) oxygenates the perfusing solution and circulates the fluid through the lumen of the mounted intestinal segment (INT) in the direction shown by the arrow. Samples of perfusing solution are removed from the top of the apparatus. The same gas mixture oxygenates and circulates the perfusing solution bathing the serosal side of the segment. The entire apparatus is placed in a water bath for temperature control.*

1 mg/ml and ceased completely at a concentration of 2 mg/ml. With the greater dose of toxin, movement of fluid into the lumen occurred after 3 h. In distal segments the lower dose of

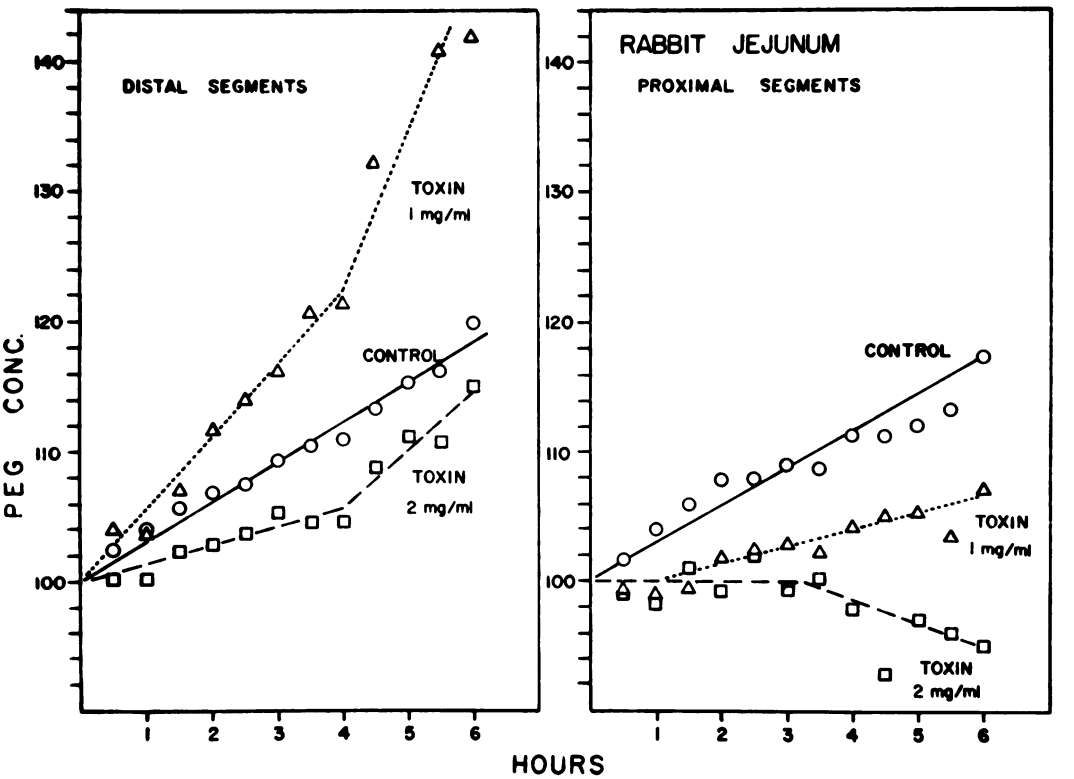

FIG. 2. *Effect of S. aureus delta-toxin on water absorption in proximal and distal segments of rabbit jejunum perfused in vivo. Polyethylene glycol (PEG) was used as a nonabsorbable reference marker with an initial concentration of 100%. Each point depicting data from segments perfused with toxin represents the mean value from eight rabbits. Each point depicting data from control segments represents the mean value from 16 rabbits. Specific activity of delta-toxin was 100 50% hemolytic doses/mg.*

delta-toxin actually increased water absorption, but toxin at 2 mg/ml did reduce water absorption for 4 h.

In contrast, rabbit ileal segments responded to delta-toxin in a consistent manner regardless of whether they were from the proximal or distal location (Fig. 3). Delta-toxin at 2 mg/ml caused an immediate cessation of water absorption that persisted during the 6-h period of observation.

Water absorption by guinea pig ileum. The aforementioned in vivo perfusions necessitated maintenance of anesthetized rabbits for several hours and resulted in a 15% loss of effort because of premature deaths. In addition, periodic contractions along the cannulated segments often impeded uniform luminal perfusion for several minutes at a time. For these reasons alternate experimental procedures were explored. The method of Darlington and Quastel maintained isolated intestinal segments in a viable state for several hours, and the small hydrostatic pressure provided by the perfusing solution kept segments fully inflated. Guinea pigs were substituted for rabbits because of convenience and to reduce expense.

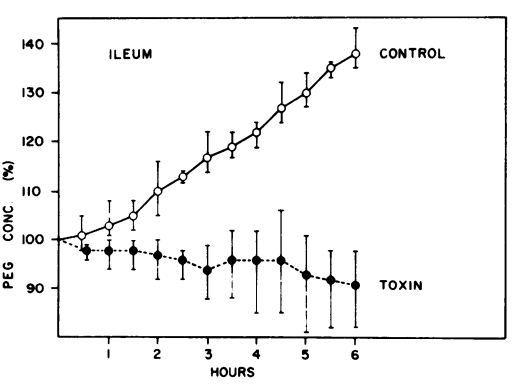

FIG. 3. *Effect of S. aureus delta-toxin on water absorption in rabbit ileal segments perfused in vivo. Polyethylene glycol (PEG) was used as a nonabsorbable reference marker with an initial concentration of 100%. Toxin concentration in perfusing solution was 2 mg/ml (specific activity, 100 50% hemolytic doses/mg). Each point represents a mean value derived from 12 rabbits.*

Guinea pig ileal segments perfused in vitro readily demonstrated a net transfer of water

from mucosa to serosa that was inhibited by delta-toxin in concentrations of 1 to 2 mg/ml (Fig. 4 and 5).

To establish a dose-response relationship, groups of ileal segments were exposed to various doses of delta-toxin. The response to a single concentration of toxin was evaluated by using five to eight animals (Fig. 6). From regression line analysis, the dose required to cause inhibition of water absorption in 50% of ileal segments was calculated to be 300 μg/ml (30

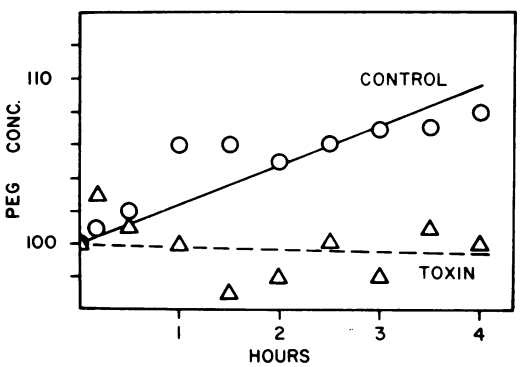

FIG. 4. *Effect of S. aureus delta-toxin on water absorption in isolated guinea pig ileal segments maintained in vitro. Polyethylene glycol (PEG) was used as a nonabsorbable reference marker with an initial concentration of 100%. Toxin concentration in perfusing solution was 1 mg/ml (equivalent to 107 50% hemolytic doses/cm² of mucosa). Each point represents mean value from four animals.*

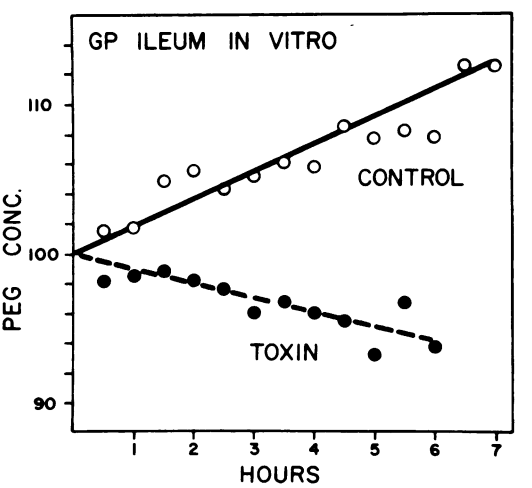

FIG. 5. *Effect of S. aureus delta-toxin on water absorption in guinea pig ileal segments perfused in vitro. Polyethylene glycol (PEG) was used as a nonabsorbable reference marker with an initial concentration of 100%. Toxin concentration in perfusing solution was 2 mg/ml (equivalent to 200 50% hemolytic doses/cm² of mucosa). Each point represents mean value derived from seven animals.*

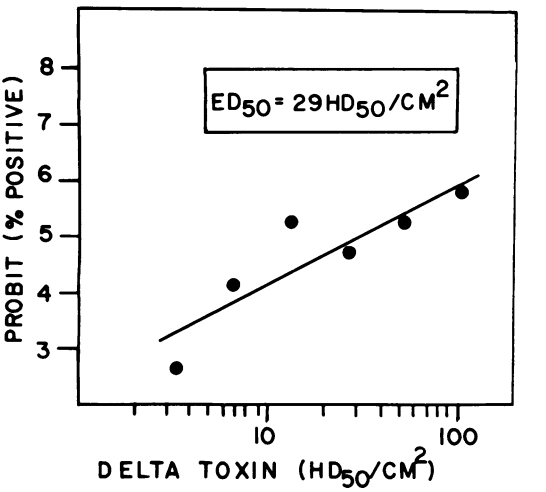

FIG. 6. *Dose-response relationship between percentage of animals demonstrating inhibition of water absorption (as probit) and concentration of delta-toxin. Effective dose 50% was calculated at 29 50% hemolytic doses per cm² of mucosa (300 μg/ml of perfusing solution).*

50% hemolytic doses/cm² of mucosa).

The degree to which water absorption was inhibited in a particular segment did not appear to be dose related. Whereas most segments, responding to delta-toxin manifested a complete inability to transport water, some segments continued to absorb water but at a markedly reduced rate. Among these latter segments, the minimal observed degree of inhibition was a 50% reduction in water absorption as compared with the control segment. Water absorption in segments evaluated as negative was indistinguishable from that of corresponding controls.

Since cholera toxin is known to interfere with water absorption in the small intestine by stimulating mucosal adenyl cyclase and thereby altering ion transport, isolated guinea pig ileal segments were examined for their response to this toxin. It was found that cholera toxin at 0.5 μg/cm² of mucosa caused inhibition of water absorption in segments perfused in vitro (Fig. 7).

S. aureus enterotoxin B, however, had no demonstrable effect on water absorption by guinea pig ileal segments when used in a dose of 5.0 μg/ml of perfusing fluid (5 μg/cm² of mucosa) (Fig. 8).

DISCUSSION

These studies demonstrated the ability of *S. aureus* delta-toxin to interfere with water absorption in rabbit jejunum and ileum perfused in vivo and in guinea pig ileum maintained in

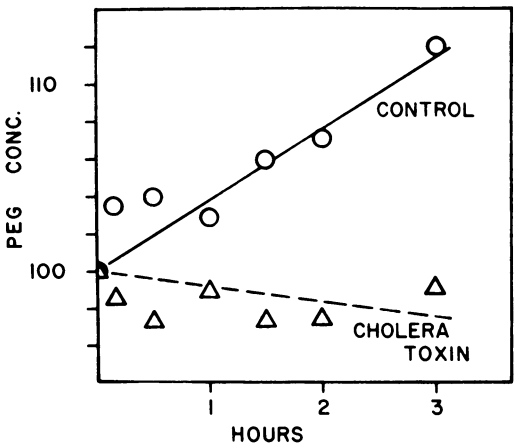

FIG. 7. *Effect of cholera toxin on water absorption in guinea pig ileal segments perfused in vitro. Polyethylene glycol (PEG) was used as a nonabsorbable reference marker with an initial concentration of 100%. Cholera toxin concentration was 0.5 μg per cm² of mucosa (0.5 μg/ml of perfusing solution). Mean values from two animals.*

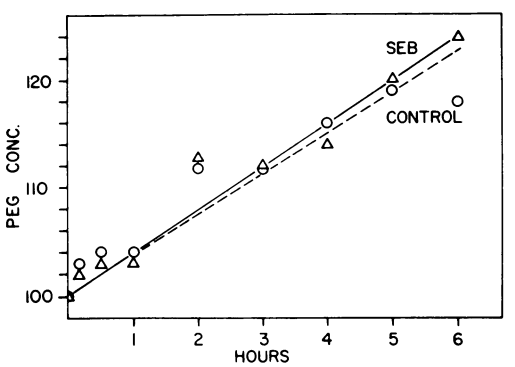

FIG. 8. *Effect of S. aureus enterotoxin B (SEB) on water absorption in guinea pig ileal segments perfused in vitro. Polyethylene glycol (PEG) was used as a nonabsorbable reference marker with an initial concentration of 100%. SEB concentration was 5.0 μg/cm² of mucosa (5 μg/ml of perfusing solution). Mean values from three animals. Controls, ○; enterotoxin B, △.*

vitro. Since movement of water across the mucosa is secondary to ion transport, these findings suggest that the primary action of delta-toxin is alteration of normal ion transport. However, measurement of specific ion transport in toxin-treated mucosa was not undertaken in this investigation.

Exposure of guinea pig ileal segments to decreasing concentrations of delta-toxin in vitro resulted in progressively fewer segments responding to toxin, yet the response in almost all affected segments was a complete cessation of water absorption. The reason for lack of graded responses is not clear. It is possible that graded responses occur over a narrow range of toxin concentration and that the minimal effective dose varies with the physiological state of the mucosa. Under these conditions graded responses might be difficult to demonstrate in groups of animals permitted free access to food and water.

It is known that the hemolytic activity of delta-toxin can be markedly influenced by the presence of various fatty acids and phospholipids (7; unpublished data). It is thus conceivable that variations in membrane lipids, or in lipids loosely associated with the mucosa, might regulate the response to a given dose of toxin by determining the proportion of toxin binding to the mucosa. If major fluctuations in lipid content among ileal segments occur, this might obscure the actual amount of toxin acting upon the mucosa and also make the existence of graded responses difficult to reveal.

In rabbit jejunum perfused in vivo, delta-toxin caused greater inhibition of water absorption in proximal segments (closest to the duodenum) than in distal segments (closer to the ileum). Water absorption in the absence of toxin was the same regardless of the relative positions of the adjacent segments. The reason for this difference in response to toxin is not known. Regional differences in physiological state, available lipids, or membrane receptor sites might account for such variation but presently have not been explored.

Delta-toxin is ordinarily characterized by its hemolytic or cytotoxic properties, but in this regard is of relatively low potency when compared with other bacterial products with similar activities. Although exposure of mucosa to large doses of delta-toxin in vitro can cause disruption of mucosal integrity, inhibition of water absorption did not appear to result solely from direct physical damage to the mucosa since histological sections prepared from toxin-perfused segments at the close of experiments revealed the mucosa intact. Furthermore, subsequent studies (11) indicated that doses of delta-toxin sufficient to increase mucosal cyclic adenosine 3',5'-monophosphate concentrations are much smaller than those required for cytotoxicity.

In our studies, *S. aureus* enterotoxin B had no effect on water absorption by guinea pig ileum in doses far in excess of an emetic dose. Sullivan and Asano (12), however, did note a brief and transient inhibition of water absorption with enterotoxin B in the rat. Huang et al. (5), using the flounder, reported alterations in

intestinal ion transport and changes in electrical properties upon exposure to staphylococcal enterotoxins, but water absorption was not specifically measured. Such differing results may reflect use of different animal species or different sources and purity of enterotoxins.

Although delta-toxin was shown to interfere with water absorption in the intestine, it cannot presently be concluded that this toxin is involved in the pathogenesis of staphylococcal enteritis. Information pertaining to toxin production in the lumen and to its specific effect on mucosa would be helpful in evaluating its role in this disease.

ACKNOWLEDGMENT

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