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Increased Cyclic Adenosine 3',5'-Monophosphate Content in Guinea Pig Ileum After Exposure to *Staphylococcus aureus* Delta-Toxin

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To compare *Staphylococcus aureus* delta-toxin with cholera toxin, which is known to increase cellular cyclic adenosine 3',5'-monophosphate (cAMP), studies were undertaken to determine the effect of delta-toxin on the cAMP content of guinea pig ileum maintained in vitro. Concentrations of delta-toxin as low as 0.40 μg/ml increased cAMP levels in guinea pig ileum after 2 h of incubation. Histological damage was seen in ileum exposed for 2 h to delta-toxin concentrations of 100 μg/ml. As little as 3 μg of delta-toxin increased vascular permeability in guinea pig skin. Permeability changes became evident within 5 min and were maximal within 6 h, whereas those produced by cholera toxin required 24 h to become maximal. Benadryl did not interfere with the ability of these toxins to alter vascular permeability. Purified egg lecithin reduced the effectiveness of delta-toxin in the skin but did not inhibit cholera toxin. Delta-toxin in concentrations as low as 0.1 μg/ml caused dislodgement of HeLa cells in tissue cultures. Therefore, delta-toxin appears unique in being the only bacterial toxin, currently known to alter water absorption in the ileum, that is capable of both increasing cAMP levels and being cytotoxic. These findings suggest a possible role for delta-toxin in the pathogenesis of staphylococcal enteritis.

Much effort has been directed toward understanding the pathogenesis of various bacterial diarrheas (3, 16). Most bacteria associated with diarrhea produce enterotoxins capable of altering fluid and electrolyte transport in the small intestine. The enterotoxin of *Vibrio cholerae* has been shown to be solely responsible for clinical cholera (20) by a mechanism involving stimulation of mucosal adenyl cyclase with subsequent elevation of cyclic adenosine 3',5'-monophosphate (cAMP) levels (4, 29, 34) which, in turn, leads to a net Cl^- secretion, a decrease in net Na^+ absorption, and a concomitant loss in water (12). The *Escherichia coli* heat-labile enterotoxin also appears to stimulate adenyl cyclase (11), but the mechanisms whereby other bacterial enterotoxins act have not been elucidated (14, 26, 36).

The ability of *Staphylococcus aureus* delta-toxin to inhibit water absorption in guinea pig and rabbit ileum (24) prompted us to compare its action with that of cholera toxin. We found that delta-toxin increased cAMP levels in guinea pig ileum and caused increased vascular permeability (presumably a cAMP-dependent response) in guinea pig skin.

MATERIALS AND METHODS

Animals. Random-bred guinea pigs of either sex, weighing 400 to 900 g (average, 600 g) and allowed food and water ad libitum, were used.

Toxins. *S. aureus* delta-toxin was produced and purified as previously described (23). In this study only the soluble form of delta-toxin with a specific activity of approximately 100 50% hemolytic doses per mg was used.

Cholera enterotoxin, lot number 0172, was prepared under contract for the National Institute of Allergy and Infectious Diseases by Richard A. Finkelstein, The University of Texas Southwestern Medical School, Dallas (13).

Ringer solution. A Ca^{2+}-free Ringer solution was used for all experimental procedures. The fluid had the following composition (grams per liter): NaCl, 7.14; KCl, 0.365; KH_2PO_4, 0.167; $MgSO_4 \cdot 7H_2O$, 0.311; $NaHCO_3$, 0.416. The solution was prepared as a 10× concentrate and was diluted just before use. The solution was equilibrated with 95% O_2 + 5% CO_2 and adjusted to pH 7.3 with 7.5% $NaHCO_3$ as necessary.

Treatment of guinea pig ileum with delta-toxin and cAMP determinations. Guinea pig ileum was incubated in vitro according to the technique of Kimberg et al. (28). Animals were anesthetized with ether, a midline incision was made, the distal 25 to

40 cm of ileum was removed after rinsing the lumen with Ringer solution to remove intestinal contents, and the cleansed ileum was cut into 1.5-cm segments. The segments were cut longitudinally along the mesenteric border and two pieces (3 cm), randomly selected, were placed in a glass vial containing 5.0 ml of Ringer solution. Various doses of delta-toxin, cholera toxin, or diluent were added as desired. Vials were incubated in a shaking water bath at 37 C while being continuously gassed with a 95% O_2 + 5% CO_2 gas mixture.

At the termination of incubation the contents of each vial were placed in a 10-ml Potter-Elvejehm mortar containing 1.0 ml of ice-cold 30% trichloroacetic acid with 3 to 5 μl of [^{3}H]cAMP solution (0.15 to 0.25 nCi) as a recovery marker. The tissues were homogenized and the homogenates were centrifuged at 1,100 $\times$ g for 15 min. The supernatant fluid was transferred to screw-cap tubes containing 0.5 ml of 0.1 N HCl. The trichloroacetic acid was removed by extracting two to five times with 10-ml aliquots of water-saturated ether, and the samples were dried at 70 C under nitrogen at reduced pressure. The residues were dissolved in 2.0 ml of 50 mM sodium acetate buffer, pH 4.0. cAMP recovery from extracted tissued was determined by counting 0.5-ml aliquots in 10 ml of preblended liquid scintillation cocktail (Research Products International Corp. Complete Counting Cocktail 3a70). Counts per minute obtained for each sample were compared with those obtained with an equal amount of unprocessed marker counted the same way. Each sample was assayed in triplicate for cAMP content by the protein binding method described by Gilman (15), using [^{3}H]cAMP assay kit no. AO (Diagnostic Products). To obtain values falling on the standard curve, samples had to be diluted in the range of 1:4 to 1:16 in acetate buffer. Most dilutions were stock rather than serial to minimize error.

Histological sections of ileum treated with delta-toxin. Guinea pig ileal segments were obtained and incubated in vitro with delta-toxin as described above. After various periods of incubation, segments were placed in 10 ml of neutral buffered formalin. Histological sections (5 μm thick) were cut and stained with hematoxylin-eosin.

Vascular permeability. A modification of Craig's method (5) was used. After clipping the ventral surface of guinea pigs, skin sites were inoculated with 0.1-ml volumes containing various doses of delta-toxin (1 μg to 1.0 mg) or other test preparations. After various intervals (5 min to 24 h), 5% (wt/vol) Evan's blue in 0.45% NaCl was given intracardially at a dose of 1.2 ml/kg. One hour later the animals were sacrificed by a blow on the head and skinned. The major and minor diameters of responses were measured, and the area was calculated by considering that lesions assumed the shape of an elipse.

Microassay for HeLa cell cytotoxicity. The cytotoxic effect of delta-toxin on HeLa cells was quantitated by a modification of a microassay previously described by Keusch et al. (27). HeLa cells were grown at 37 C in Eagle minimal essential medium plus Earle balanced salts (Flow Laboratories) with 100 U of penicillin G per ml, 100 μg of streptomycin per ml, 50 μg of chlortetracycline per ml, 10 μg of polymyxin B per ml, 10% fetal calf sera (FCS; Flow Laboratories), and 2.25 g of $NaHCO_3$ per liter under an atmosphere of 10% CO_2. Cells were grown in 32-oz (about 960-ml) glass prescription bottles. Upon obtaining a confluent monolayer, cells were trypsinized (with a solution containing 0.25% trypsin, 0.47% sodium citrate, and 0.075% KCl), counted in a Neubauer chamber, and suspended in the above growth media to the desired concentration. Cells were planted in microplates (Micro Test II tissue culture plate, Falcon Plastics) at a density of 20,000 cells/well. The following day cells were washed twice with 0.1-ml aliquots of growth media without FCS and exposed to 0.1 ml of the same serum-free medium containing appropriate amounts of delta-toxin. Serum was avoided at this stage since phospholipids present in sera inhibit the activity of delta-toxin (7). After 15 min of incubation, wells received 0.1 ml of medium with or without 10% FCS. Incubation was continued for another 24 h at 37 C, and the wells were washed twice with 0.1-ml aliquots of medium. Trypsin solution (0.05 ml) was added to each well and incubated 10 min at 37 C, and 0.1 ml of medium was added. Cells remaining in the wells were suspended by agitation and counted in a Neubauer chamber. The percentage of HeLa cells detached by each concentration of delta-toxin was determined by comparing cell counts in control wells with counts in toxin-treated wells.

RESULTS

Effect of delta-toxin and cholera enterotoxin on cAMP concentration in guinea pig ileum. Preliminary efforts to measure cAMP content of mucosa were hampered because guinea pig ileal mucosa cannot be stripped easily from the underlying muscularis. Attempts to relate cAMP content to protein content using mucosal scrapings proved erratic since the amount of extraneous tissue removed together with mucosa could not be regulated. Although the amount of cAMP in mucosal scrapings was approximately 70% of the total cAMP recoverable from equal lengths of entire ileum, for greater consistency it was decided to relate cAMP concentrations to unit length by using entire ileal segments.

Exposure of guinea pig ileal segments to delta-toxin for 2 h in vitro resulted in elevation of cAMP levels in eight out of nine animals tested (Table 1). Ileum from the single animal not responding significantly (no. 8) was exposed only to the lowest concentration of delta-toxin used in this study. Representative portions of ileum from all animals were treated with cholera toxin which served as a positive control. There was an average 1.49-fold increase in cAMP content in ileum from five animals

TABLE 1. *cAMP content of guinea pig ileum after treatment with S. aureus delta-toxin or cholera enterotoxin*

Animal no.	Control (pmol of cAMP/cm)	Delta-toxin treated			Cholera toxin treated[a]	
		Delta-toxin concn	pmol of cAMP/cm	Fold increase	pmol of cAMP/cm	Fold increase
1	418	1.0 mg/ml	735	1.76	519	1.24
2	379	1.0 mg/ml	509	1.34	588	1.55
3	789	1.0 mg/ml	1,256	1.59	1,476	1.87
4	454	1.0 mg/ml	553	1.22	867	1.91
5	1,002	1.0 mg/ml	1,551	1.55	1,462	1.46
				Avg: 1.45 ($P = 0.03$)[b]		
5	1,002	0.4 mg/ml	1,128	1.12		
6	505	0.4 mg/ml	694	1.37	1,025	2.03
				Avg: 1.25 ($P = 0.14$)[b]		
5	1,002	40 μg/ml	958	0.96		
6	505	40 μg/ml	717	1.42		
				Avg: 1.19 ($P = 0.28$)[b]		
6	505	0.4 μg/ml	1,101	2.18		
7	245	0.4 μg/ml	422	1.72	340	1.39
8	214	0.4 μg/ml	242	1.13	263	1.22
9	637	0.4 μg/ml	1,285	2.02	1,498	2.35
				Avg: 1.76 ($P = 0.025$)[b]		Avg: 1.67 ($P < 0.005$)[b]
4 (Dead tissue)	956	1.0 mg/ml	989	1.03	916	0.96

[a] At a concentration of 10 μg/ml.
[b] Statistics performed by using one-sided student's *t* test.

treated with 1 mg of delta-toxin per ml and an average 1.76-fold increase in four animals exposed to 0.4 μg of toxin per ml. Ileum from guinea pig 5 responded to a toxin concentration of 1 mg/ml, less definitively to 400 μg/ml, and not at all to 40 μg/ml, whereas tissue from animal 6 manifested an increase in cAMP content upon exposure to delta-toxin over the range of 400 to 0.4 μ/ml. Treatment of segments with 10 μg of cholera toxin per ml resulted in an average 1.67-fold increase in cAMP content for the nine animals used. Although considerable animal-to-animal variation in cAMP content of untreated control segments was noted, this did not preclude demonstration of a subsequent response to these toxins. A portion of normal ileum from animal 4, held overnight at 4 C and appearing nonviable by trypan blue staining, contained a higher level of cAMP than its viable counterpart but showed no further increase after exposure to delta-toxin or cholera toxin.

Guinea pig ileal segments, exposed to either 1 mg or 0.4 μg of delta-toxin per ml in vitro and periodically assayed for cAMP, revealed that at least 1 h was required before an increase in cAMP content was discernible (Fig. 1 and 2).

Histological changes in toxin-treated ileum. Ileal segments placed in various concentrations of delta-toxin and maintained in vitro were, at intervals, fixed in formalin and sectioned.

Segments exposed to 40 μg of toxin per ml for 2 h appeared similar to control segments maintained for the same period. The villi were long and slender with epithelium intact (Fig. 3a, b). Ileum exposed to 100 μg of toxin per ml possessed normal-appearing villi after 30 min, but by 2 h the villi were short, swollen, and without

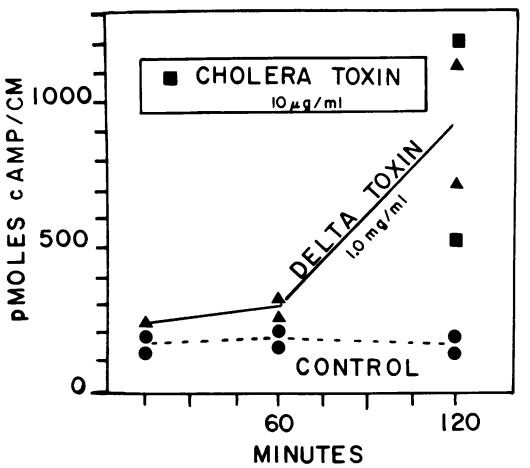

FIG. 1. *Changes in cAMP content of guinea pig ileal segments exposed to 1.0 mg of S. aureus delta-toxin per ml. Representative segments, treated with 10 μg of cholera toxin per ml, served as positive controls and were assayed only after 2 h. Each point is derived from two 1.5-cm lengths of ileum, all obtained from a single animal.*

crests (Fig. 4a, b). Similar changes were noted within 30 min in ileum treated with 1 mg of delta-toxin per ml (Fig. 5a, b).

Vascular permeability changes in guinea pig skin. Skin sites, prepared by using various concentrations of delta-toxin or cholera enterotoxin, were elicited by the intracardiac administration of Evan's blue. The area of increased capillary permeability, delineated by leakage of dye from the vasculature, was manifest as a blue zone when viewing the under-surface of the skin. In addition to some animal-to-animal variation, the size of skin responses to a particular dose of toxin was found to vary depending upon location of the site; therefore attempts were made to make comparisons on one animal and to place sites equidistant from the midline.

As little as 3 μg of delta-toxin increased vascular permeability as evidenced by producing noticeable skin bluing. In general, larger doses evoked greater responses (Fig. 6). Sites inoculated with toxin in excess of 100 μg also demonstrated a central region of blanching. In such sites blanching occurred within 5 min and appeared as a white area visible through the intact skin even before administration of Evan's blue. Later, when viewed from the underside, the central region appeared hemorrhagic, edematous, and devoid of dye, but surrounded by a much larger zone of blue.

In studies where dye was given various intervals after preparation of skin sites, it became evident that delta-toxin caused maximal effects within 6 h, whereas the size of skin responses produced by cholera toxin continued to increase beyond this period (Fig. 7). It should be noted that the time intervals stipulated refer to the interval between toxin inoculation and dye administration; another hour elapsed before the animals were sacrificed and the skin sites were examined.

Chromatographically pure egg lecithin, a known inhibitor of delta-toxin (22), reduced the activity of delta-toxin but did not inhibit cholera toxin (Table 2). With large doses of delta-toxin, lecithin more readily eliminated the blanching response than the skin bluing response. The antihistaminic benadryl hydrochloride, given to animals (50 mg/kg intraperitoneally) 1 h before preparation of skin sites, did not reduce the ability of delta-toxin or cholera toxin to alter capillary permeability (Table 3), nor did preparation of skin sites with 100 μg of histamine hydrochloride lead to skin bluing.

HeLa cell cytotoxicity. The ability of delta-toxin to dislodge HeLa cells in tissue culture is shown in Fig. 8. Cells were more readily affected when cultured in the absence of serum

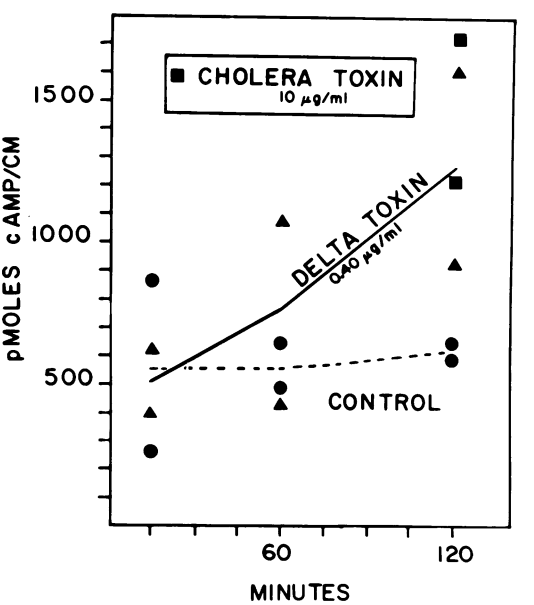

FIG. 2. *Changes in cAMP content of guinea pig ileal segments exposed to 0.4 μg of S. aureus delta-toxin per ml. Representative segments, treated with 10 μg of cholera toxin per ml, served as positive controls and were assayed only after 2 h. Each point is derived from two 1.5-cm lengths of ileum, all obtained from a single animal.*

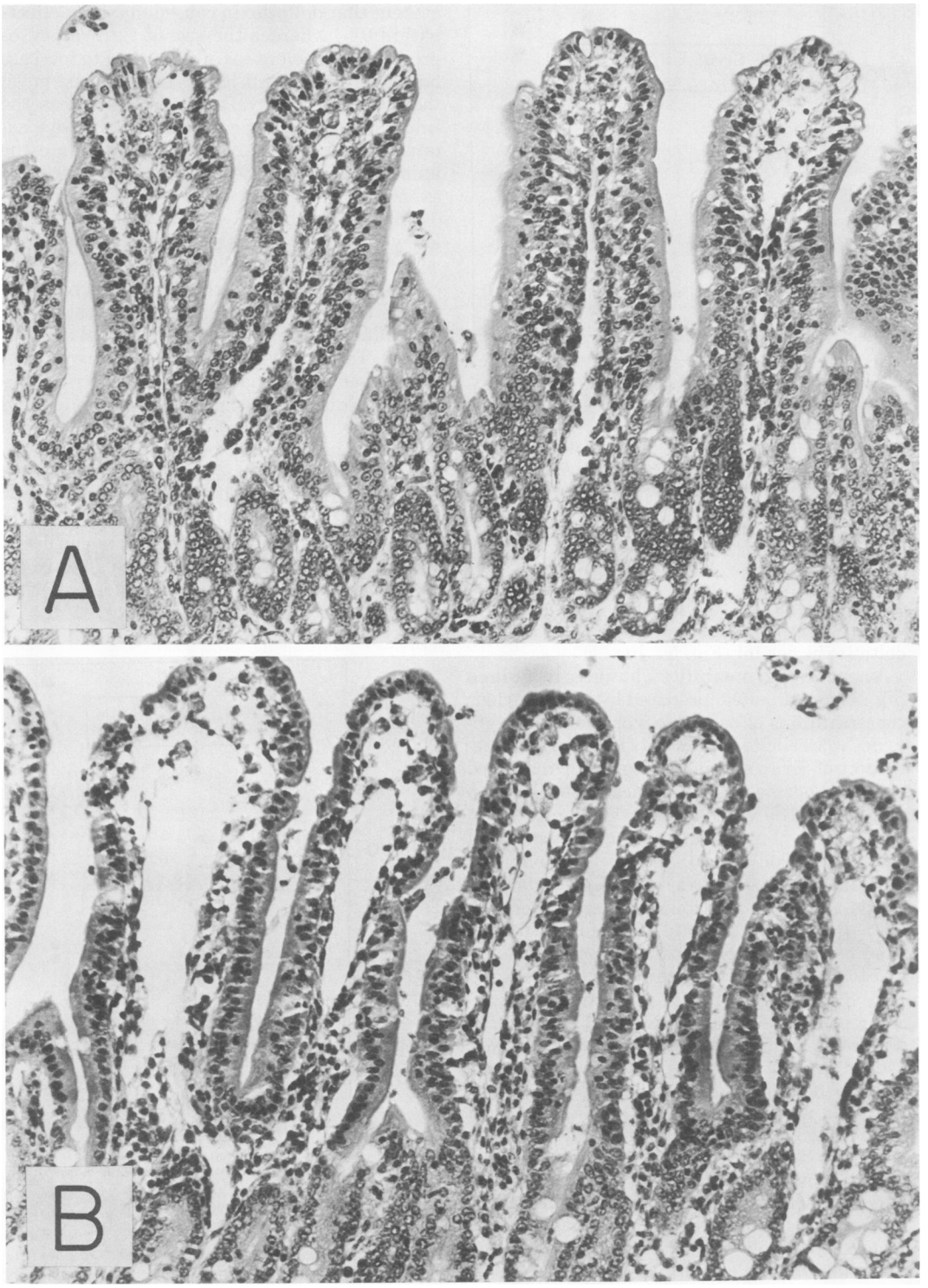

FIG. 3. *Histological sections of guinea pig ileum maintained in vitro for 2 h. (A) Control; (B) S. aureus delta-toxin, 40 μg/ml. Hematoxylin-eosin stain.*

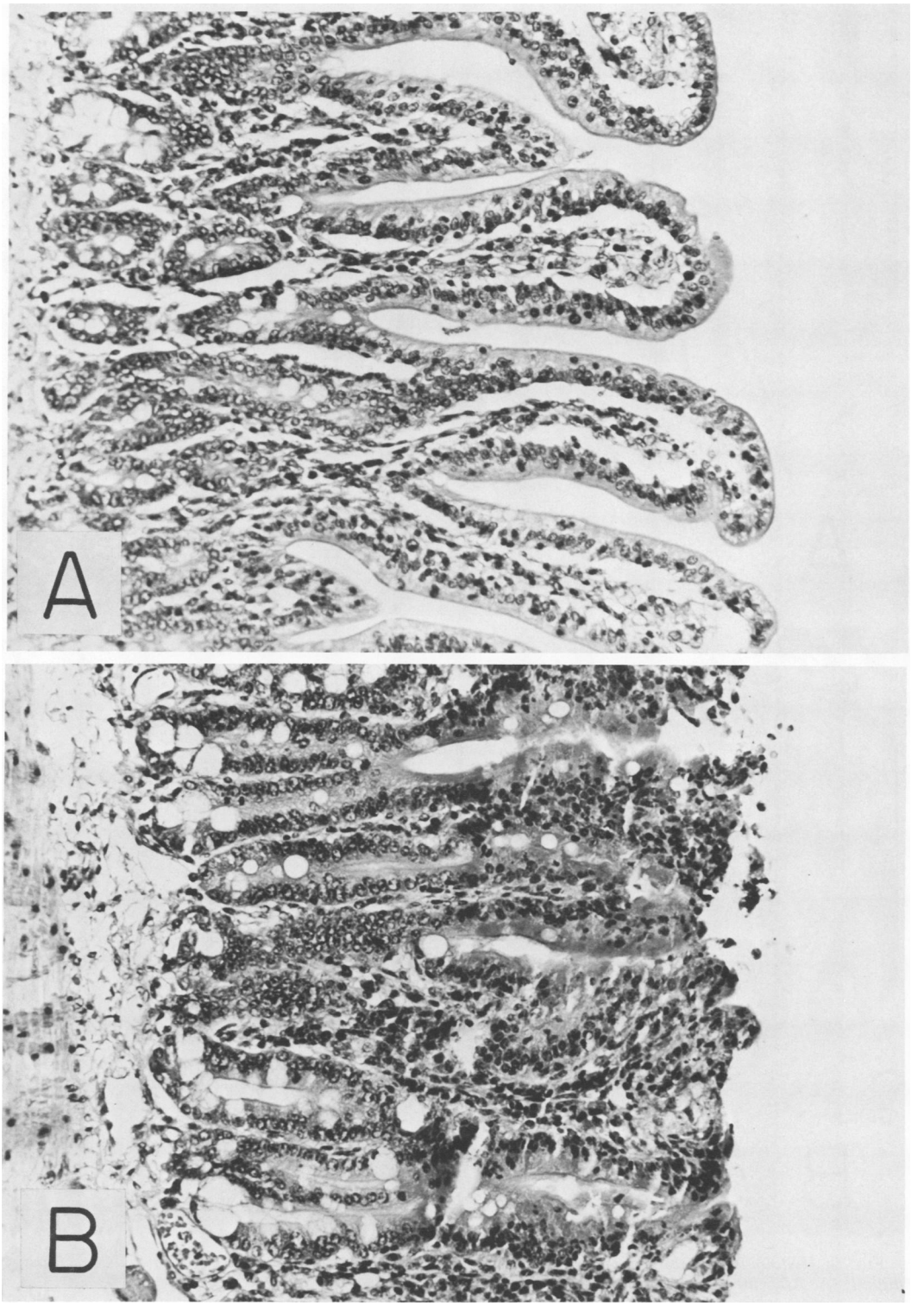

FIG. 4. *Histological sections of guinea pig ileum maintained in vitro in presence of S. aureus delta-toxin, 100 μg/ml. (A) After 30 min; (B) after 2 h. Hematoxylin-eosin stain.*

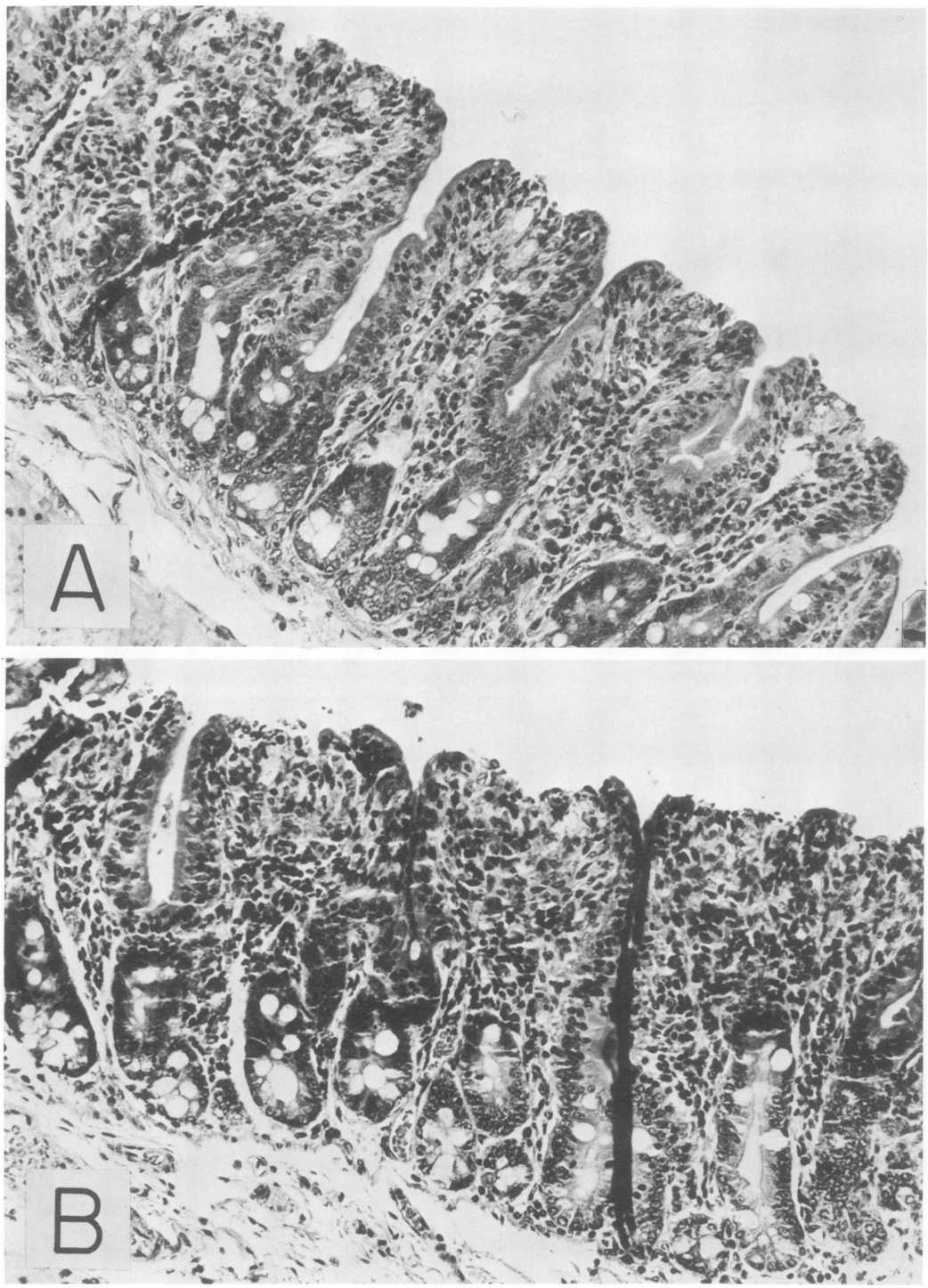

FIG. 5. *Histological sections of guinea pig ileum maintained in vitro in presence of S. aureus delta-toxin, 1 mg/ml. (A) After 30 min; (B) after 2 h. Hematoxylin-eosin stain.*

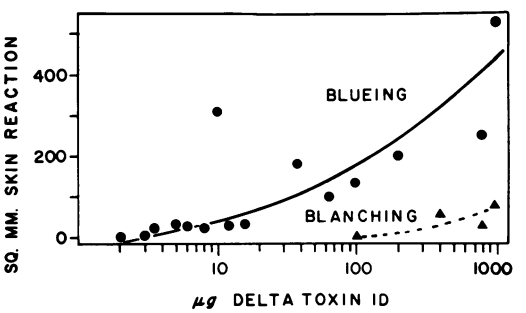

FIG. 6. *Increased vascular permeability in guinea pig skin after intradermal inoculation of S. aureus delta-toxin. Evan's blue was administered 22 h after preparation of skin sites, and skin responses were measured 1 h later. The areas of skin bluing and of blanched zones were determined separately. Each point represents values derived from one to six animals.*

than in medium containing FCS. In toxin concentrations exceeding 30 μg/ml evidence of cytotoxicity could be observed microscopically as early as 15 min after contact with toxin, and in the range of 100 to 1,000 μg/ml this toxicity was manifest as complete lysis of the cell population.

DISCUSSION

Bacterial enterotoxins, which cause alterations in fluid and electrolyte transport in the gut, have been segregated by Keusch and Donta into two catagories: those which increase cAMP concentrations by stimulating adenyl cyclase, and those which are cytotoxic (25). *V. cholerae* enterotoxin and *E. coli* heat-labile enterotoxin have been shown to affect such cAMP-dependent reactions as steroidogenesis in Y1 adrenal cells (8, 9), morphological changes in Chinese hamster ovary cells (17), increased lipolysis in fat cells (11, 38), and increased vascular permeability in skin (1, 5, 10). In the intestine, cholera toxin increases the transmural potential difference, the short-circuit current, and the net Cl^- secretion while simultaneously decreasing the net Na^+ absorption (12). These effects can be mimicked by exogenously added cAMP. Neither cholera toxin nor *E. coli* heat-labile enterotoxin causes histological damage to intact ileal or jejunal mucosa or cytotoxicity to HeLa cells in culture (3, 25). On the other hand, *Shigella dysenteriae* enterotoxin and *Clostridium perfringens* enterotoxin alter water and electrolyte transport in the gut (32, 35, 36), but do not stimulate adenyl cyclase or cause increased vascular permeability in rabbit skin (26, 35). Whereas both are cytotoxic for HeLa cells (25) and crude preparations of *C. perfringens* enterotoxin have been reported to destroy villi crests in rat ileum (32), *S. dysenteriae* enterotoxin has no destructive effect on rabbit jejunum (36).

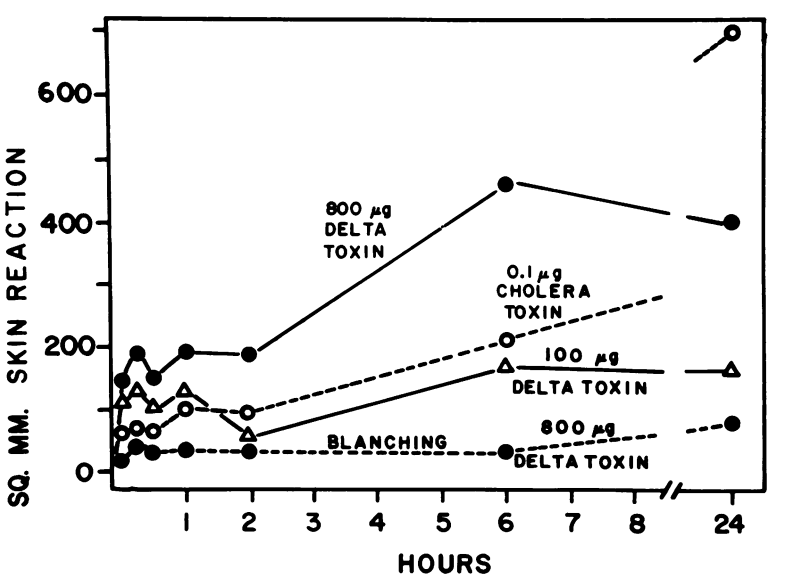

FIG. 7. *Development of skin reactions (increased vascular permeability) after preparation of sites with S. aureus delta-toxin or cholera toxin. The time indicated refers to intervals between preparation of skin sites and administration of Evan's blue. Lesions were measured 1 h after dye inoculation and the areas were calculated. Central zones of blanching occurred only in sites prepared with 800 μg of delta-toxin and are shown separately. Each point represents data from two guinea pigs.*

TABLE 2. *Effect of lecithin on guinea pig skin reactions to S. aureus delta-toxin and cholera enterotoxin*[a]

Dose of toxin	Dose of lecithin	Area of bluing (mm^2)	Area of blanching (mm^2)
Delta-toxin			
1.0 mg	None	251	129
1.0 mg	0.25 mg	276	20
1.0 mg	0.50 mg	471	0
1.0 mg	1.0 mg	38	0
1.0 mg	2.0 mg	0	0
100 μg	None	204	0
100 μg	25 μg	294	0
100 μg	50 μg	345	0
100 μg	100 μg	311	0
100 μg	200 μg	55	0
Cholera toxin			
0.1 μg	None	466	0
0.1 μg	200 μg	566	0
0.1 μg	1 mg	539	0
None	200 μg	0	0
None	1 mg	0	0

[a] Evan's blue was administered 2 h after preparation of skin sites with delta-toxin or 24 h after preparation of sites with cholera toxin or lecithin only.

TABLE 3. *Effect of pretreatment with benadryl on guinea pig skin reactions to S. aureus delta-toxin and cholera enterotoxin*[a]

Skin test dose	Area of skin reaction (mm^2)	
	Untreated animals	Animals pretreated with benadryl
Delta-toxin:		
1.0 mg	530 (85)[b]	659 (44)[b]
100 μg	138	333
50 μg	198	224
5 μg	33	55
Cholera toxin		
0.1 μg	223	431

[a] Evan's blue was administered 2 h after preparation of skin sites; reactions were measured 1 h later.

[b] Number in parentheses refers to blanched area.

S. aureus delta-toxin is similar to cholera toxin and *E. coli* heat-labile enterotoxin in its ability to elevate ileal cAMP levels and in its ability to increase vascular permeability in guinea pig skin. This increase in vascular permeability is assumed to be a cAMP-mediated response; if true, this suggests that delta-toxin may increase cAMP content of tissues other than intestinal mucosa. However, in contrast to cholera toxin, the ability of delta-toxin to increase vascular permeability is inhibited by lecithin. This differential effect may simply reflect the existence of different membrane binding sites for the two toxins: one subject to blockage by phospholipids, the other not. Whether lecithin or other phospholipids can also prevent elevation of cAMP content in ileum exposed to delta-toxin has not as yet been investigated. The possibility that increased vascular permeability resulted from the local release of histamine would seem to be precluded by the observation that skin reactions were not inhibited in animals pretreated with benadryl and by the fact that skin sites prepared with histamine, even in doses sufficient to kill, did not initiate the skin-bluing response.

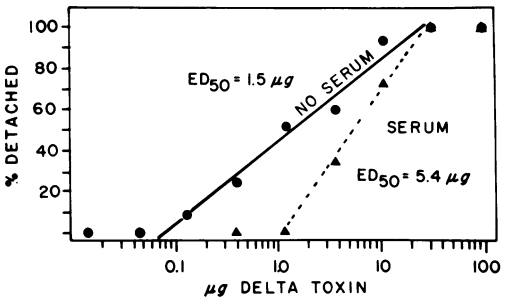

FIG. 8. *HeLa cell toxicity of S. aureus delta-toxin in the presence or absence of serum. Effective dose for 50% detachment was 5.4 and 1.5 μg, respectively.*

With respect to HeLa cell toxicity, delta-toxin resembles the enterotoxins of *S. dysenteriae* and *C. perfringens*, but the slope of the dose-response relationship with delta-toxin is markedly greater than that reported by Keusch and Donta for the other toxins (25). Delta-toxin, in high concentrations, also resembles *C. perfringens* enterotoxin by its ability to disrupt the integrity of villi crests (32). However, delta-toxin differs from the other four enterotoxins by being able to lyse a wide variety of erythrocytes and by apparently being nonantigenic (18, 30, 31, 33).

Although some investigators believe that *S. aureus* enterotoxins may cause diarrhea (2), definitive evidence is lacking that staphylococcal enterotoxins act like the enterotoxins of other bacterial species. We have been unable to show that *S. aureus* enterotoxin B alters fluid absorption in guinea pig ileum (24). Enterotoxin B has been reported by Sullivan and Asano (37) to cause a transient alteration in water absorption in rat ileum, and Huang et al. (21) noticed that this toxin initiated changes in ion transport and electrical properties in flounder intestine. However, no effect on Y1 adrenal cells could be produced by staphylococcal entero-

toxins A or B (9), nor has cell cytotoxicity been demonstrated (19).

There can be little doubt that staphylococcal enterotoxins are responsible for emesis seen in cases of staphylococcal food poisoning, but this clinical entity is quite distinct from staphylococcal enteritis. Craig excluded staphylococcal enterotoxins from a discussion of enterotoxic enteropathies (6) because he considered the clinical manifestations of staphylococcal food poisoning (nausea, vomiting, and occasional diarrhea) to result from these toxins acting on the central and autonomic nervous systems. For these reasons he viewed the staphylococcal enterotoxins as "neurotoxins."

Although the experimental evidence demonstrating the ability of delta-toxin to inhibit water absorption and to increase cAMP levels in the gut is consistent with the concept that the toxin may play a role in the pathogenesis of staphylococcal enteritis, these observations do not constitute proof of the hypothesis. Information concerning intraluminal production, inactivation, binding, and distribution of delta-toxin must be obtained before a clearer appreciation of its role in the pathogenesis can be determined. Furthermore, the possible role of other staphylococcal products in this disease cannot be overlooked.

In our studies, considerable animal-to-animal variation in ileal cAMP content was noted (Table 1). In part, this may have resulted from animals having free access to feed. Similar animal-to-animal variation has been reported by Kimberg et al. in stripped rabbit ileum (29). Nevertheless, further increases in cAMP content were readily demonstrable after exposure to delta-toxin or cholera toxin, the latter serving as a positive control.

Although our data indicate the ability of delta-toxin to increase cAMP in guinea pig ileum, the mechanism whereby this is accomplished is not known. In general, cellular cAMP levels can be elevated either by stimulation of adenyl cyclase, thus leading to greater production of the product, or by inhibition of cyclic nucleotide phosphodiesterase, which results in reduced destruction of the nucleotide. Whether delta-toxin acts by either of these mechanisms remains to be determined. Cholera toxin binds to mucosal cell membranes and somehow stimulates adenyl cyclase, and this leads to increased intracellular concentrations of cAMP, which in turn alters ion transport. Water movement, a passive phenomenon, is then dependent upon ion transport. It does not necessarily follow that delta-toxin behaves in the same manner as cholera toxin. It is possible that delta-toxin inhibits water absorption by interfering with normal ion transport through a direct action on the cell membrane. The observed increase in cAMP levels could be coincident with such an effect and may not be the immediate cause of altered ion movement. Attempts to gather information pertinent to these questions by studying the effect of delta-toxin on such cAMP-mediated phenomena as morphological changes in Chinese hamster ovary cells and steroidogenesis in Y1 adrenal cells are currently underway and will be reported elsewhere.

Although high concentrations of delta-toxin (100 to 1,000 μg/ml) disrupted mucosal integrity (Fig. 4 and 5), it is unlikely that such physical damage alone could account for the observed inhibition of water absorption (24). Three observations argue against this possibility. Water absorption was notably reduced in perfused ileal segments before histological damage was seen in comparable ileal segments exposed to toxin in vitro. In addition, water absorption was inhibited with toxin concentrations less than those required to disrupt villi crests. Finally, ileal segments perfused with toxin in vivo or in vitro, and demonstrating inhibition of water absorption, revealed no evidence of histological damage when examined at the conclusion of experiments.

In contrast to the other organisms whose enterotoxins are capable of increasing cellular cAMP, *S. aureus* is distinctly invasive and capable of producing a variety of disease states throughout the body. A toxin capable of altering cAMP levels, when produced in sites other than the intestinal lumen, might cause widespread disturbances in tissue functions. The fact that delta-toxin is commonly elaborated by *S. aureus* strains, is rather nonspecific with respect to cells attacked, and is seemingly nonantigenic, is additional reason for suspecting that this toxin may be responsible for certain systemic manifestations seen during severe staphylococcal infections even in individuals possessing antibody to numerous products of the organism.

ACKNOWLEDGMENTS

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