Staphylococcal Enterotoxins Fail to Disrupt Membrane Integrity or Synthetic Functions of Henle 407 Intestinal Cells

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The potential cytotoxic activity of purified staphylococcal enterotoxins for mammalian cells was evaluated. The effects of staphylococcal enterotoxins A (SEA) and B (SEB) on cell membrane integrity as measured by leakage of labeled cytoplasmic constituents ([^{3}H]uridine), amino acid transport (lysine and amino-isobutyric acid), and macromolecular synthesis (protein, ribonucleic acid, and deoxyribonucleic acid) was evaluated for a human intestinal epithelial cell (Henle 407). No evidence of cytotoxicity by any of these criteria could be detected for cell monolayers incubated with SEA for periods of between 30 min and 24 h. Purified staphylococcal hemolysins (alpha- and delta-toxins) were shown to exert cytotoxicity by the leakage and amino acid uptake assays. In efforts to detect synergistic effects between enterotoxin and the staphylococcal cytotoxins, membrane functions were evaluated after sequential or combined treatment with enterotoxin and alpha-toxin or with enterotoxin and delta-toxin. In no instance could a contribution to cytotoxicity by the staphylococcal enterotoxin be detected. That the assays were sufficiently sensitive to detect synergistic effects was shown by the greater than additive effects achieved with a combination of alpha- and delta-toxins. The data, contrary to previous reports, showed that staphylococcal enterotoxins did not behave as bacterial cytotoxins.

Several antigenically distinct staphylococcal enterotoxins are produced by a significant percentage of *Staphylococcus aureus* clinical isolates. The toxins cause severe food poisoning in humans. Symptoms of this clinical entity include vomiting, diarrhea, and nausea, usually within 8 h after ingestion (2), and the toxin serotypes produce the same clinical syndrome. Although enterotoxins A (SEA), B (SEB), and C are distinct proteins, they have similar physiochemical properties (1). SEA is most frequently associated with human food poisoning outbreaks. Furthermore, this toxin serotype is most readily available in highly purified form (18). Therefore, SEA was chosen for this study, although some experiments using SEB were also included.

The biochemical mechanism by which staphylococcal enterotoxin acts is unknown. One of several mechanisms suggested is a direct cytotoxic effect of ingested toxin on gastrointestinal tissues. Observations that support this view include acute exogenous gastritis observed in outbreaks of staphylococcal food poisoning (11), lesions of the small intestine observed in rhesus monkeys given SEB by mouth (8), and changes in intestinal transport of fluids and electrolytes as a result of the administration of enterotoxins (6, 19). These data suggest that staphylococcal enterotoxins may disrupt normal intestinal physiology but do not describe what changes, if any, occur in intestinal mucosal cells. Opportunity for study of toxin action is provided by in vitro experimental models.

Using an established tissue culture line of embryonic intestinal mucosa (Henle 407), Schaffer et al. (15, 16) and Schaffer (14) observed a cytopathic effect and a significant reduction in cellular protein of monolayers treated with enterotoxin for periods of between 24 and 48 h. Although microscopically visible cytopathic changes and apparent cessation of cell growth suggested a direct cytotoxic action of enterotoxin on intestinal epithelial cells, these observations were not conclusive. There have been no subsequent studies on the putative cytotoxic action of staphylococcal enterotoxins by using sensitive and quantitative assays currently available to detect mammalian cell damage. The in vitro assay developed by Thelestam et al. (20–22), which quantitatively measures leakage of low-molecular-weight compounds from previously labeled mammalian cells, and an assay developed by Duncan and Buckingham (3), which measures inhibition in amino acid or sugar uptake as a result of subtle membrane damage, are extremely sensitive indicators by which cytotoxicity may be assessed. Thus, these assays were used to evaluate cell damage induced by SEA and SEB. In addition, we also measured several metabolic parameters of macromolecular syn-

thesis in efforts to detect changes produced as a result of enterotoxin interaction with the cells.

Since no definitive evidence exists that staphylococcal enterotoxins are in fact cytotoxins, we included as positive controls two staphylococcal toxins which are recognized cytotoxic agents. In addition, these staphylococcal toxins were used in experiments in which treatment of mammalian cells with either alpha- or delta-toxin was combined either simultaneously or sequentially with staphylococcal enterotoxin. The rationale for such experiments was to detect effects of enterotoxin which might not be detected by treatment with the enterotoxin alone. A synergistic effect on cells by more than a single toxin of staphylococcal origin was a possibility which required testing.

The data which were obtained, however, showed unequivocally that SEA and SEB did not behave as cytotoxic protein toxins on the Henle 407 cell line. Enterotoxin caused no alteration in membrane integrity or function, and cellular macromolecular synthesis was unaffected by treatment with toxin at concentrations exceeding by several orders of magnitude those required for induction of emesis in humans (1).

MATERIALS AND METHODS

Isotopes. Radioisotopes were obtained from New England Nuclear Corp., Boston, Mass.: [α-methyl-^{3}H]aminoisobutyric acid (AIB; specific activity, 10 Ci/mmol); [α-1-^{14}C]AIB (specific activity, 51.6 Ci/mmol); L-[G-^{3}H]lysine (specific activity, 4.1 Ci/mmol); and [5,6-^{3}H]uridine (specific activity, 40.8 Ci/mmol).

Toxins. Staphylococcal alpha-toxin was obtained from Sidney Harshman, Vanderbilt University, Nashville, Tenn. Purified toxin contained 16,000 hemolytic units per mg of protein. Alan Bernheimer, New York University School of Medicine, kindly supplied purified delta-toxin. The delta-toxin preparation contained 100 hemolytic units per mg of protein. SEA and SEB were provided by Leonard Spero, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Md., and Merlin Bergdoll, Food Research Institute, University of Wisconsin, Madison. The enterotoxins had been purified by the method of Schantz et al. (17, 18). The enterotoxin preparations were free of contaminating hemolysins.

Tissue culture and tissue culture medium. The Henle 407 human intestinal epithelial cell line (ATCC CCL6) was obtained from the American Type Culture Collection, Rockville, Md. This cell line was established originally by W. Henle from jejunum and ileum of a 2-month-old human embryo. 10T1/2 fibroblasts of C3H mouse origin were obtained from George Todaro, National Cancer Institute, Bethesda, Md. Tissue culture medium was obtained from GIBCO Laboratories, Grand Island, N.Y. Growth medium consisted of Hanks balanced salts solution (HBSS), basal Eagle medium (BME), and 10% fetal calf serum. Tissue culture plastic supplies were obtained from the Costar Division of Bellco Glass, Vineland, N.J.; both 6-well

(no. 3506) and 24-well (no. 3524) cluster dishes were used.

[^{3}H]uridine leakage assay. The assays described originally by Thelestam et al. (20–22) were used to detect cytotoxicity. Henle 407 human epithelial cells and 10T1/2 mouse fibroblasts were cultured as monolayers in 24-well tissue culture plates in BME plus 10% fetal calf serum as the growth medium. Cells were grown to less than total confluence before use in experiments. [^{3}H]uridine labeling of cells was accomplished by removing growth medium from the monolayers and replacing it with fresh BME containing 10 μCi of [^{3}H]uridine per ml for 2 h followed by a 2-h chase with medium free of radiolabel. The cells were washed three times with tris(hydroxymethyl)aminomethane-buffered saline (pH 7.5) followed by incubation with or without toxin in the buffer for 30 min. The supernatant was aspirated, and radioactivity in a 0.1-ml portion in 10 ml of Thrift Solve (Kew Scientific, Columbus, Ohio) was counted to determine the quantity of uridine which leaked from the cells. Maximal release of cellular label was determined by disrupting the cells with 0.1% Triton X-100.

The amount of [^{3}H]uridine released from the cells as a consequence of specific membrane damage was calculated according to the formula:

$$\% \text{ release} = \frac{\text{cpm released} - \text{cpm spontaneously released}}{\text{total cpm} - \text{cpm spontaneously released}} \times 100$$

Amino acid uptake assay. Duncan and Buckingham (3) showed that inhibition of hexose or amino acid uptake was a more sensitive indicator of membrane damage than assays based on leakage of intracellular components. Henle 407 cells were grown to near confluence in six-well (35-mm diameter) plastic tissue culture dishes. BME was aspirated, and each well was rinsed two times with HBSS. The plates were floated on a water bath at 37°C, and 2 ml of [^{14}C]AIB (0.5 μCi/ml in 1.0 mM AIB) or L-[^{3}H]lysine (1 μCi/ml in 1.0 mM L-lysine) in HBSS was added to the monolayers in each well for 20 min. Preliminary experiments indicated that the uptake of both amino acids had attained saturation within this 20-min period. At the conclusion of incubation, the labeled amino acid solution was aspirated, the dish was placed on ice, and each well was rinsed quickly three times with 3 ml of cold phosphate-buffered saline (PBS; pH 7.4). Cells were solubilized in 1.5 ml of 0.2 N NaOH, and 0.5 ml was removed for protein determination (10). A 1.0-ml sample neutralized with HCl was added to a scintillation vial containing 12 ml of Kewsolve (Kew Scientific) and counted in a Packard model 2125 scintillation spectrophotometer.

Several experiments involved use of two amino acids simultaneously, in which case both [^{14}C]AIB at 0.5 μCi/ml and L-[^{3}H]lysine at 1.0 μCi/ml were added to the incubation medium. The window settings for discriminating ^{14}C and ^{3}H in double-labeling experiments were determined as described by Beckman Instruments, Inc. (technical report 915-NUC-76-77).

SEA effects on cells. SEA was dissolved in PBS at 200 times the desired final concentration. At the beginning of each experiment, a 10-μl portion of SEA

was added to each well containing 2 ml of tissue culture medium and mixed well. Cells were incubated at 37°C for the indicated period in a 5% CO_2 atmosphere before rinsing in HBSS and processing for evaluation of amino acid transport as described above.

Macromolecular synthesis by cells treated with SEA. Henle 407 cells grown for 3 days in 24-well tissue culture dishes were used for ascertaining de novo protein, ribonucleic acid (RNA), or deoxyribonucleic acid (DNA) synthesis. Spent medium was aspirated, and fresh medium containing SEA or a recognized inhibitor of macromolecular synthesis as a positive control was added to the wells. *Pseudomonas* exotoxin A was used as an inhibitor of protein synthesis (12), actinomycin D was used as an inhibitor of RNA synthesis, and mitomycin C was used as an inhibitor of DNA synthesis. To determine protein synthesis, [³H]leucine (2 µCi/ml) was added for 90 min; RNA synthesis was determined after 24 h of incubation with [³H]uridine at a concentration of 1 µCi/ml; and DNA synthesis was determined after 4 h of incubation with 1 µCi of [³H]thymidine per ml. All radiolabeled solutions were added in fresh BME with 10% fetal calf serum which also contained the appropriate radiolabel and toxin at the concentration noted in Table 6. Cells in each well were dissolved in 0.1% sodium dodecyl sulfate and precipitated with an equal volume of 10% trichloroacetic acid. The resulting precipitate was washed with 5% trichloroacetic acid, dissolved in 1 N NaOH, and evaluated by liquid scintillation counting.

Statistical analysis. Statistical analysis for experiments with equal numbers of replicates was done by using analysis of variance as described by Hicks (5). Analysis of data with unequal replicates was done by using the General Linear Models program of the Statistical Analysis System computer package at the University of Cincinnati Computer Center. As described by Hicks (5), the "interaction" term referred to in Tables 2 and 3 is a measure of the change in response of one factor under the influence of a second factor. Specifically, a statistically significant interaction was interpreted as cell damage due to a combined toxin treatment greater than the sum of effects of the toxins administered separately, i.e., a synergistic effect. For data statistically significant as measured by analysis of variance or the general linear model, the means which were statistically significant were determined by Duncan's multiple-range test.

RESULTS

Synergistic action of staphylococcal alpha- and delta-toxins. The effects of staphylococcal alpha-toxin, delta-toxin, SEA, and SEB on leakage of [³H]uridine from prelabeled cells are shown in Table 1. Only delta-toxin was overtly cytotoxic as measured by increased leakage of [³H]uridine by 10T1/2 fibroblasts or Henle 407 cells. A similar leakage assay (data not shown) using cells prelabeled with [α-³H]-AIB detected damage induced by staphylococcal alpha-toxin as well as the delta-toxin. However, with either assay, no leakage of uridine or AIB

TABLE 1. *[³H]uridine leakage from prelabeled cells exposed to staphylococcal toxins*[a]

Cell type	Toxin (µg/ml)	Uridine leakage (% maximum)
10T1/2 mouse fibroblast	Enterotoxin A (10)	2.8 ± 2.0
	Enterotoxin A (20)	−0.1 ± 1.6
	Enterotoxin A (100)	4.4 ± 1.5
	Enterotoxin B (100)	3.0 ± 2.3
	Delta-toxin (1.0)	3.0 ± 1.2
	Delta-toxin (2.5)	8.8 ± 6.6[b]
	Delta-toxin (5.0)	18.8 ± 5.0[b]
	Delta-toxin (25.0)	64.4 ± 14.4[b]
	Alpha-toxin (3.2)	5.5 ± 3.5
	Alpha-toxin (32.0)	6.8 ± 2.7
Henle 407 human intestinal epithelial	Enterotoxin A (10)	1.8 ± 1.3
	Enterotoxin A (20)	2.7 ± 0.6
	Enterotoxin A (100)	2.8 ± 1.5
	Enterotoxin B (100)	4.2 ± 1.8
	Delta-toxin (1.0)	2.8 ± 1.1
	Delta-toxin (2.5)	0.2 ± 3.2
	Delta-toxin (5.0)	3.8 ± 4.5
	Delta-toxin (25.0)	19.9 ± 12.1[b]
	Alpha-toxin (0.3)	2.2 ± 1.6
	Alpha-toxin (32)	3.0 ± 1.4

[a] Each value is the mean of 6 to 12 determinations ± standard deviation. Henle 407 cells or 10T½ mouse fibroblasts were grown to near confluence on 24-well tissue culture plates. Spent medium was aspirated and replaced with fresh medium containing 1 µCi of [³H]uridine per ml. A 2-h incubation was followed by a 2-h chase with fresh medium without radiolabeled components. The cells were washed three times with tris(hydroxymethyl)aminomethane-buffered saline followed by incubation with toxin in the buffer for 30 min at 37°C. Sephadex G-25 gel chromatography revealed that cells labeled by this technique leaked materials of low molecular weight (<2,500) that therefore represented free uridine and oligonucleotides.

[b] Statistically significant ($P < 0.05$).

greater than control values was induced by SEA.

The assay of Buckingham and Duncan (3), which measures uptake of amino acids by tissue culture cells after toxin treatment, was also used in efforts to detect cytotoxicity. The amino acids lysine and AIB were chosen for study since they represent amino acid species taken into mammalian cells via different transport systems (4). Although independent transport systems were not demonstrated specifically for Henle 407 cells, our experiments showed that cold L-lysine in excess did not inhibit uptake of labeled AIB significantly, and, conversely, unlabeled AIB inhibited lysine uptake only slightly (data not shown). Thus, the two transport systems appeared to be independent in Henle 407 cells.

It was also important to evaluate the potential cytotoxicity of SEA and SEB together with the known cytotoxins staphylococcal alpha- and

delta-toxins. Therefore, as positive controls in the assays, the effects on AIB and lysine transport after treatment of Henle 407 cells with alpha- or delta-toxin alone or both toxins in combination are shown in Table 2. Delta-toxin did not substantially reduce transport of either AIB or lysine except at the highest dose tested (10 μg/ml). Alpha-toxin treatment of cells reduced transport of both amino acids at concentrations of 3 μg/ml or greater. Combinations of alpha- and delta-toxins produced significant decreases in amino acid uptake at concentrations which, when administered singly, had no measurable inhibitory effect. For example, alpha- or delta-toxin at 1 μg/ml was not inhibitory for amino acid transport. However, combined treatment with both toxins, each at that concentration, substantially reduced uptake of AIB (30%) and lysine (53%). Similar synergistic effects by alpha- and delta-toxins were evident at concen-

trations of 1 μg of alpha-toxin and 5 μg of delta-toxin per ml or 0.5 μg of alpha-toxin and 5 μg of delta-toxin per ml. The interaction of the effects of the alpha- and delta-toxins was statistically significant ($P < 0.0001$). These observations demonstrated the extreme sensitivity of the uptake assay for detection of subtle cell membrane perturbation and also showed that the assay could detect a synergistic action of two cytotoxic agents used at levels below threshold.

Absence of cytotoxicity of staphylococcal enterotoxin. The effects of a 24-h incubation with SEA at concentrations up to 10 μg/ml on uptake of AIB and lysine are shown in Table 3. Enterotoxin alone had no significant effect on AIB uptake at the doses tested. Cells treated with enterotoxin for 24 h together with one of the hemolytic toxins for the last 30-min period showed decreases in transport of AIB and lysine which could be accounted for solely by the ac-

TABLE 2. *Effect of combined staphylococcal hemolytic toxin treatment on amino acid uptake by Henle 407 cells[a]*

Alpha-toxin (μg/ml)	Amino acid uptake (% of control) with delta-toxin at:							
	None		1.0 μg/ml		5.0 μg/ml		10.0 μg/ml	
	AIB	Lysine	AIB	Lysine	AIB	Lysine	AIB	Lysine
None	100.0 ± 3.4[b]	100.0 ± 3.2[b]	89.3 ± 2.5	101.2 ± 3.0	107.9 ± 10.5	104.2 ± 6.0	81.1 ± 5.9	89.3 ± 8.3
0.5	94.6 ± 4.6	99.9 ± 4.2	80.3 ± 4.1	94.4 ± 5.0	66.9 ± 2.0	80.9 ± 2.2	74.5 ± 6.1	82.9 ± 8.0
1.0	89.0 ± 5.7	104.0 ± 13.2	30.3 ± 3.9	53.4 ± 4.6	14.4 ± 0.8	34.5 ± 0.8	73.5 ± 12.7	Not done
3.0	38.1 ± 5.4	52.0 ± 8.4	36.2 ± 8.6	55.3 ± 10.1	15.0 ± 2.8	36.8 ± 3.5	11.0 ± 0.7	32.9 ± 0.4

[a] Nearly confluent monolayers of Henle 407 cells in 35-mm wells were rinsed twice with HBSS. Toxin(s) at the concentrations noted was added in 2 ml of HBSS for 30 min and aspirated; then 2 ml of labeled amino acid solution containing 1 mM AIB (1 μCi/ml) and 1 mM lysine (1 μCi/ml) was added for a 20-min incubation at 37°C. Wells were rapidly washed three times with ice-cold PBS, and cells were dissolved in 1 ml of 0.1 N NaOH. Statistical analysis showed significant interaction of the combined alpha- and delta-toxin treatment on AIB uptake ($P < 0.0001$) and lysine uptake ($P = 0.0003$). The values listed are the means of 3 to 12 determinations ± standard deviation.

[b] Normal control values for AIB and lysine uptake.

TABLE 3. *Effect of prolonged SEA pretreatment of Henle 407 cells on amino acid uptake[a]*

Hemolytic toxin (μg/ml)	Amino acid uptake (% of control) with SEA at:					
	None		1.0 μg/ml		10.0 μg/ml	
	AIB	Lysine	AIB	Lysine	AIB	Lysine
None	100.0 ± 2.1	100.0 ± 1.8	94.2 ± 4.6	99.7 ± 7.7	99.3 ± 3.2	96.4 ± 8.3
Alpha (1.0)	95.9 ± 7.5	101.2 ± 8.6	86.1 ± 6.6	102.3 ± 4.4	76.6 ± 7.1	88.4 ± 2.0
Alpha (3.0)	39.2 ± 12.3	56.7 ± 8.8	36.9 ± 11.9	59.3 ± 10.7	35.6 ± 11.3	57.6 ± 10.2
Delta (1.0)	89.4 ± 5.7	99.8 ± 5.7	90.1 ± 1.9	95.0 ± 3.0	91.0 ± 2.7	103.0 ± 2.5
Delta (5.0)	102.9 ± 4.1	94.8 ± 2.8	91.9 ± 1.7	92.0 ± 3.0	89.5 ± 3.1	88.1 ± 6.8
Delta (10.0)	993.4 ± 2.0	81.9 ± 2.0	90.6 ± 6.6	71.9 ± 5.0	96.0 ± 5.1	82.3 ± 5.2

[a] SEA in 10 μl of PBS was added to 2 ml of BME plus 10% fetal calf serum in 35-mm wells of Henle 407 cell monolayers 24 h before evaluating amino acid transport. After the 24-h preincubation period. The cells were rinsed twice with HBSS. Toxin(s) at the concentrations listed was added in 2 ml of HBSS for 30 min and aspirated, then 2 ml of labeled amino acid solution containing 1 mM AIB (1 μCi/ml) and 1 mM lysine (1 μCi/ml) was added for 20 min of incubation at 37°C. Protein and radioactivity were determined as described in footnote *a*, Table 2. The values listed are the means of three to six determinations ± standard deviation. Statistical analysis indicated no significant interaction for combined enterotoxin and hemolytic toxin treatment ($P > 0.01$).

tivity of the hemolytic toxin. No synergism between the enterotoxin and the hemolytic toxins was evident.

On the basis of these data, we conclude that SEA caused no overt membrane damage of mammalian cells or modification of the membrane transport systems evaluated. The enterotoxin also did not predispose the plasma membrane of Henle 407 cells to subsequent damage by other staphylococcal toxins.

As another index for detecting cytotoxicity of SEA, macromolecular synthesis by the cell cultures exposed to the toxin was assessed. SEA had no effect on synthesis of protein, RNA, or DNA when incubated with Henle 407 cells for periods of between 2 and 24 h (Table 4). Thus, it may be concluded that SEA, even at concentrations exceeding physiological doses, does not alter macromolecular synthesis of the intestinal epithelial cells.

DISCUSSION

Our observations show clearly that two staphylococcal enterotoxin serotypes exert no measurable direct cytotoxic action on the mammalian cell cultures tested. Assays of membrane leakage and membrane functional integrity (i.e., intact amino acid transport systems) demonstrate that staphylococcal enterotoxin does not qualify as a microbial cytotoxin. Studies revealing normal macromolecular synthesis by mammalian cells after exposure to high concentrations of enterotoxin also do not support a mechanism involving direct toxicity to gastrointestinal tissues. It was also established that enterotoxin does not act synergistically to augment inhibitory effects of staphylococcal alpha- or delta-toxin on AIB or lysine uptake by Henle 407 cells. The evidence

for absence of synergy is compelling, since the assay used was shown to be sufficiently sensitive to detect synergism between alpha- and delta-toxins. The data presented in this report showing that staphylococcal enterotoxins do not behave as typical cytotoxins alone or in combination with known cytotoxins of staphylococcal origin are significant, for they mandate that other than direct mechanisms of toxicity be evaluated. In spite of considerable efforts, the biochemical basis of staphylococcal food poisoning remains an unresolved issue.

In addition to the recognized role of staphylococcal enterotoxin in food-associated gastroenteritis, enterotoxin has been detected in staphylococcal lesions. In a clinical study, approximately 50% of S. aureus isolates from patients with skin lesions were found to be enterotoxin producers (24). Josefcyzk (7) reported that staphylococcal infections frequently result in detectable levels of serum antitoxin. This suggests that enterotoxin is disseminated systemically from staphylococcal lesions. Thus, consideration for a role of enterotoxin in staphylococcal infections becomes potentially significant. Indeed, enterotoxin administered intravenously to monkeys produces profound effects, including emesis, diarrhea, and, at higher doses, shock, fever, and death (1). Thus, staphylococcal enterotoxins may exert important effects during infection not appreciated because these entities are currently classified as enterotoxins.

The recent recognition that enterotoxins act as potent immunomodulating agents (13, 23) at extremely low concentrations (9) raises the possibility that they may act via mediation of immunological phenomena and not via a directly expressed cytotoxicity.

TABLE 4. Effect of SEA on synthetic capabilities of Henle 407 cells[a]

| Toxin or inhibitor | Macromolecular synthesis (% of control) | | | | | |
| | [³H]leucine | | [³H]uridine | | [³H]thymidine | |
	3 h	24 h	2 h	24 h	4 h	24 h
None (PBS)	100 ± 14	100 ± 17		100 ± 9	100 ± 15	100 ± 18
SEA						
0.1 µg/ml	100 ± 9	88 ± 9	99 ± 8	103 ± 4	100 ± 25	
1.0 µg/ml	84 ± 12	96 ± 9	95 ± 6	102 ± 3	89 ± 27	89 ± 41
10 µg/ml	94 ± 37	88 ± 7	98 ± 1	108 ± 6	73 ± 24	110 ± 48
Pseudomonas toxin						
10 µg/ml	30 ± 7[b]					
50 µg/ml	11 ± 4[b]					
Actinomycin D, 1.0 µg/ml				10 ± 1[b]		
Mitomycin C, 30 µg/ml					26 ± 2[b]	4 ± 1[b]

[a] Nearly confluent Henle 407 cells were treated with toxin for the times shown by aspirating spent medium and replacing with fresh BME containing 1 µCi of the indicated label per ml and toxin at the concentration listed. Incorporation into macromolecules was determined as described in Materials and Methods.

[b] Significantly different from control (PBS).

ACKNOWLEDGMENT

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