phages, may depend on the continued presence in our lymph node cell suspensions of phagocytic reticulo-endothelial cells (about 3%). Fishman's data support this conjecture; he found that rabbit lymph node cells, unlike those of the rat, respond to direct addition of antigen with appreciable antibody titers.

Summary. Primary immunization of lymphoid cells by exposure to homologous skin within diffusion chambers was demonstrated by the ability of these cells to produce a transfer reaction in the donor's skin. Addition of peritoneal exudate or alveolar macrophages to lymph node cell suspensions resulted in significant enhancement of sensitization in 5 of 11 experiments. There was no enhancement in 4½ of 11 experiments, and in 1½ sensitization was greater in the absence of macrophages.

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Received May 26, 1964. P.S.E.B.M., 1964, v117.

Diarrhea in Cecectomized Rabbits Induced by Staphylococcal Enterotoxin.* (29510)

T. HAYAMA† AND H. SUGIYAMA

Food Research Institute and Department of Microbiology, University of Chicago, Chicago, Ill.

The ingestion of preformed staphylococcal enterotoxin is the cause of staphylococcal food poisoning. The essential features of this illness are nausea, vomiting, abdominal cramping and diarrhea with shock-like collapse in severe cases. The emetic response is used for laboratory detection of enterotoxin (1) but the diarrheal aspects have not been extensively studied. Diarrhea can be observed in monkeys fed highly purified enterotoxin near the threshold emetic dose and in cats given intravenous challenges, but the response is not consistent. Similarly, mice and rabbits injected parenterally have proved not to be satisfactory in the study of enterotoxin-induced diarrhea.

Nausea, vomiting and gastrointestinal re-

* Supported by grant from U. S. Public Health Service, EF-77.

† Permanent address: Laboratory of Pharmacology and Toxicology, Inst. of Food Microbiology, Chiba Univ., Japan.

actions after X-irradiation bear some resemblance to the symptoms caused by staphylococcal enterotoxin. Among the commonly used laboratory rodents, the rabbit and guinea pig are less susceptible to diarrhea and death following irradiation injury than the mouse and rat. The correlation between the relatively large cecum of rabbit and guinea pig and their tolerance to irradiation effects has been pointed out and the suggestion made that the large cecum plays a protective role against irradiation-induced diarrhea in these animals(2).

The present communication is concerned with the effect of the removal of the cecum of rabbit on the diarrhea induced by staphylococcal enterotoxin.

Methods. New Zealand, male, albino rabbits of 1.2 to 2.5 kg body weight were used, although most experiments were done with animals close to 2 kg. Attempts were made initially to prepare totally cecectomized rabbits but the poor post-operative course necessitated a change to partial cecectomy. The appendix and all of the cecum except the proximal 3 segments were taken out after ligation of the attached blood vessels. Post-surgical diarrhea developed in most animals; except for the few that died from severe diarrhea, complete recovery usually occurred within 10 days. At this time the stools were formed although the fecal droppings were of slightly softer consistency than without cecectomy. Rabbit pellets (Rockland Farm) were fed *ad libitum*; the diet of young animals (less than 1.7 kg) was supplemented with vegetables. After an initial weight loss, the animals gained weight during the latter part of the recovery period.

Highly purified staphylococcal Enterotoxin B(3) was dissolved in pyrogen-free saline for intravenous injection. Dosage was in μg enterotoxin per animal. The intragastric 50% emetic dose for monkeys of this preparation was 30 μg/animal at the time of these experiments.

The cecectomized rabbits were challenged with enterotoxin after 4 consecutive days of normal bowel movements. The floor of the cage below the wire mesh screen was cleaned immediately before enterotoxin administration. Periodic examinations of the stools were made after challenge to determine latency and type of diarrhea.

All precautions were taken to use only animals completely recovered from the surgical treatment, but a few of the cecectomized rabbits developed diarrhea spontaneously and died after 1-2 days with a purging diarrhea. The diarrhea induced by enterotoxin lasted 24 hours or less; thus, only those animals which recovered within this period were considered to have responded specifically to enterotoxin challenge and deemed reliable. Although these animals recovered from the enterotoxin-induced diarrhea a few died several days later with other symptoms.

Antagonists against the biologically active amines were used to study possible protection against enterotoxin-induced diarrhea in cecectomized rabbits. Atropine sulfate, pyribenzamine hydrochloride and lysergic acid diethylamine (LSD_{25}) were used as anticholinergic, antihistaminic and antiserotonin drugs, respectively. These drugs were administered intravenously 30 minutes before enterotoxin challenge and refer to the salts given on mg/kg body weight basis.

Results. The intravenous injection of highly purified enterotoxin is ineffective in provoking diarrhea in normal rabbits. Appendectomy did not significantly alter the responsiveness but cecectomy resulted in 100% response to 100 μg per animal (approximately 50 μg/kg). The cumulative data of animals prepared and tested over several months are shown in Table I.

Diarrhea consisted of the passage of one or more fluid stools with abundant mucous.

TABLE I. Diarrheal Response of Rabbits to Intravenous Enterotoxin.

Animal type	Enterotoxin, μg/animal	No. with diarrhea /No. tested
Normal	100	0/4
	200	1/9
Appendectomy	100	1/2
	200	0/2
Cecectomy	20	0/2
	50	2/4
	100	13/13

TABLE II. Development of Resistance of Cecectomized Rabbits to Enterotoxin-Induced Diarrhea After Weekly Challenges of 100 μg/Animal.

Challenge	No. with diarrhea/No. tested	Latency, hr
1st	13/13*	1¾ (1½–2)†
2nd	11/11	2½ (1½–3¾)
3rd	4/10	3¾ (2½–4½)
4th	1/6	3

* From Table I. † Range in latencies.

TABLE III. Effect of Atropine and Pyribenzamine on Enterotoxin-Induced Diarrhea in Cecectomized Rabbits. Drugs i.v. 30 min before enterotoxin.

Drug	Enterotoxin, μg/animal	No. with diarrhea/No. tested
Atropine (1 mg/kg)	200	1/6
	500	2/2
Pyribenzamine (2 mg/kg)	200	1/3
	500	2/3

All rabbits that responded developed diarrhea after similar latency periods, the average being 105 minutes. About one-third of the animals recovered from the diarrhea within 6 hours; otherwise the bowel movements were normal within 24 hours. No significant difference in sensitivity was observed between cecectomized rabbits of different ages.

The animals included in Table I were given enterotoxin for the first time. Successive weekly challenges resulted in the development of resistance. Cecectomized rabbits that developed diarrhea after 100 μg enterotoxin (Table I) were rechallenged with the same dose 1 week later. Third and fourth challenges were given to rabbits that were in apparent good health (Table II).

Complete protection against enterotoxin-induced diarrhea in cecectomized rabbits was not obtained with the amine antagonists employed. Atropine and pyribenzamine had definite but incomplete protection (Table III). LSD_{25} effects were of low order and the significance was questionable.

Administration of enterotoxin to cecectomized rabbits by the intragastric route was uniformly unsuccessful in provoking diarrhea. Doses up to 2 mg of enterotoxin were tried.

Discussion. Intravenous injection of a highly purified staphylococcal enterotoxic preparation into cecectomized rabbits provoked diarrhea with doses which were ineffective for normal rabbits. The relatively large dosage (compared to intragastric emetic dose for monkeys) and the required parenteral route of challenge fail to settle the question whether enterotoxin *per se* and not some residual contaminating material is the agent inciting the diarrhea. However, the large dosage seems to be a species function; this enterotoxic sample is uniformly lethal for monkeys above 30 μg/kg, i.v., while 100 μg/kg by the same route is not fatal to rabbits(4). The enterotoxin sample is of high purity, and although its intragastric emetic activity for monkeys has decreased over the years, it is still used as a "standard" enterotoxin in this laboratory. Serological gel-diffusion tests indicate the presence of enterotoxin antigen and only a trace amount of a second antigen. The development of resistance after successive enterotoxin injection seems significant in ascribing the diarrhea to enterotoxin. Moreover, the latency for the diarrheal response is what might be expected with enterotoxin as the responsible substance.

The basis for the greater sensitivity of cecectomized rabbits to diarrhea following intravenous injection of highly purified staphylococcal enterotoxin is not understood. The mucoid-watery fecal excretions indicate the importance of increased mucous secretion and net movement of water into the intestinal lumen. The cecum is an important organ of digestion for the rabbit(5); material from the small intestine is mixed in the cecum and released into the colon periodically(6,7). The absence of the cecum would allow direct entry of the intestinal contents from the small to the large bowel so that hypermotility, excessive mucous and water secretion along the intestinal tract could result in diarrhea. The gastrointestinal tract above the cecum appears to be an important site of action of intravenously administered enterotoxin; otherwise, the removal of the cecum would not be expected to predispose the animal to enterotoxin challenge. On the other hand, purified enterotoxin has no demonstrable effect on the smooth muscle of isolated small intestine preparations(1).

Summary. Cecectomized rabbits (removal of appendix and all of cecum except proximal 3 segments) were challenged intravenously with highly purified staphylococcal enterotoxin. Diarrhea was induced in cecectomized rabbits in doses ineffective for normal rabbits. Average latency was 1¾ hours with recovery from diarrhea within 24 hours. Successive weekly injections of enterotoxin resulted in development of resistance. Pretreatment of cecectomized rabbits with atropine or pyribenzamine gave definite but incomplete protection against enterotoxin-induced diarrhea.

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Received May 26, 1964. P.S.E.B.M., 1964, v117.

Relationship of Food Intake to the Effect of Cortisone Acetate on Skin Wound Healing. (29511)

G. DIPASQUALE AND B. G. STEINETZ (Introduced by W. M. Govier)
Department of Physiology, Warner-Lambert Research Institute, Morris Plains, N. J.

Glucocorticoids have been reported to inhibit wound healing in laboratory animals(1-6) by suppressing formation of granulation tissue(7,8), collagen(2,9,10) and acid mucopolysaccharides(11-13). The effect of cortisone acetate may not be immediate but may follow a "lag" period(14). Some investigators have been unable to demonstrate histologically faulty wound healing, fibroblastic proliferation(15) or differences in granulation collagen(8).

Much of the confusion may have developed from use of incomparable dose levels of steroids and from failure to control obvious anorexic effects of glucocorticoids. Accordingly, effects of graded doses of cortisone acetate on skin wound tensile strength were reevaluated utilizing the technique of pair-feeding to control food consumption.

Materials and methods. One hundred and forty-four male Wistar rats weighing 170 ± 10 g were individually housed and arranged in 9 groups consisting of 16 animals each as indicated in the Tables. Groups A, B-1, C-1, D-1, E-1 received Rockland ground food and tap water *ad libitum.* Group A was the absolute control; whereas the other groups received respectively, 1, 2, 4, 8 mg of cortisone acetate s.c. per rat per day. Each cortisone treated animal had a corresponding pair-fed control (Groups B-2, C-2, D-2, E-2).

On day 1 the rats were anesthetized with ether, the skin of the dorso-lumbar region was shaved with electrical clippers and a wound approximately 1 inch in length was incised. The wound was closed with 3 Michel wound clips which were removed 48 hours later. Eight rats from each group were killed 3 days and the remaining eight 9 days after wounding.

Body weights were recorded on days 1, 3, 6, 9. Food consumption of cortisone acetate treated animals was recorded daily. Skin wound tensile strength was measured on day 3 and day 9.

The apparatus used to measure the wound tensile strength consisted of 2 alligator clips, a ball-bearing pulley system, plastic volumetric cylinders and a constant rate of flow water source. After the rat was killed one clip was attached to one side of the wound