The existence or importance of autoantibody in the pathogenesis of the renal lesion is still open to question but the present experiment suggests that antibodies transmitted through colustrum do not mediate the disease. It might also be expected that if sensitized cells were the mediator of tissue damage that it would be unlikely that a sufficient amount of such cells were able to traverse the placenta barrier or be transmitted through colustrum.

Summary. (1) Three sheep developed a fatal nephritis in 45 to 54 days when immunized with heterologous GBM and Freund's adjuvant in the last third of their pregnancy. (2) Immunization of the mothers or suckling their colustrum (in 2 of the 4 lambs) did not produce a renal lesion in the lambs. (3) It appears that the agent which produces a fatal nephritis in the mother was either not transmitted *in utero* or in the colustrum or it was incapable of affecting the fetal or neonatal kidney. (4) The above observations are consistent with the hypothesis that this sheep nephritis is produced by an autoimmune mechanism mediated by sensitized cells and/or autoantibody.

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Effect of Staphylococcal Enterotoxin on Dermal Reactivity to Epinephrine.* (27937)

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Recently it was reported that certain strains of *Staphylococcus aureus* contain a toxic product with biologic properties resembling those of bacterial endotoxins(1-6). Staphylococcal extracts alter dermal reactivity of the rabbit to epinephrine, cause hemorrhagic necrosis of sarcoma 180 of mice, are lethal to adrenalectomized mice, and cause death of 10-day-old chick embryos(1-6). Viable or killed staphylococci also produce some of these effects(1,5). The heat stability of the active principle of the extracts was reminiscent of staphylococcal enterotoxin. Accordingly, the present investigation was carried out to determine whether purified preparations of enterotoxin also alter dermal reactivity to epinephrine of rabbit and guinea pig.

Materials and methods. Partially and highly purified enterotoxins prepared from the S-6 strain of *Staphylococcus aureus* were employed. Preparations 7A, 8A, 9B, and 94 were prepared according to the methods of Bergdoll *et al.*(7), and supplied by Dr. Bergdoll of the University of Chicago. Preparations D2 and D6 were prepared by Dr. E. J. Schantz of Fort Detrick, according to somewhat modified procedures. The toxin content of the preparations was measured by the Oudin single diffusion method in tubes; purity was calculated as the ratio of the specific

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TABLE I. Effect of Intravenous Injection of Enterotoxin on Dermal Reactivity of Rabbits to Epinephrine.

Preparation of enterotoxin	Approx. % purity	μg/rabbit	Total positive /Total sites
8A	20	1	1/2
9B	80	1	1/2
D2	96	1	2/2
D6	96	2	6/7
		.2	3/4
		.02	4/4
Saline		—	0/2

activity thus obtained to that of a highly purified preparation judged by several criteria to represent essentially pure toxin. Albino rabbits, weighing approximately 2 kg, and guinea pigs weighing approximately 200 g, were used. The toxins were given intravenously and epinephrine in aqueous solution was injected into the shaved abdominal skin. Alternatively, toxin was administered intradermally, and after 4 hours epinephrine was administered into the same skin sites. The resulting reactions were read 18 hours after the injections and were considered positive if the lesions measured more than 50 mm^2; the lesions appeared either red or bluish and resembled those observed after injection of endotoxin from gram-negative bacteria and epinephrine(1,3). Lipopolysaccharides were kindly supplied by Professor O. Westphal, Max-Planck Institute, Freiburg, Germany, and by Dr. E. Ribi, Nat. Inst. of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Mont. Pyrogen-free test tubes, pipettes, syringes, and needles were used. Phosphate buffer (pH 7.3) prepared with pyrogen-free distilled water was used as diluent. Representative experiments were carried out in both laboratories with essentially identical results.

Results. In exploratory experiments it was found that intravenous injection of staphylococcal enterotoxin (10 μg/kg) followed immediately by intradermal injection of epinephrine (100 μg) resulted in positive skin reactions at the epinephrine sites in 2 of 11 rabbits. The reactions reached their maximum severity in about 18 hours and were similar to those produced by intradermal injections of staphylococcal extracts and suspensions(1). Subsequent work revealed that, when the interval between injections was lengthened to 18 hours, the incidence of positive reactions was increased. Under these conditions 4 preparations of staphylococcal enterotoxin were all active in eliciting the reaction (Table I). Enterotoxin preparation D6 produced positive reactions in amounts as small as 0.02 μg.

The effects of intradermal injection of enterotoxin were also investigated. Enterotoxin was injected intradermally in a volume of 0.1 ml, and 4 hours later 100 μg of epinephrine (0.1 ml) was injected into the same site. Results obtained with preparation 9B in rabbits are recorded in Table II. Amounts as small as 0.0001 μg elicited positive reactions at 2 of 5 skin sites tested. Six preparations of staphylococcal enterotoxin were all active in producing the reaction (Table III).

The effects of enterotoxin administered intradermally into guinea pigs were also investigated, and the results obtained with 4 preparations of enterotoxin are recorded in Table IV. Enterotoxin in amounts as small as 0.01 μg elicited positive reactions. Intraperitoneal injection of 10 μg of enterotoxin into guinea pigs followed in 18 hours by intradermal injection of epinephrine (10 μg), did not result in positive skin reactions.

Additional studies were carried out to char-

TABLE II. Effect of Enterotoxin 9B on Dermal Reactivity of Rabbit to Epinephrine.

Amt of enterotoxin (μg/0.1 ml intradermally)	No. of skin sites				
	Strongly positive	Moderately positive	Weakly positive	Negative	Total
10	2	0	1	0	3
1	17	5	2	0	24
.1	8	1	1	1	11
.01	4	4	2	1	11
.001	5	2	1	3	11
.0001	2	0	0	3	5

TABLE III. Effect of Intradermal Enterotoxin on Dermal Reactivity of Rabbit to Epinephrine.

Preparation of enterotoxin	Approx. % purity	μg of enterotoxin injected: 10 — No. of dermal sites developing indicated degrees of reaction ++	+	—	1 — ++	+	—	0.1 — ++	+	—
D2	96	2	1	0	22	2	0	9	1	1
7A	20				2	1	0	5	1	0
8A	20				2	0	1	2	1	0
9B	80	3	0	0	3	2	0	7	2	2
D6	96				5	0	4			
94	94	2	0	0	2	0	0			

acterize the altered reactivity to epinephrine elicited by staphylococcal enterotoxin. Intradermal injection of 100 μg of serotonin failed to elicit a reaction in skin sites of 4 rabbits previously injected with 1 μg of enterotoxin 9B. The same animals were then given 100 μg of epinephrine into skin sites prepared in the same way, and 3 of the 4 rabbits developed positive reactions. The results are similar to those obtained previously with staphylococcal extracts(1). The effect of heating the enterotoxin was studied, and it was observed that enterotoxin yielded positive epinephrine reactions in rabbits after it had been held 15 min at 60°C or 100°C.

Antiserum prepared against the highly purified D2 enterotoxin was tested for neutralization of the epinephrine effect. Intravenous or intradermal injection of enterotoxin mixed and incubated with anti-enterotoxin caused epinephrine reactions as did controls without antiserum, although the antiserum contained precipitating antibodies for enterotoxin.

Studies in which more than one skin site per animal were injected with 1 to 10 μg of enterotoxin or diluent and followed after 4 hours by epinephrine revealed that occasional positive reactions occurred at control sites injected with diluent and epinephrine, probably due to spread of toxin. The distance between the toxin sites and the diluent sites, and whether they were on the same or opposite sides of the animal appeared to have no effect on the incidence of reactions. Epinephrine injected immediately after administration of toxin did not produce reactions at control sites.

TABLE IV. Effect of Intradermal Injection of Enterotoxin on Dermal Reactivity of Guinea Pigs to Epinephrine.

Enterotoxin preparation intradermally	Approx. % purity	Total positive/Total guinea pigs: μg/0.1 ml 10	1	0.1	0.01
8A	20	3/6	4/6	1/6	2/6
9B	80	3/6	2/6	3/6	2/6
D2	96	4/6	4/6	3/6	4/6
D6	96	3/6	2/6	1/6	2/6
Saline			0/6		

Local Shwartzman reactions were not observed in a group of rabbits that received intradermal preparatory doses of enterotoxin ranging from 10 μg to 0.01 μg followed after 18 hours by an intravenous provoking dose of 100 μg of enterotoxin. Shwartzman-like reactions were produced in 2 of 4 rabbits when a preparatory dose of 100 μg enterotoxin was given intradermally and a provoking dose of *Escherichia coli* lipopolysaccharide was given intravenously 18 hours later. With a preparatory dose of 10 μg of enterotoxin, one of 10 rabbits gave positive results. None of 4 rabbits gave Shwartzman reactions when the toxins were given in the reverse order.

Rabbits were tested with intradermal enterotoxin and epinephrine, and the following day were injected intravenously with *Salmonella enteritidis* endotoxin (2 μg/kg) and intradermally with epinephrine (100 μg) at a previously uninjected site. Positive reactions with both toxins were obtained in 11 animals, 2 rabbits reacted only to enterotoxin, and 4 rabbits only to endotoxin. These results suggest that the susceptibilities of rabbits to the 2 toxins are independently determined.

Discussion. The present investigation has revealed that partially and highly purified preparations of staphylococcal enterotoxin alter the dermal reactivity of rabbits and guinea pigs to epinephrine. The question arises whether the epinephrine effect is due to enterotoxin itself or to some other component present in the purified preparations. If the epinephrine effect were due to a hitherto unrecognized component of purified enterotoxin, it would have to be present in all preparations examined thus far, and, to account for the high activity, in high concentration. On the other hand, if the epinephrine effect is in fact due to enterotoxin, a new basis will be provided for biological assay of this elusive toxin, and inferences may be drawn regarding its mode of action.

The altered reactivity to epinephrine induced by the enterotoxin preparations in rabbits resembles that produced by staphylococcal extracts, by viable or killed staphylococci, and by endotoxins from gram-negative bacteria. Although similarities are evident in the biological activities of enterotoxin and endotoxin, differences are also apparent. Thus it would appear that although enterotoxin is active as a preparatory dose in the Shwartzman reaction, it differs from endotoxin in being inactive as a provoking dose. The available chemical and physical data also reveal no similarities between classical endotoxin and the staphylococcal enterotoxin, and accordingly it is suggested that the latter product should not be referred to as endotoxin.

Summary. Intravenous injection of 6 preparations of the S-6 type of staphylococcal enterotoxin in rabbits resulted in the appearance of dermal lesions at the site of subsequent intradermal injections of epinephrine. Intradermal injection of staphylococcal enterotoxin followed by administration of epinephrine into the same sites also resulted in the appearance of dermal lesions in both rabbits and guinea pigs. Enterotoxin was effective in amounts as small as 0.0001 μg. The toxic factor was not destroyed by heating for 15 min at 60°C or 100°C. Antiserum failed to neutralize the toxic effect.

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Induced Drinking in Dogs: Comparative Effects of Hypertonic Sodium Chloride and Sorbitol. (27938)

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Work on the hypothalamus of various mammals(1-5) indicates the existence of an area whose stimulation leads to drinking, which is responsive to osmotic stimuli, and whose destruction profoundly inhibits water intake. Substances which remain osmotically

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