Effects of Enterotoxin B on Intestinal Transport *in Vitro** (34060)

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(Introduced by Tomoaki Asano)
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The epidemiology and symptomatology of food poisoning arising from ingestion of staphylococcal enterotoxins have been well documented (1). The chemical properties of the toxins have also been elucidated (2). However, there is as yet little specific knowledge about the mechanism of action in regard to the induced diarrhea. Although it is possible to reproduce the entire pattern of human symptoms only with primates and monkeys, using purified enterotoxin, it is possible to demonstrate the inhibitory action of enterotoxin B on the intestinal transport in the small intestine of the rat *in vitro*.

Method. Female rats of Wistar strain weighing between 150 and 200 g were used. Under light ether anesthesia the middle section of the small intestine was excised and made into an everted sac about 25 cm in length in a similar manner to that already reported (3). One milliliter of the control Ringer's fluid was put inside the sac (serosal fluid) and both ends were tied with silk thread. The sac was incubated in approximately 200 ml of either the control Ringer's fluid or the Ringer's fluid containing enterotoxin B at 2 mg/liter (mucosal fluid) at 37° for 1 hr. A stream of gas mixture of 95% O_2 and 5% CO_2 was bubbled through the mucosal fluid.

At the end of incubation the sac was gently blotted on wet filter paper and the serosal fluid was drained into a weighing bottle after cutting one end. The length of the sac was measured. The final volume of the serosal fluid was determined by weighing the fluid collected. The preliminary study showed that the specific gravity of the solution was close to 1 and the error introduced in the estimation of volume was negligible.

The following chemical analyses were performed on the final serosal fluid: (1) osmolarity with Mechrolab vapor pressure osmometer with urea solution as the standard; (2) Na and K with Beckman DU-2 spectrophotometer with flame attachment; (3) Cl with Cotlove chloridometer; (4) glucose with Glucostat (Worthington Biochem. Corp.); (5) lactic acid with Lactate Test Pack (Calbiochem).

The net transport of solutes referred to unit length of intestine was calculated as $(C_fV_f - C_iV_i)/L$, where C and V indicate concentration of solutes and volume of serosal solution respectively, suffixes f and i stand for the final and initial values, and L stands for the length of intestine in centimeters. The net transport of water was identified with the net volume transport calculated as $(V_f - V_i)/L$. The concentration of solutes in the "moving solution" was calculated as $(C_fV_f - C_iV_i)/(V_f - V_i)$.

The composition of the control Ringer's fluid was as follows: NaCl 130 mM, KCl 5 mM, $NaHCO_3$ 10 mM, $CaCl_2$ 1.3 mM, NaH_2PO_4 1.2 mM, and glucose 10 mM. The pH of the fluid was approximately 7.0 after equilibration with 5% CO_2. The toxin Ringer's fluid was made by adding enterotoxin B directly to the control Ringer's fluid at 2 mg/liter.

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TABLE I. Effect of Enterotoxin B on Transport.

A Final serosal concentration (mean + SE)

Treatment	No. of samples	Osmolarity (mOsm/liter)	(mM) Na	K	Cl	Glucose	Lactate
Control	8	271 ± 1	134 ± 1	3.65 ± 0.13	76.8 ± 1.5	33.8 ± 1.1	80.2 ± 1.6
Toxin 2 mg/liter	8	264 ± 1	140 ± 1	3.98 ± 0.10	82.2 ± 2.1	25.8 ± 1.0	76.4 ± 2.3

B Net transport (mean ± SE) (mucosal to serosal +)

	Water μl/cm-hr	(μmole/cm-hr) Na	K	Cl	Glucose	Lactate
Control	61.0 ± 5.6	7.61 ± 0.66	0.13 ± 0.01	1.20 ± 0.35	3.44 ± 0.25	9.62 ± 0.49
Toxin 2 mg/liter	22.9 ± 3.5	2.71 ± 0.38	0.05 ± 0.01	−0.46 ± 0.28	1.27 ± 0.13	5.02 ± 0.41

The oxygen consumption and lactic acid production under the influence of enterotoxin were determined using a standard Warburg manometer (GME). A small piece of intestine 1–2 cm in length was excised from the middle section and was made into a sheet by cutting longitudinally. The intestinal wall was transferred to a Warburg flask which contained 3 ml of either the control Ringer or that containing enterotoxin. Four pieces of intestinal wall were obtained from a single rat and two were used for the control and the other two for the toxin Ringer. The gas phase was replaced with oxygen. The oxygen uptake was followed for 1 hr and at the end an aliquot of Ringer's fluid was collected for lactic acid production. The intestinal wall was dried under an infrared lamp and the result was expressed on the dry weight basis. The compositon of Ringer's fluid used for manometry was as follows: NaCl 140 mM, KCl 5 mM, $CaCl_2$ 1.3 mM, NaH_2PO_4 1.2 mM, Na_2HPO_4 2.0 mM, and glucose 10 mM. The toxin Ringer's fluid was made by adding enterotoxin B directly to the control Ringer at 2 and 20 mg/liter.

Results. Table IA shows the osmolarity and the concentrations of solutes in the final serosal fluid. In the control the osmolarity is very slightly less than the Ringer's fluid (276 mOsm/liter) indicating a net fluid absorption is nearly iso-osmotic fashion. The toxin seems to lower the osmolarity still further. The lactic acid concentration in the control is higher and consequently that of chloride is lower than those already reported in a similar experiment (4). This may be due to slight hypoxia resulting from inadequate oxygen supply. The effect of toxin as shown in the final concentration is not too salient. However, there are drastic reductions in the net transports as shown in Table IB. In the control series the magnitude of the individual net transport, such as water and Na, are similar to a former report (5). The net water transport is inhibited to nearly one third of the control and similar inhibition is seen in the transport of Na, K, and glucose. The chloride transport is inhibited to such an extent that the normally occurring net absorption is reversed in direction resulting in net secretion. The lactic acid transport is least affected.

Table II shows the results of metabolic measurement. The oxygen uptake for the control is similar to formerly reported values (6). Under the influence of enterotoxin the oxygen uptake does not seem to differ from that of the control, at 2 mg/liter as well as at 20 mg/liter. Also in the lactic acid production there seems to be no difference.

Discussion. It seems well established in this study that purified enterotoxin inhibits the net absorption of water and solutes in the small intestine *in vitro,* and the evidence could be presented to account for diarrhea, one of the symptoms arising from ingestion of

TABLE II. Effect of Enterotoxin B on Metabolism (Mean ± SE).

Treatment	No. of expt.	Oxygen uptake QO_2 μl/hr-mg dry	Lactic acid production μmole/hr-mg dry
Control	10	3.86 ± 0.14	0.38 ± 0.09
Toxin 2 mg/liter	10	4.37 ± 0.23	0.37 ± 0.02
Control	10	4.77 ± 0.27	0.34 ± 0.03
Toxin 2 mg/liter	10	5.26 ± 0.18	0.32 ± 0.02

enterotoxin in humans. If the molecular weight of enterotoxin B is assumed to be 35,000 (2), the concentration of 2 mg/liter corresponds to $6 \times 10^{-8}M$. The inhibition as revealed by reduction of the water transport to one third of the control value at this low concentration testifies that enterotoxin B is a specific and powerful inhibitor of intestinal transport. The mechanism for the inhibitory action cannot be elucidated from the present result, but a few points seem to deserve a comment.

Although it was reported that enterotoxin induced swelling of mitochondria in the epithelial cell in the small intestine in monkeys orally administered the toxin (7), there seems no discernible change in the oxygen uptake and lactic acid production in the present study. Therefore the inhibition on transport must be concluded not to be mediated through metabolic disturbances but through more direct action on the transport process.

It is interesting to note that there seem to be graded responses to enterotoxin in the net transport of different solutes. In order to demonstrate the graded response more clearly, the concentration of a solute in the so-called "moving solution" or "absorbate" was calculated for individual experiments and the averages are shown in Table III. As noted earlier (5), the concept of the concentration in the "moving solution" has no physical basis, since the net transport is the algebraic sum of influx and efflux, and furthermore it is by no means clear whether water and solutes proceed through the same route in the membrane. Nevertheless, the realtive inhibition on a solute transport with respect to that of water is clearly discernible in terms of the concentration in the "moving solution."

It is evident that the concentration of Na, K, and glucose in the "moving solution" in the toxin-treated series is almost identical to that of the control series. This indicates that the degree of inhibition is similar among the transports of Na, K, glucose and water. Since a close correlation has been repeatedly demonstrated between the transports of Na and water (8), of glucose and Na (9, 10), and of glucose and water (11), it is not surprising to find the same degree of inhibition among them. At present it is not possible to decide whether this inhibition resulted from the inhibition on active transport processes or from alterations of passive membrane characteristics.

In this regard it is of interest to note that the chloride concentration became negative and that of lactate became greater than the control value. This behavior may be interpreted as resulting from severe inhibition of chloride influx (from mucosal to serosal) and relative increase of lactate influx to such an extent that it accounts for the net secretion of chloride. Since the chloride transport is assumed to be passive in the small intestine at least *in vitro* (12), this interpretation can be substantiated if the toxin reduces the passive permeability to chloride at the brush border of the epithelial cell. The lactate, which is produced in the epithelial cell, is

TABLE III. Concentration in "Moving Solution" (Mean ± SE).

Treatment	(mM)				
	Na	K	Cl	Glucose	Lactate
Control	126 ± 3	2.24 ± 0.20	19.1 ± 5.6	57.8 ± 2.6	164 ± 8
Toxin 2 mg/liter	119 ± 3	2.13 ± 0.51	−32.3 ± 15.2	59.3 ± 5.4	239 ± 23

hardly expected to be affected by the permeability change.

It cannot be decided at present whether the net chloride secretion observed under enterotoxin is due to an active process or due to the altered electrochemical potential difference under the influence of the toxin.

Summary. The effect of enterotoxin B on intestinal transport was tested using an everted sac of the middle section of the small intestine in rats. Severe inhibition at the toxin concentration of 2 mg/liter was observed on the net transport of water, Na, K, Cl, glucose and lactate. The degree of inhibition was, however, graded; whereas the transports of water, Na, K and glucose were reduced in the same degree the chloride transport was severely inhibited so that the normally occurring net absorption turned into a net secretion. The lactic acid transport was least affected.

The metabolic indices, oxygen uptake and lactic acid production, were not influenced by enterotoxin at the concentration of 2 mg/liter as well as 20 mg/liter.

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