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Sensitivity of Thorotrast-Treated Monkeys to Staphylococcal Enterotoxin.* (28398)

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Preliminary evidence has been adduced that pretreatment of monkeys with stabilized colloidal thorium dioxide (Thorotrast)† sensitizes the animals to the emetic action of staphylococcal enterotoxin. Previously untreated monkeys not vomiting to an ED_{50} dose (eliciting vomiting in 50% of animals tested) of enterotoxin were challenged one week later with the same enterotoxin dose after intravenous injection of Thorotrast 18 hours previously. All of 7 such animals vomited, even though the dose without Thorotrast had been ineffective previously and some resistance to the enterotoxin should have developed as a consequence of the previous enterotoxin feeding(1).

The present communication extends the initial observation. Thorotrast pretreatment rendered monkeys hypersusceptible to the emetic action of both of the antigenic types of staphylococcal enterotoxin tested. The degree to which this colloidal agent enhanced the sensitivity to these distinct enterotoxins is reported.

Materials and methods. Young rhesus monkeys *(Macaca mulatta)* of 1.5 to 2.4 kg body weight were employed. These animals were conditioned to laboratory environment for about one month and had not been exposed experimentally to enterotoxin previously.

Enterotoxin was fed by stomach tube in a volume of 50 ml of water and the animals observed for emesis continuously over a 5-hour period. Thorotrast, at a level of 3 ml per kg body weight, was injected intravenously 4 or 18 hours prior to the challenge with enterotoxin. Thorotrast alone did not provoke vomiting. Wherever possible control monkeys administered enterotoxin alone were used; they were of the same shipment as the experimental animals.

The enterotoxin preparations used were lyophilized samples which had previously been assayed in monkeys. In accordance with a recent proposal for the designation of staphylococcal enterotoxins(2), the naming of the preparations used has been changed from that employed in previous communications from this laboratory. Enterotoxin A (previously called 196E type) was of approximately 20% purity with an ED_{50} level of 50 μg per animal by the intragastric route. Enterotoxin B (formerly called S-6 type) was of estimated 95% purity with a 50% emetic dose of 20 μg per animal. Although the S-6 strain produces both Enterotoxins A and B, only the B remains at this stage of purification. These enterotoxins, while producing the same symptoms, do not cross-react serologically.

Results. Intravenous injection of Thorotrast 18 hr before intragastric challenge with enterotoxin increased the sensitivity of the monkeys to the emetic action of Enterotoxins A and B (Table I). The data represent totals obtained from several different tests using different shipments of animals. The dosage of Enterotoxin B required to provoke

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† Testagar & Co., Inc., Detroit, Mich.

TABLE I. Emetic Response of Thorotrast-Treated Monkeys (3 ml/kg, i.v.) to Intragastrically Administered Staphylococcal Enterotoxins A and B.

Enterotoxin challenge		No. vomiting/No. tested Thorotrast treatment None	4 hr	18 hr
	(μg)			
A	50*	1/2	—	—
"	20	0/3	—	—
"	10	1/6	1/3	3/6
"	5	0/3	3/6	5/6
B	20	3/5	—	—
"	5	0/10	1/3	8/14
"	1	—	1/2	5/10
"	0.5	—	—	1/6

* ED_{50} for large number of monkeys = 50 μg; for B = 20 μg.

vomiting in 50% of the animals was reduced to 1 μg per animal from 20 μg for the untreated group.

The number of animals tested with Enterotoxin A was limited and the actual ED_{50} dose for the Thorotrast treated (18 hours) was not actually determined. However, extrapolation from the data is possible. Five μg of enterotoxin elicited vomiting in 5 of 6 animals of the experimental group. From the number of vomiting episodes per animal (2 to 4 times for each responsive animal) and the proportion of reacting animals it is reasonable to suggest that 2.5 μg instead of 5 μg is nearer to the ED_{50} level. Thus, monkeys pretreated with Thorotrast 18 hr previously are approximately 20 times more sensitive to the emetic stimulus of both Enterotoxins A and B.

The incidence of vomiting in monkeys treated with Thorotrast 4 hr before intragastric administration of enterotoxin is not significantly different from that evoked by the same enterotoxin dose in monkeys pretreated 18 hours prior to the enterotoxin feeding. However, when the animals vomited with the shorter pretreatment schedule, fresh blood was observed in the vomitus of all but one of the 6 reacting monkeys. Complete recovery was normal. In contrast, bloody vomitus was not observed in any of the animals with the longer interval. Bloody vomiting is an extremely rare occurrence in monkeys fed these enterotoxin preparations and can be regarded as a more severe response.

The latency period (interval between enterotoxin feeding and initial vomiting response) was not greatly different after Thorotrast pretreatment.

Discussion. Previously untreated monkeys given a small dose of staphylococcal enterotoxin are significantly less sensitive to the emetic action of the homologous antigenic enterotoxin 24 hours later. The rapidly developed tolerant state is temporary in duration and is distinct from the permanent type of immunity. Some similarity of this induced tolerance to enterotoxin and the non-specific resistance to infections and endotoxins stimulated by the endotoxins of the Gram negative bacteria(3,4) has been suggested(1).

A recent report shows that highly purified staphylococcal enterotoxin given intravenously or intradermally alters the dermal reactivity to epinephrine to elicit a lesion similar to that produced by bacterial endotoxin and epinephrine(5). Intravenous administration of a highly purified enterotoxin into cats produces a biphasic pyrogenic response and leukopenia, similar to those produced by intravenous endotoxin(6). Intravenous injection of staphylococcal enterotoxin into rabbits greatly enhances the lethal effect of the lipopolysaccharide bacterial endotoxin (Sugiyama, to be published).

Several different manifestations of bacterial endotoxin activity are accentuated by the use of Thorotrast in rabbits. The tolerance to the pyrogenic action of endotoxin is abolished(7), lethal effect is increased(8), and natural immunity to the Shwartzman phenomenon is overcome(9). The present observations with Thorotrast and staphylococcal enterotoxin further suggest similarities in certain biological activities of these 2 toxins of different origin. It should be emphasized that the studies with monkeys were concerned with the intragastric challenge of the enterotoxin. This route of administration assures that the active material is enterotoxin and not some residual contaminating substance(10).

The mechanism whereby Thorotrast renders the monkeys more susceptible to staphylococcal enterotoxin can only be conjectured. The phagocytic activity of the reticuloendo-

thelial system is depressed by colloidal materials(11); the reticuloendothelial system is involved with removal of bacterial endotoxin from the circulation(12). These observations give credence to the suggestion that the greater sensitivity of Thorotrast-treated animals to bacterial endotoxins is the result of blockade of the reticuloendothelial system and consequent deleterious effect on the removal and/or detoxification of the subsequently administered endotoxin.

A similar mechanism may be used to explain the present results. No information is currently available on the absorption of enterotoxin after intragastric administration. But if the above explanation is correct, the emesis stimulated by feeding of enterotoxin probably involves the appearance of enterotoxin or some toxic material in the circulatory system.

Observations made with materials such as colloidal thorium dioxide are difficult to interpret since little is known of the other physiological changes induced by these agents(13). Direct or indirect sensitization of the emetic receptor sites for enterotoxin (14,15) would produce the observed results. If antibodies contribute to the normal resistance to enterotoxin, their depletion by Thorotrast, as indicated by the reduction in serum opsonins after injection of colloids(16), must be considered.

Summary. Monkeys pretreated with stabilized colloidal thorium dioxide (Thorotrast) are more sensitive to the emetic action of intragastrically administered staphylococcal enterotoxin. The amounts required to elicit vomiting in 50% of the animals tested was reduced to about one-twentieth of that required for untreated monkeys when Thorotrast was given 18 hours before the enterotoxin challenge. The degree of sensitization was the same for both of the 2 different antigenic types of enterotoxin tested. Fresh blood was observed in the vomitus when the interval between Thorotrast and enterotoxin was reduced to 4 hours.

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