easily manipulated environmental factor is of obvious practical interest, but it also constitutes a requisite for a basic analysis of functions, within the range in which they vary under ordinary circumstances.

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Staphylococcal Enterotoxin: Increased Vomiting Incidence in Monkeys Following Subemetic Doses of Dihydroergotamine.* (23916)

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Very little definitive information is available on mode and site of action of staphylococcal enterotoxin. It has been suggested that action of enterotoxin is peripheral rather than central and that it affects either the sensory nerve endings or the smooth muscle of small intestine(1). Experiments with isolated intestinal strips show no direct stimulatory action of enterotoxin (unpublished, 2). The action of enterotoxin upon some specific area of the central nervous system has also been suggested(3). The results with animals subjected to various denervation and surgical procedures have been interpreted as indicating the site of action in the forebrain(4), but enterotoxin induced vomiting in chronic, totally decorticate cats with the third ventricle and surrounding structures intact(5). The

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rapid "neutralization" of toxic manifestations in pseudomembranous enterocolitis by corticotrophin (ACTH) is indicative of a relationship between enterotoxin and the adrenal cortical hormones(6). The small, but definite, elevation of serum glutamic oxalacetic transaminase of monkeys following oral administration of enterotoxin suggests cellular damage at some site (to be published).

The present communication is concerned with attempts to influence by administration of dihydroergotamine methanesulfonate (DHE-45) and other drugs the incidence of emesis in monkeys fed enterotoxin. Should a compound decrease the incidence of vomiting, then a reasonable basis is provided for investigating the possibility that the drug and enterotoxin had opposite pharmacological activities. Conversely, should a drug treatment increase the number of monkeys which vomit, the possibility would exist that the drug and the enterotoxin had similar pharmacological actions. Experiments of this nature may provide a clue to mode and site of action of staphylococcal enterotoxin.

Materials and methods. Partially purified enterotoxin preparations dissolved in 50 ml of water were fed to *Macaca mulatta* by stomach tube. Vomiting in these monkeys within a 5 hr period was considered a positive response to the enterotoxin. The animals were employed in groups of 6. The control group received toxin alone and the test group received the same dose of same toxin preparation in addition to the drug being tested. Since monkeys were fed enterotoxin samples several times and varied considerably in their sensitivity to enterotoxin, the animals for each experiment were selected so the reactivity to enterotoxin of the test and control groups of monkeys were as nearly equal as possible(7). When monkeys were reused in experiments involving the same drug, they were placed as equally as possible in both control and test groups. The enterotoxin samples were derived from 3 enterotoxigenic strains of *Staphylococcus*. Toxin preparations were in varying stages of purification. Most feeding experiments were performed with relatively crude concentrates, but one experiment was done with a preparation of 20-25% purity(8). The dosage of toxin used was one considered capable of inducing emesis in one to 3 of the 6 control animals used in that experiment. Relatively large amounts of drugs were employed to accentuate any differences in responsiveness of monkeys to enterotoxin. If the drug had emetic activity of its own the dosage used was one which was subemetic.

TABLE I. Effect of Dihydroergotamine Methanesulfonate on Vomiting in Monkeys Fed Staphylococcal Enterotoxin.

Enterotoxin		No. vomiting/No. tested		
Strain	mg/monkey	Control*	Test†	Drug‡
196	11.4	3/6	5/6	0/1
"	"	1/6	"	0/6
"	2.3	2/6	3/6	
"	1.14	3/6	5/6	
"	.515	2/6	3/6	
"	4.6	1/6	4/6	
"	"	"	2/6	
8	17.68	3/6	6/6	0/6
S-6	24.0	2/6	4/6	"
"	.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 .01.

Results. The number of monkeys vomiting in the group treated with dihydroergotamine methanesulfonate (DHE-45) was approximately twice that of control group receiving enterotoxin alone. DHE-45 was injected subcutaneously in dose of 1.5 mg/animal (average wt about 3.5 kg) 45-60 minutes before the toxin was fed. A total of 60 monkeys in both control and test groups was used. None of 25 monkeys vomited when the same quantity of DHE-45 was given subcutaneously and 50 ml of water was fed. The results are presented in Table I.

The data of Table I were obtained through oral administration of enterotoxin prepared from 3 strains of *Staphylococcus*. The increase in vomiting incidence with the toxin from all 3 strains is of interest since there is evidence that enterotoxin from different strains is antigenically different(7,9).

No significant difference in incidence of vomiting in monkeys fed enterotoxin was observed in a limited number of similar experiments involving other types of drugs. Diphenhydramine (benadryl) was used for its antihistaminic effect; apomorphine because

its site of emetic action has been established. Veriloid (alkaloid of *Veratrum viride*) was included since the site of emetic action of the veratrum alkaloids is not the central nervous system but seems to be associated with the nodose ganglion(4). The possible effect of ganglionic blockage was tested by use of tetraethylammonium chloride (Etamon chloride). Atropine was employed as an example of a parasympathetic nervous system depressant. The possible prophylactic effect of the adrenal cortical hormones (in the form of adrenal cortical extracts) was investigated because ACTH has been reported to alleviate the toxic symptoms of pseudomembranous enterocolitis(6).

Since ergot alkaloids and their dihydrogenated derivatives possess emetic property, the possibility that increased incidence of emesis among DHE-45 treated animals might be due to summation of 2 similar pharmacological activities was investigated. So far no adrenolytic activity of enterotoxin as exhibited by DHE-45(10) has been found. Enterotoxin does not inhibit relaxing effect of epinephrine on isolated small intestine of the rabbit and does not affect the action of epinephrine in antagonizing the contraction of the rat uterus stimulated by acetylcholine. Enterotoxin does not inhibit the hyperglycemia in rabbits which follows subcutaneous injection of epinephrine. No distinctive blood pressure change in cats under chloralose anesthesia has been observed following injection of enterotoxin samples.

DHE-45 is also a serotonin antagonist(11). Serotonin antagonism can be demonstrated with some enterotoxin samples on the isolated oestrous rat uterus preparation, but this reaction does not seem specific to the enterotoxin itself.

Discussion. The number of monkeys which vomit after oral administration of staphylococcal enterotoxin is significantly increased by subcutaneous injection of subemetic doses of dihydroergotamine methanesulfonate. Since only partially purified preparations of enterotoxin were used, it is possible that the agent responsible for the observed results may be some substance other than the enterotoxin. However, the monkey feeding test is the most specific of the tests for detection of enterotoxin in untreated crude staphylococcal culture filtrates. Presumably, substances other than enterotoxin present in these filtrates are rendered innocuous or are not absorbed; they do not provoke emesis. Thus, it is probably the enterotoxin itself which plays a role in increasing the incidence of emesis in monkeys treated with subemetic quantity of DHE-45.

The results suggest a summation of effects of 2 emetic compounds. The subemetic quantity of DHE-45 may give a stimulus below the threshold necessary to evoke vomiting; the enterotoxin may give a similar subthreshold stimulus in animals which do not vomit with enterotoxin alone. The sum of these 2 subthreshold stimuli may then be sufficient to produce the emetic response. Available information is insufficient to consider possible potentiation effects.

10 μg of essentially pure enterotoxin cause vomiting in monkeys after oral administration (8). This high toxicity and symptoms of staphylococcal food poisoning suggest the nervous system as site of action. If it is a central nervous system toxin, the passage through the blood-brain barrier of a polypeptide of molecular weight of 20-25,000 must be considered. On the other hand, if the results reported in this paper are due to summation of effects of 2 compounds acting on the same site, then the site of emetic action of enterotoxin may be the medullary emetic chemoreceptor trigger zone. This suggestion follows from the demonstration(4) that dogs with chronic ablation of medullary emetic chemoreceptor trigger zone do not vomit after intravenous injection of Hydergine, a mixture of dihydrogenated ergot alkaloids.

A practical application of the observations with DHE-45 may be possible. Repeated feedings of enterotoxin to monkeys result in development of resistance to the toxin. This resistance may become so great that the animals are no longer usable for experiments with practicable doses of enterotoxin. Since subemetic quantity of DHE-45 increases the number of positive reactions in the partially resistant monkeys, a greater number of feeding tests on them may become possible by use of the drug.

Summary. Subcutaneous injection of sub-

emetic dose of dihydroergotamine methanesulfonate increases the number of *Macaca mulatta* which vomit following oral administration of staphylococcal enterotoxin. The possibility that increased incidence of vomiting might be due to summation effects of 2 similar pharmacological compounds has been considered, but no adrenolytic effect of enterotoxin corresponding to that of DHE-45 has been found. The other drugs tested have not significantly affected the incidence of emesis in monkeys following feeding of enterotoxin.

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Food Utilization and Weight Gain in Hypophysectomized Rat as Function of Handling. (23917)

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Previous studies have shown that normal young rats handled or gentled by the experimenter gain significantly more weight and length than unhandled control animals(1-9). Furthermore, these effects of handling appear to be a function of better food utilization rather than increased intake(4,6,7). The literature on this subject has recently been reviewed in detail(10).

This paper reports the effects of handling on weight gain, skeletal growth, food utilization and body composition in the young hypophysectomized rat. It is the first of a series of investigations designed to study the mechanism of various effects of handling in experimental animals.

Methods. Twenty-eight male hypophysectomized rats of Sprague-Dawley strain, obtained from Hormone Assay Laboratories, (Chicago), were used. Hypophysectomy was performed on nineteenth day of age. Animals were weighed and then divided at random into 2 equal groups: control and experimental. Beginning on twenty-first day of age, each animal of experimental group was handled individually by one of us (L.B.) for 10 minutes every day at the same time for 6 weeks. Handling consisted of holding the animal in one hand, and stroking it with the other from head to tail repeatedly, with animal facing experimenter. Control animals were never touched after their original placement in cages. A weighed amount of food in petri dish was placed in each cage daily and leftover food weighed daily to determine daily food consumption. The diet used was of the following content and proportion: 480 g canned dog food (Pard®), 2 slices of crumbled whole wheat bread, 480 ml skim milk, and 2.5 ml Vi-Daylin®* (a liquid multi-vitamin preparation). These ingredients were thoroughly mixed and proved suitable for these animals (tender-jawed following hypophysectomy), and food loss by spillage from petri dish was

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