STAPHYLOCOCCUS ENTEROTOXIN*: A REVIEW

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Dr. M. A. Barber (1914) made several visits to a farm in the Philippine Islands and three times was a victim of attacks of gastroenteritis. On one visit he drank fresh milk from two cows on the farm with no bad results. He observed that all of these attacks followed the ingestion of fresh sweet cream after an interval of $1\frac{3}{4}$ to 2 hours. Milk was drawn directly into clean bottles and brought to the laboratory keeping it in contact with the ice in a refrigerator. The milk of the two cows was kept separate. Dr. Barber drank 30 ml of the cream from one of the cows. This sample had not been above refrigerator temperature and no symptoms followed. However, the bottle from which the sample was taken was left at room temperature of from 28-30°C for 5 hours and a second dose of 40 ml of mixed milk and cream was taken. About $1\frac{3}{4}$ hours afterwards a decided nausea and diarrhea set in which lasted several hours. Two other volunteers were similarly made ill. The white staphylococcus from the suspected cow was inoculated into a flask containing preserved milk from Norway and the flask incubated at 36.5°C for about $8\frac{1}{2}$ hours. An uninoculated control flask from the same tin, also incubated, was proved by plate cultures to be sterile. Fifty ml from the flask inoculated with the white staphylococcus caused symptoms in $1\frac{3}{4}$ hours consisting of cramps, faintness, nausea and diarrhea. Kittens, pups, and monkeys showed no symptoms upon ingestion of milk cultures containing the white staphylococcus. Barber ascribes this illness to a toxin elaborated by a white staphylococcus which occurred in almost a pure culture in the udder of a cow. The fresh milk was harmless and the toxin was produced in effective quantities only after the milk stood for some hours at room temperature. Although the term "toxin" is used by Barber he did not demonstrate the poison in filtrates of cultures and as far as the author is aware, it was not until 1929 that toxin was demonstrated in sterile filtrates of staphylococcus (Dack *et al.*, 1930). Had experimental animals reacted to staphylococcus enterotoxin as does man, this type of food poisoning would have been recognized in outbreaks occurring in the 1880's.

In 1946 a project was set up at the Food Research Institute of the University of Chicago to study the production of staphylococcus enterotoxin, its chemical nature, and its mode of action in the body. Strain 196E was first used in this work. This strain came from an outbreak of food poisoning. Subsequently, a strain, S-6, was isolated by Evans from frozen shrimp which was not involved in an outbreak of food poisoning (Evans, 1948). This strain appeared to produce a very potent enterotoxin and for this reason it was chosen for study.

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As the study progressed it was learned that the enterotoxin produced by Strain 196E was different from that produced by Strain S-6. At a meeting of the American Society for Microbiology in 1961, Casman, Bergdoll and Robinson (unpublished) proposed a nomenclature for the antigenically different enterotoxins. The 196E strain produces an enterotoxin in common with the majority of strains isolated from food poisoning outbreaks. It has been proposed that this enterotoxin be called Enterotoxin A. The S-6 strain produces a small amount of Enterotoxin A which is lost in the purification procedure for the enterotoxin from this strain. The majority of enterotoxin produced by the S-6 strain is antigenically different and it was proposed that this enterotoxin be called Enterotoxin B. There is evidence that still another type of enterotoxin is produced by Strain 137 but until more is known of this enterotoxin a designation has not been given for it.

The amount of Enterotoxin B produced by staphylococci under optimum conditions in the laboratory varies from undetectable to 200–300 μg per ml of culture supernatant when measured by oral administration to monkeys in terms of the production of emesis. Enterotoxin A appears to give identical symptoms as B in the monkey. Specific antibodies to Enterotoxin B have been prepared in the Food Research Institute laboratories from purified enterotoxin (Bergdoll, Surgalla *et al.*, 1959). An antibody to Enterotoxin A prepared by serum absorption techniques has been reported by Casman (1960) of the Food and Drug Administration laboratories. Most of the enterotoxin producing strains isolated from foods implicated in food poisoning outbreaks appear to produce principally Enterotoxin A although Dr. Kikuo Fujiwara (1961) of Chiba University, Japan, isolated 5 strains of staphylococci from foods implicated in food poisoning outbreaks that produced Enterotoxin B. It is the author's understanding that these five strains were isolated from foods from different outbreaks but obtained at the same food store. Therefore, the strain may represent a common contaminant in this store. The majority of strains which produce Enterotoxin B also produce Enterotoxin A in much smaller amounts. Foods inoculated with the Enterotoxin B, S-6 strain and incubated at times and temperatures similar to those encountered in food poisoning outbreaks resulted in the production of only Enterotoxin A. However, when the foods were mixed in a Waring blender with water and incubated with shaking after inoculation with S-6 strain, Enterotoxin B was produced. It has not been established why Enterotoxin B was not produced when grown in foods under natural conditions but it may have been due either to poorer growth under these conditions or the difference may be in aeration (Bergdoll, 1962). Since specific antiserum to Enterotoxin A has not been available a monkey feeding test has been developed for determining differences in enterotoxin. Monkeys become resistant to enterotoxin and the resistance is assumed to be due to the development of specific antibodies. Therefore, monkeys resistant to one type of enterotoxin should not be resistant to a type that is immunologically different. Monkeys resistant to Enterotoxin A are susceptible when fed Enterotoxin B, and monkeys which are resistant to Enterotoxins A and B should be susceptible to enterotoxin produced by Strain 137 as indicated in Table 1.

Purified enterotoxin is necessary for a study of the chemical nature of enterotoxins. Therefore, studies have been concentrated on the production and purification of enterotoxins. Enterotoxin has been produced in a relatively simple medium simplifying its recovery from culture supernatants. A medium most commonly used in our laboratories is an enzyme hydrolyzed casein (Protein Hydrolysate Powder, PHP) supplied by Mead Johnson and Co., Evansville, Indiana. The hydrolyzed casein medium is supplemented

Table 1. Feeding of enterotoxin from heterologous types to monkeys resistant to a single enterotoxin type

Monkeys	Enterotoxin A		Enterotoxin B		Strain 137	
	MED*	Results**	MED	Results	MED	Results
Enterotoxin A resistant	10	3/6	2	2/6	Not fed	
Enterotoxin A resistant	30	3/6	Not fed		3	5/6
Enterotoxin B resistant	12	9/16	200	4/16	Not fed	
Enterotoxin B resistant	Not fed		100	3/12	2	4/12

* Minimum emetic doses.

** Monkeys vomited/monkeys fed.

with niacin and thiamine. Equally good enterotoxin has been produced from enzyme hydrolyzed caseins from other sources when supplemented with other ingredients and are more economical than the PHP in the production of large quantities. The added ingredients, however, make it more difficult to recover Enterotoxin B from the culture supernatant by the ion exchange procedure (Bergdoll *et al.*, 1961). Substitute media are used in the production of Enterotoxin A because no interference is encountered using trichloroacetic acid as a precipitant for the Enterotoxin A. The amounts of Enterotoxin B produced have been variable. Studies with single colony isolates indicate that enterotoxin production varied from colony to colony as indicated in Table 2.

Antiserum has been added to culture plates for selecting colonies that produce enterotoxin in varying amounts (Sugiyama *et al.*, 1960). The maintenance of strains of high enterotoxin production has been overcome by drying selected cultures on porcelain beads and storing them at 4°C (Hunt *et al.*, 1958). Using cultures maintained on porcelain beads no change in enterotoxin production has been observed over a period of 18 months storage.

Several procedures were investigated in the course of purifying Enterotoxin B and partially purifying Enterotoxin A. Enterotoxin A has been purified to approximately 70% purity by the following steps: 1) removal from the culture supernatant by precipitation with trichloroacetic acid, pH 3.0, 2) absorption of the enterotoxin by cation exchange resin, Amberlite XE-64 pretreated, 0.02 M sodium phosphate, pH 6.2, 3) precipitation from 40% ethanol at -13°C and 4) chromatography on carboxymethyl cellulose using sodium acetate buffers.

Table 2. The production of enterotoxin.* Effect of colony selection

S-6 colony	Enterotoxin (μ g/ml)**
AMIF	190
No. 7	184
No. 14	49
No. 19	95
No. 27	30

* 18 hr incubation at 37°C on gyrotary shaker, 2% Protein Hydrolysate Powder+0.04 mg thiamin and 1.2 mg niacin per liter.

** Enterotoxin determined by gel-diffusion method.

Enterotoxin B was purified by the following procedures: 1) precipitation at pH 3.5 with phosphoric acid (H_3PO_4), 2) alumina absorption, 3) ethanol precipitation, 4) cation exchange resin Amberlite XE-64 chromatography, 5) starch electrophoresis, 6) ethanol precipitation and 7) freeze drying (Bergdoll, Sugiyama *et al.*, 1959).

The properties of Enterotoxin A have not been determined. Some of the properties of Enterotoxin B have been investigated (Hibnick and Bergdoll, 1959). It is a protein with a molecular weight of approximately 23,000 and an isoelectric point at pH 8.5. Enterotoxin B is resistant to proteolytic enzymes, chymotrypsin and pepsin above pH 2.5 (Bergdoll, unpublished). It is water soluble and heat coagulable. The approximate amino acid composition is listed in Table 3.

Table 3. Approximate amino acid analysis of Enterotoxin B*

Amino acid	Amino acid Residue/ 100 g sample	Amino acid	Amino acid Residue/ 100 g sample
Alanine	1.3	Methionine	2.5
Arginine	2.5	Phenylalanine	5.7
Aspartic acid	19.1	Proline	1.7
Glutamic acid	9.3	Serine	6.1
Glycine	2.8	Threonine	5.0
Histidine	3.0	Tryptophan	0.8
Isoleucine	4.0	Tyrosine	10.7
Leucine	7.3	Valine	5.8
Lysine	17.1	Amide N	1.4

* Determined by microbiological techniques.

The monkey assay has been used for the major portion of the work at the Food Research Institute. Samples are fed in solution usually 50 ml to young *Macaca mulatta* by catheter. Animals are observed for 5 hours after feeding. Vomiting is accepted as a positive reaction to enterotoxin. The method is only qualitative, is expensive, but is useful for circumventing other biologically active substances produced by the staphylococcus. Investigators may be misled in assaying enterotoxin when control cultures are inadequate or because purified enterotoxin was not used for test purposes. This is particularly the case where parenteral injections are given to experimental animals. One worker has proposed the use of nematodes to determine enterotoxin (Del Valle, 1960). Initial studies with these worms indicated that a certain type of coiling reaction occurred when the nematodes came in contact with solutions containing enterotoxin. When other investigators were unable to duplicate these observations it was discovered that the coiling reaction was not specific for enterotoxin since purified enterotoxin solutions had no effect on these worms. The specific reaction used for detecting enterotoxin is its reaction with its antibody. The method currently in use to measure Enterotoxin B is based on the Oudin simple gel-diffusion technique (Bergdoll, Surgalla *et al.*, 1959). In this test an agar column containing enterotoxin antiserum is layered with the enterotoxin solution. As the enterotoxin diffuses into the agar layer a band of precipitate is formed. The front of this band moves down the agar column at a rate corresponding to the concentration of enterotoxin and the concentration of its antibody. This method was adapted as a quantitative determination for enterotoxin B as follows: The standard

curve was made by plotting the log of the enterotoxin concentrations ($\mu\text{g/ml}$) against the distance that the enterotoxin anti-enterotoxin band moved in millimeters in the agar column in 7 days (Fig. 1).

Reliability of the test is affected by the solution in which the enterotoxin is dissolved and it is recommended that a standard gel-diffusion buffer be employed. The test is best carried out when the lowest concentration of antibodies that will give an easily visible band is used and enterotoxin concentration is such that the band moves 4-10 mm in 5-7 days. Typical examples of this test are given in Table 4.

Table 4. Measurement of Enterotoxin B by gel-diffusion technique
Effect of pH on production of enterotoxin*

pH	Dilution of bacterial culture supernatant**	Band movement (mm)				Enterotoxin ($\mu\text{g/ml}$)
		Dilution no.				
		1		2		
5.0	1:16	7.6,	7.6	8.1,	8.1	204
6.0	1:20	8.5,	8.5	8.5,	8.5	288
7.0	1:20	8.1,	8.1	7.9,	7.7	259
8.0	1:16	7.8,	7.8	7.8,	7.8	197

* 18 hrs incubation at 37°C on gyrotary shaker; 2% Protein Hydrolysate Powder + 0.04 mg thiamin and 1.2 mg niacin per liter.

** Dilutions made with 0.02 M sodium phosphate, pH 7.4 (0.85% saline).

The mode and site of action of staphylococcus enterotoxin has been studied with the purpose of searching for a new biological assay for enterotoxin. In the author's laboratory enterotoxin is sought by feeding experiments in monkeys. Experiments with isolated intestinal strips show no stimulatory action of enterotoxin (Sugiyama, unpublished). The action of enterotoxin in the central nervous system upon some specific area is suggested (Dolman, 1943). A rapid "neutralization" of toxic symptoms in pseudo-membranous enterocolitis by corticotropin (ACTH) is indicative of a relationship between enterotoxin and adrenocortico hormones (van Prohaska *et al.*, 1956). Pathological changes have not been found in the gastrointestinal tract of animals subjected to a single challenge of enterotoxin. However, gastroscopic and biopsy study of a group of humans suffering from staphylococcus food poisoning showed definite pathology (Palmer, 1951). A short lived progression of changes resulting from the death of the glandular cells at the junction of the glands and foveolae resulted in exfoliation of the foveolar layer and localized erosion. Regeneration was rapid and the abnormality disappeared within 92 hrs. These results need confirmation.

The continued presence of enterotoxin in the gastrointestinal tract results in severe lesions. The pathology of the disease in chinchillas fed antibiotic containing diet is essentially identical to the human pseudomembranous enterocolitis (Bennett *et al.*, 1956). Chinchillas fed several doses of purified Enterotoxin B during a course of a day developed severe and lethal enterocolitis. A dose of 0.4 mg of this enterotoxin divided into 4 doses produces the characteristic changes of loss of mucosa and ulceration (Warren *et al.*, unpublished).

Fig. 1. Standard curve for determination of enterotoxin B. Standard antigen : enterotoxin of an estimated "95% " purity. Standard antibody : 3-fold dilution of serum obtained from rabbits immunized with "70% " enterotoxin. Time of band development : 7 days.

Table 5. Effect of dihydroergotamine methanesulfonate on vomiting in monkeys fed staphylococcal enterotoxin

Enterotoxin		No. vomiting/No. tested		
Type	mg/monkey	Control*	Test**	Drug*
A	11.4	3/6	5/6	0/1
"	"	1/6	"	0/6
"	2.3	2/6	3/6	
"	1.14	3/6	5/6	
"	0.515	2/6	3/6	
"	4.6	1/6	4/6	
"	"	"	2/6	
"	17.68	3/6	6/6	0/6
B	24.0	2/6	4/6	"
"	0.05	1/6	3/6	"
Totals		19/60	40/60	0/25

* Enterotoxin alone.

** DHE-45, 1.5 mg subcut. + enterotoxin.

* DHE-45, 1.5 mg subcut. χ^2 probability less than 0.01.

An attempt was made to influence by administration of dihydroergotamine methanesulfonate (DHE-45) and other drugs the incidence of emesis in monkeys fed enterotoxin. If a compound decreased the incidence of vomiting then a reasonable basis is provided for investigating the possibility that the drug and enterotoxin had opposite pharmacological activity. Conversely, however, should a drug treatment increase the number of monkeys which vomit the possibility would exist that the drug and the enterotoxin had similar pharmacological activities. Partially purified enterotoxin preparations dissolved in 50 ml of water were fed by Sugiyama (1958) to *Macaca mulatta* by stomach tube. Vomiting in these monkeys within a 5-hour period of observation was considered a positive response to the enterotoxin. The animals were used in groups of six. The control group received enterotoxin alone and the test group received the same dose of the same toxin preparation in addition to the drug being tested. Because monkeys were fed enterotoxin samples repeatedly and they varied in their sensitivity to enterotoxin the animals for each experiment were selected so that the reactivity to enterotoxin of the test and control groups of monkeys were as nearly equal as possible (Surgalla *et al.*, 1953). When monkeys were re-used in experiments with the same drug they were placed as equally as possible in both control and test groups. Enterotoxin preparations were used which were in various stages of purification. Most experiments being performed with relatively few crude concentrates but one experiment was done with a preparation of 20-25% purity (Bergdoll *et al.*, 1952).

The dosage of toxin used was considered capable of inducing emesis in some 1-3 of the 6 control animals used in the experiment. The results are shown in Table 5 (Sugiyama *et al.*, 1958).

From these experiments it is apparent that subcutaneous injection of a subemetic dose of dihydroergotamine methanesulfonate increases the number of *Macaca mulatta* which vomit following oral administration of staphylococcal enterotoxin. This work suggests that vomiting is due to a summation of the effects of two similar pharmacological compounds. However, no adrenolytic or antiserotonin effect is produced by enterotoxin corresponding to that of DHE-45.

Antiemetics against staphylococcal enterotoxin induced emesis were studied by Sugiyama *et al.* (1960). Two tranquilizing drugs were studied by these investigators. Antiemetics effective in dogs against emetics whose site of action is the chemoreceptor trigger zone (CTZ) (Palmer, 1951) were tested: chlorpromazine (Glaviano and Wang, 1955; Brand *et al.*, 1954), perphenazine (Rosenkilde and Govier, 1957; Wang, 1958), cyclizine lactate (Ballinger and Borison, 1957). Reserpine was studied although reports on its ability to protect against apomorphine induced vomiting in dogs are contradictory (Ballinger and Borison, 1957; Malhotra and Sidhu, 1956). Nonspecific protection by depression of the medullary reticular emetic center was tested by determining change in sensitivity of monkeys to orally administered copper sulfate which evokes vomiting by peripheral action of the gastrointestinal tract (Wang and Borison, 1951). The results of these experiments are given in the Tables 6 and 7.

From these tables it is apparent that perphenazine and reserpine have significant antiemetic action against staphylococcal enterotoxin in induced emesis in monkeys. Chlorpromazine and cyclizine lactate have little or no protective action. Perphenazine gave the best protection and was effective at 50 $\mu\text{g/kg}$ intravenously. It prevented vomiting when administered 45 minutes after the enterotoxin was fed. Pretreatment of the monkeys was necessary for protection with reserpine. The effective levels for the drugs did not greatly depress the medullary vomiting center.

Table 6. Antiemetic activity of reserpine (1 mg/kg, i. v., 18 hrs previously) against vomiting provoked by orally administered staphylococcal enterotoxin and copper sulfate

Emetic		No. vomiting/No. fed	
Enterotoxin type	mg/monkey	Control	Drug treated
A	1.5	6/6	1/6
"	2.5	4/6	2/6
"	0.75	2/6	1/6
B	0.1	3/6	0/6
"	0.25	4/6	2/6
Totals		19/30	6/30
CuSO ₄ •5H ₂ O	320	10/10	9/10

Table 7. Antiemetic activity of perphenazine (50 µg/kg) against vomiting provoked by orally administered staphylococcal enterotoxin and copper sulfate

Emetic		No. vomiting/No. fed		Perphenazine
Enterotoxin type	mg/monkey	Control	Drug treated	
A	5.0	5/6	0/6	Immediate*
"	1.5	5/6	0/5	"
"	3.0	2/5	0/6	45 min. after**
B	2.0	2/6	0/6	Immediate
"	10.0	3/6	1/6	"
"	0.05	4/6	1/6	"
"	0.5	4/6	0/6	45 min. after
Totals		25/41	2/41	
CuSO ₄ •5H ₂ O	160-320	9/9	7/9	60 min. before*

* Intramusc., immediately before feeding enterotoxin.

** Intrav., 45 min. after feeding enterotoxin.

* Intramusc., 60 min. before feeding each animal predetermined threshold dose.

A medullary emetic center (MEC) which lies in the tractus solitarius and its nucleus and the dorsolateral border of the lateral reticular formation is needed for vomiting with all emetics (Borison and Wang, 1953). This center is neither motor nor sensory but coordinates the activity which constitutes emesis. Each type of emetic has its own primary locus of action. Vomiting following oral administration of copper sulfate results from gastrointestinal irritation. The centrally acting emetics such as apomorphine and Hydergine initiate vomiting by acting at the chemoreceptor trigger zone (CTZ) which lies in or is immediately adjacent to the area postrema on the floor of the 4th ventricle (Wang and Glaviano, 1954).

Sugiyama *et al.* (1961) found that bilateral destruction of the area postrema on the floor of the 4th ventricle, CTZ, makes rhesus monkeys completely refractory to the emetic action of staphylococcal enterotoxin. The surgically induced resistance is effective

against both Enterotoxins A and B. Vagotomy at the level of the diaphragm gave protection in all animals but in some protection was only partial. The incomplete protection against enterotoxin induced emesis in some of the vagotomized monkeys may be due to incompleteness of the vagotomy. If this reasoning is sound a common basis of emesis prevention following staphylococcal enterotoxin feedings may account for the similarity of vomiting response of area postrema ablated and vagotomized animals.

The situation would then be similar to the protection accorded monkeys by either CTZ ablation or vagotomy against the early emesis following a single total-body X-irradiation of 1200 r (Brizzee, 1956). However, rhesus and cynomolgous monkeys fail to vomit in response to emetics known to act on the CTZ of dogs and cats (Brizzee *et al.*, 1955).

The site of emetic action of enterotoxin in cats being challenged by parenteral administration is different from that in monkeys. The results with cats subjected to various denervation and surgical procedures indicated that the viscera innervated by the vagus nerve was an important site of action of enterotoxin (Bayliss, 1940). However, the possible greater importance of a forebrain site was emphasized in the re-evaluation of the data (Borison and Wang, 1953). The destruction of the CTZ of cats does not prevent enterotoxin-stimulated vomiting. Transthoracic vagotomy raised the threshold for enterotoxin induced vomiting but the combination of vagotomy and spinal cord transection at T1 or T3 seemed to confer better protection (Clark *et al.*, 1961; Clark, 1962).

The different results obtained in the monkeys and in the cats may reside in species difference. For example, CTZ destruction in cats does not confer protection against emesis induced by radiation or by nitrogen mustard while in the dogs this procedure gives complete protection (Borison, 1957; Borison *et al.*, 1958). The possible difference resulting from the route of challenge cannot be eliminated at the present time. Although fever is not an important syndrome in staphylococcus food poisoning, the intravenous injection of highly purified enterotoxin into cats elicits a typical pyrogenic response. Moreover, an early leucopenia after intravenous administration of enterotoxin into cats occurs (Clark, 1962); monkeys fed enterotoxin do not develop leucopenia.

The evidence for a forebrain site of emetic action in cats is inconclusive. Cats with chronic midbrain transection continued to show a pyrogenic but not an emetic response. However, lateral ventricular injection of enterotoxin does not cause vomiting although the pyrogenic response can be evoked by this route (Clark, 1962).

Sugiyama *et al.* (unpublished) have found that previously unused monkeys given their first experimental feeding of staphylococcal enterotoxin become significantly less sensitive to its emetic action within 24 hours. The refractory state develops rapidly and is essentially specific to the antigenic type to enterotoxin used for the initial feeding. A higher degree of resistance develops against one homologous antigenic type of enterotoxin than against the other. This refractory state is of temporary duration. The tolerance against the emetic action does not protect against all the actions of enterotoxins since animals not vomiting to several multiples of the original emetic dose may show other signs of illness. The early developed resistance has some features of the non-specific resistance induced by bacterial endotoxin and other bacterial products. An example is shown in Table 8.

Another similarity between enterotoxin and endotoxin (LPS) toxin is the increase in the sensitivity of monkeys to enterotoxin induced emesis after reticuloendothelial system blockade by Thorotrast (colloidal thorium dioxide) (Sugiyama *et al.*, unpublished).

Table 8. Resistance (no vomiting) developed in previously unused monkeys 24 hr after initial intragastric administration of Enterotoxins A and B

Enterotoxin			<u>Number vomiting</u> <u>Number fed</u>
First feeding	Second feeding		
Type	Type	×MED*	
A	A	5	0/2
"	"	10	0/2
"	"	15	1/1
"	"	20	1/2
"	B	2	1/4
"	"	3	1/1
"	"	4	3/3
B	"	2	0/2
"	"	4	0/2
"	"	5	0/1
"	"	10	0/4
"	"	20	0/2
"	"	40	0/2
"	A	4	1/1

* Number of minimal emetic doses if monkeys do not develop resistance from the first feeding of enterotoxin.

These observations suggested a study of a possible interaction of enterotoxin and endotoxin. Enterotoxic preparations of at least 20% purity have no apparent effect in mice after intraperitoneal injection. If enterotoxin is administered to mice 4 hr preceding a challenge with a dose of LPS which will cause death in a small proportion of mice, a significantly larger number of deaths occurs (Sugiyama, unpublished).

Four enterotoxic preparations ranging from 20–95% purity were assayed in mice by giving graduated enterotoxin doses intraperitoneally followed in 4 hr with intraperitoneal doses of LPS. The relative potency of the enterotoxin preparations as LPS sensitizing agents correlated with the potency of the enterotoxins as measured by feeding monkeys thus indicating that the substance effective in sensitizing the mice to LPS is the enterotoxin. The use of this method for the quantitative assay of partially purified enterotoxin is promising.

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