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Analysis of Unidirectional Fluxes of Sodium During Diarrhea Induced by *Clostridium perfringens* Enterotoxin in the Rat Terminal Ileum

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Net intestinal transport of sodium in vivo, in control and enterotoxin (*Clostridium perfringens*)-treated rats, was resolved into two unidirectional fluxes, influx from and efflux into the lumen of the terminal ileum. In rats treated with the toxin, sodium influx remained similar to control values even during fluid and electrolyte loss to the lumen. Net loss of sodium was shown to be due to nearly a twofold increase in sodium efflux to the lumen in toxin-treated animals. There was only slight histopathological damage to the mucosa, especially noticeable at the tips of villi.

Crude cell-free extract from sporulating cultures of *Clostridium perfringens* and purified protein toxin from these extracts have been shown to be capable of inducing significant transport alterations in the terminal ileum of rats (4). Previous studies with cholera toxin (5) and staphylococcal enterotoxin (6) have shown that normal absorption of glucose occurs during outpouring of fluid and electrolytes due to the action of the toxins. This observation has been interpreted to imply that normal absorptive capacities are functional during these syndromes and therefore net secretion is likely due to toxin-induced increases in serosal to mucosal fluxes. A previous study in this laboratory (4) indicates that normal net glucose absorption does not occur during fluid and electrolyte loss in the terminal ileum due to *C. perfringens* enterotoxin. By using glucose absorption as an indication of operative absorptive mechanisms, it would appear that net losses in this case may be due to decreased absorption rather than increased secretion. To determine what flux alterations caused the transport patterns observed, net sodium transport was resolved into its two component fluxes and evaluated under control and experimental conditions.

MATERIALS AND METHODS

Purified enterotoxin from *C. perfringens* was kindly supplied by Charles L. Duncan of the Food Research Institute, University of Wisconsin, Madison. Male Wistar strain rats weighing between 200 and 360 g were used. The anesthesia of animals and operation procedures were as previously described (4). Pure toxin was suspended in 1 ml of glucose Ringer solution (160 erythemal units/ml) and incubated in the terminal ileum 20 min before perfusion. In controls glucose Ringer solution was incubated in place of the toxin suspension.

Perfusion solution containing ^{14}C-labeled polyethylene glycol as a nonabsorbable marker was prepared as described (4) with the addition of sufficient $^{22}NaCl$ (Amersham/Searle) to enable an accurate differential determination of ^{14}C and ^{22}Na with a Beckman LS 250 liquid scintillation system. Total Na concentration was determined by flame photometry (Beckman DU2 spectrophotometer with flame attachment).

Unidirectional fluxes of sodium were calculated from values obtained for luminal Na concentration and luminal ^{22}Na concentration by means of the following equation:

$$J_s^1 = \frac{(J_s^2 - J_s^1) \ln P_{so}/P_s}{\ln A/A_o}$$

where the symbols have the following meanings: J_s^1, influx of Na from the intestinal lumen to the blood of the animal; J_s^2, efflux of Na from the blood to the lumen; $J_s^1 - J_s^2$, net Na flux; P_{so}, total amount of ^{22}Na in the lumen at the beginning of the period; P_s, total amount of ^{22}Na in the lumen at the end of the period; A_o, total amount of Na in the lumen at the beginning of the period; and A, total amount of Na in the lumen at the end of the period. P_{so}, P_s, A_o, and A are calculated from the initial volume and the end volume as well as the concentrations of Na and ^{22}Na at the beginning and end of the period (usually 15 min).

Since $J_s^1 - J_s^2 = A - A_o$, J_s^1 can be calculated. Since the net flux is known, J_s^2 is also calculated.

Histological sections were taken at the conclusion of an experiment and prepared as described.

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RESULTS

Figure 1 shows net sodium transport in seven control and seven toxin-treated animals. Net sodium absorption in controls is significantly reversed to net sodium loss to the lumen in animals exposed to pure toxin in agreement with previous observations (4).

Figures 2 and 3 each show a typical experiment from the control and experimental groups in Fig. 1, which were resolved into the component unidirectional fluxes. Figure 2 shows influx and efflux of sodium and the net Na transport rate at 15-min intervals during a typical control experiment. It can be seen that the net transport of Na is resolved into two large fluxes moving in opposite directions. Figure 3 shows fluxes in a rat exposed to pure toxin. Unlike controls, the elevated rate of efflux consistently exceeded the simultaneous influx rate, causing a net extrusion of sodium into the lumen throughout the experiment.

Table 1 is a numerical tabulation of control and experimental net water, net sodium, and unidirectional sodium fluxes. It can be seen that water transport was reversed from net absorption in control to net secretion in toxin-treated animals. A similar significant reversal can be seen in net sodium transport. Of great importance is the fact that the reversal of net Na

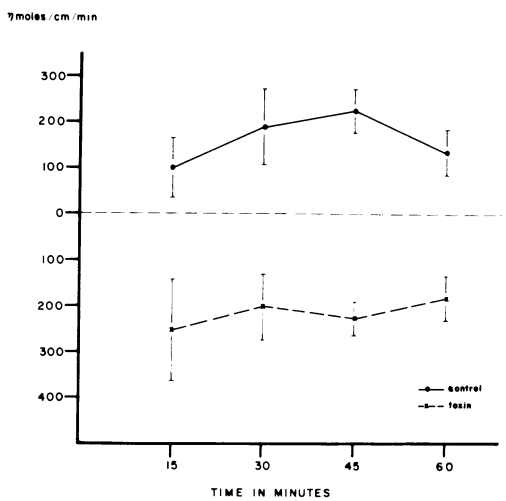

FIG. 1. *Net transport of sodium in control and experimental animals used in unidirectional sodium flux determinations. Transport on the ordinate is plotted against time on the abscissa. Values given are means ± standard error (n = 7). The unit of transport is nanomoles per centimeter per minute. Positive values indicate absorption, and negative values indicate secretion into the lumen.*

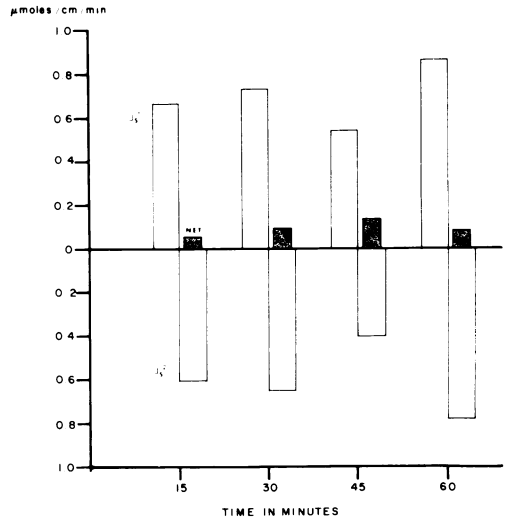

FIG. 2. *Net sodium transport resolved into unidirectional components in a typical control experiment. Transport on the ordinate is plotted against time on the abscissa. The unit of transport is micromoles per centimeter per minute. Net sodium flux (Net) is resolved into influx (J_s^1) and efflux (J_s^2). Positive values indicate absorption, and negative values indicate secretion into the lumen.*

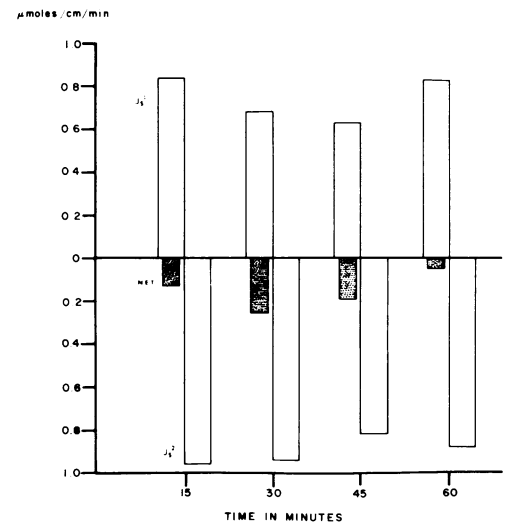

FIG. 3. *Net sodium transport resolved into unidirectional components in a typical pure toxin experiment. Transport on the ordinate is plotted against time on the abscissa. The unit of transport is micromoles per centimeter per minute. Net sodium flux (Net) is resolved into influx (J_s^1) and efflux (J_s^2). Positive values indicate absorption while negative values indicate secretion into the lumen. Toxin concentration was 160 erythemal units/ml.*

TABLE 1. *Unidirectional and net sodium fluxes during control and pure toxin experiments*[a]

Flux	Transport			
	15[b]	30[b]	45[b]	60[b]
H_2O				
Control	0.75 ± 0.29[c]	1.57 ± 0.43[d]	0.92 ± 0.28[c]	1.39 ± 0.21[d]
Toxin	−1.30 ± 0.34	−0.75 ± 0.33	−1.48 ± 0.32	−1.20 ± 0.20
Na J_s^1				
Control	531.5 ± 78.0[g]	720.1 ± 82.4[g]	552.0 ± 82.7[e]	717.2 ± 49.4[f]
Toxin	707.8 ± 44.8	845.7 ± 55.3	879.8 ± 77.6	861.4 ± 40.2
Na J_s^2				
Control	431.7 ± 95.0[c]	533.2 ± 46.1[c]	325.8 ± 70.6[c]	581.8 ± 38.4[c]
Toxin	1014.0 ± 69.5	1047.4 ± 36.6	1107.7 ± 67.8	1042.9 ± 51.7
Net				
Control	99.8 ± 95.0[e]	187.0 ± 82.6[d]	226.7 ± 45.6[c]	135.3 ± 49.2[c]
Toxin	−253.3 ± 109.6	−202.0 ± 73.1	−227.9 ± 37.7	−181.6 ± 46.2

[a] Values are means ± standard error (n = 7 in all cases); influx (M-S) is positive. Water movement is expressed as microliters per centimeter per minute; sodium transport is expressed as nanomoles per centimeter per minute.

[b] Minutes.

[c] $P < 0.001$.

[d] $P < 0.01$.

[e] $P < 0.02$.

[f] $P < 0.05$.

[g] Not significant.

transport is accounted for by nearly a twofold increase in the efflux, not by a diminution in the influx. The influx remained unchanged in toxin-treated animals and even showed a slight increase at later periods.

It is of interest to compare these unidirectional flux values to ones reported elsewhere. Curran et al. (2) reported a mean sodium influx (M-S) in the terminal ileum of rats of 750 nmol per cm per min and a mean sodium efflux of 608 nmol per cm per min. Our flux values obtained for controls are similar to those reported by them (Table 1).

Figure 4 shows a tissue section taken at the conclusion of a perfusion experiment in which the intestine was exposed to pure toxin and in which net secretion of water and sodium occurred throughout the experiment. Compared to effects induced by crude cell-free extract reported earlier (4), essentially normal villus morphology is apparent. However, the columnar epithelium at the tips of villi often appeared to be flattened to low cuboidal in shape and had prominent extrusion zones which were frequently open. Numerous free cells can be seen in the lumen near the villi tips.

DISCUSSION

The mechanism of diarrhea induced by bacterial toxins has been attributed to the enhanced secretory process, not to the inhibited absorptive process in the intestinal epithelium. This contention has been based upon observations that during copious fluid accumulation in the lumen induced by cholera toxin, the sodium efflux was increased, while sodium influx and net glucose absorption remained unchanged (3).

The effect of toxin from *C. perfringens* is unlike that of cholera in that the toxin inhibited glucose absorption, as revealed in a previous study (4), although the effect on Na fluxes is similar to those already reported for cholera toxin. The discrepancy between inhibited glucose absorption and unaltered Na influx is acute because it has been well established that Na and glucose are taken up in epithelial cells by coupled transport (1). This discrepancy can be resolved by assuming two components in the Na influx, one purely diffusive and the other concerned with coupled transport with glucose. If it is further assumed that the *C. perfringens* toxin inhibits coupled transport of Na and glucose and at the same time increases the permeability of the epithelium in general, a situation can be obtained in which a decline of Na influx component due to coupled transport can be compensated by a rise in the diffusive component of the Na influx. Since the flux values obtained by isotopic methods cannot be resolved into components, there is no support

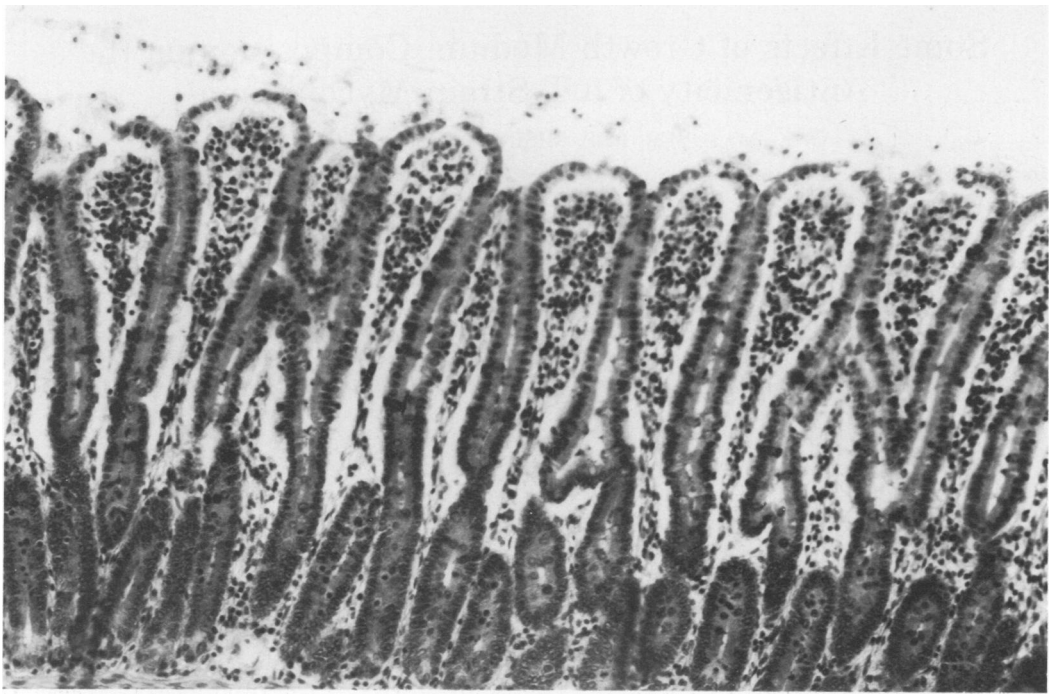

FIG. 4. *Morphology of the rat ileum after exposure to C. perfringens enterotoxin (see text). Section was exposed to pure toxin (160 erythemal units/ml) throughout the incubation and perfusion period (80 min total). Magnification: ×200.*

for this speculation at present. The histopathological evidence does not contradict the notion of a permeability rise in general. The present data indicate an interesting property of *C. perfringens* toxin and should serve to stimulate further study on this material.

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