

Effect of Enterotoxin B on Human Volunteers

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A dosage of 20 to 25 μg of pure enterotoxin B can produce clinical manifestations of enterotoxemia in man, who seems to be more sensitive than monkey.

Human volunteers have been used for the detection of enterotoxin in foods and bacterial culture supernatant fluids (1-3), but no studies have been reported on the amount of toxin required to cause illness in man. One attempt in the Food Research Institute to determine the toxicity of enterotoxin B in two human volunteers, first with 1 μg and then 10 μg , were unsuccessful (Sugiyama, *unpublished data*). An estimate of the toxicity of enterotoxin A for man determined from

TABLE 1. Effect of enterotoxin B on humans

Time (PM)	Volunteer ^a		
	1	2	3
12:00	Lunch	Lunch	
1:00	Toxin dose (50 μg) ingested ^b	Toxin dose (50 μg) ingested	Toxin dose (50 μg) ingested
1:30	No reaction	No reaction	Lunch; no reaction
3:00	No reaction	No reaction	Feeling sick in stomach
3:40	No reaction	No reaction	First vomiting
4:00	No reaction ^c	Feeling sick	Nausea
4:20	No reaction	Feeling sick	Second vomiting
4:45	No reaction	Nausea and abdominal cramping	Nausea and abdominal cramping
5:00	Nausea and retching	First diarrhea motion; no vomiting	Third vomiting and first diarrhea motion
5:10	First vomiting	Abdominal cramping	Nausea and abdominal cramping
5:20	Second vomiting	Second motion but no vomiting	Fourth vomiting and second diarrhea motion
5:30	Third vomiting and abdominal cramping		
5:45	Fourth vomiting and first diarrhea motion	Diarrhea stopped; feeling relieved but very weak	No more vomiting and diarrhea; feeling relieved but very weak
10:00	Vomiting and diarrhea motion continued simultaneously every 0.5 hr or so up to 10 PM; thereafter, every 1 hr or so up to 1:00 AM		
1:00 AM	No more vomiting and diarrhea motion, but feeling very weak		

^a Volunteer 1 was a 35-year-old, 145-lb male; 2 was a 26-year-old, 135-lb male, and 3 was a 19-year-old, 120-lb female.

^b Dehydrated enterotoxin B preparation (S6-KF-51B of about 50% purity) was dissolved in distilled water just prior to ingestion.

^c At 3 hr after the first dose (50 μg), volunteer 1 accidentally took an additional dose of 850 μg of the toxin preparation.

the toxin content in cheese consumed by human volunteers indicated that less than 1 μg of this toxin might cause illness in sensitive individuals (Bergdoll, unpublished data).

In our studies, an enterotoxin B preparation (S6-KF-51B with about 50% purity) was tested on three human volunteers (two males and one female). The enterotoxin was extracted and partially purified from *Staphylococcus aureus* strain S-6 by the procedure described elsewhere (5). At zero-time, each of the three volunteers ingested 50 μg of the toxin preparation dissolved in distilled water. All three volunteers showed typical symptoms of staphylococcal enterotoxemia. The case history of each volunteer is summarized in Table 1.

The results indicate a direct relationship between the time of onset of symptoms and the body weight or age or both. Volunteer 3 (weighing 120 lb) showed the first symptoms during the 3rd hr from zero-time, whereas in the cases of volunteers 1 (145 lb) and 2 (135 lb), the first symptoms were noticed during the 6th and 4th hr, respectively. However, such a correlation may not be significant, since a wide variation in the sensitivity of individuals to staphylococcal food poisoning is known to exist (4). As expected, volunteer 1, who had consumed a much larger dose, experienced more severe vomiting and diarrhea and suffered for a longer period of time than the others. The volunteers, when medically examined during the illness, had normal temperatures and blood pressures, and their vomitus

and stools were free of blood and mucus. The symptoms wore off completely in a short course of time, and the volunteers soon returned to a normal state of health.

Although a small number of volunteers were tested in this study, the data obtained seem to indicate that a dosage of 20 to 25 μg of pure enterotoxin B can produce a syndrome compatible with enterotoxemia in man. Also, the same enterotoxin preparation was tested previously on monkeys and fish for comparative studies. The results show that man is more sensitive to enterotoxin B (0.4 $\mu\text{g}/\text{kg}$ of body weight) than rhesus monkeys [0.9 $\mu\text{g}/\text{kg}$ (5)]. Much higher doses (up to 200 μg) of the same toxin preparation were found to have no effect when tested on tropical fish, *Tilapia zilli* (H. Raj and J. Liston, Bacteriol. Proc., p. 65, 1962).

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