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THE EFFECT OF ENTEROTOXIN ON DERMAL REACTIVITY TO EPINEPHRINE

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Fort Detrick, Maryland

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V. Bokkenheuser
M.A. Cardella
E.A. Gorzynski
G.G. Wright
E. Nater

Medical Investigation Division
DIRECTOR OF MEDICAL RESEARCH

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Three of the authors of this report, V. Bokkenheuser, E.A. Gorzyski, and E. Neter, are associated with the Departments of Bacteriology and Pediatrics, University of Buffalo Medical School; and the Laboratory of Bacteriology, Children's Hospital, Buffalo, New York. G.G. Wright and M.A. Cardella are at Biological Laboratories, Fort Detrick.

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ABSTRACT

Intravenous injection of six 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 micrograms. The toxic factor was not destroyed by heating for 15 minutes at 60° or 100°C. Antiserum failed to neutralize the toxic effect.

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TABLES

I. INTRODUCTION

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 ten-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 of the rabbit to epinephrine.

II. 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 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 four 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, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana. 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.

III. 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 inflammatory 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, if the interval between injections was increased to 18 hours, the incidence of positive reactions was increased. Under these conditions four preparations of staphylococcal enterotoxin were all active in eliciting the reaction, as shown in Table I. Enterotoxin preparation D6 produced positive reactions in amounts as small as 0.02 micrograms.

TABLE I. EFFECT OF INTRAVENOUS INJECTION OF ENTEROTOXIN ON THE DERMAL REACTIVITY OF RABBITS TO EPINEPHRINE

Preparation of Enterotoxin	Approx. Purity	Micrograms per Rabbits	Total Positive / Total Sites
8A	20%	1	1/2
9B	80%	1	1/2
D2	96%	1	2/2
D6	96%	2	6/7
		0.2	3/4
		0.02	4/4
Saline		-	0/2

The effects of intradermal injection of enterotoxin were also investigated. Enterotoxin was injected intradermally in a volume of 0.1 milliliter, and four hours later 100 micrograms of epinephrine (0.1 ml) was injected into the same site. Results obtained with preparation 9B in rabbits are recorded in Table II. It is evident that amounts as small as 0.0001 microgram elicited positive reactions at two of five skin sites tested. Six preparations of staphylococcal enterotoxin were all active in producing the reaction, as shown by the results recorded in Table III.

TABLE II. EFFECT OF ENTEROTOXIN 9B ON DERMAL REACTIVITY OF RABBIT TO EPINEPHRINE

Amount of Enterotoxin, μg/0.1 ml intradermally	Number of Skin Sites				
	Strongly Positive	Moderately Positive	Weakly Positive	Negative	Total
10	2	0	1	0	3
1	17	5	2	0	24
0.1	8	1	1	1	11
0.01	4	4	2	1	11
0.001	5	2	1	3	11
0.0001	2	0	0	3	5

TABLE III. EFFECT OF INTRADERMAL ENTEROTOXIN ON DERMAL REACTIVITY OF RABBIT TO EPINEPHRINE

Preparation of Enterotosin	Approx. % Purity	Micrograms of Enterotoxin Injected								
		10			1			0.1		
		No. of dermal sites developing indicated degrees of reaction								
		++	+	-	++	+	-	++	+	-
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			

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

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 — Micrograms/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		

Additional studies were carried out to characterize the altered reactivity to epinephrine elicited by staphylococcal enterotoxin. Intradermal injection of 100 micrograms of serotonin failed to elicit a reaction in skin sites of four rabbits injected previously with one microgram of enterotoxin 9B. The same animals were then given 100 micrograms of epinephrine into skin sites prepared in the same way, and three of the four rabbits developed positive reactions. The results are similar to those obtained previously with staphylococcal extracts.[2] 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 minutes at 60° 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 antienterotoxin 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 one to ten micrograms of enterotoxin or diluent and followed after four 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.

Local Shwartzman reactions were not observed in a group of rabbits that received intradermal preparatory doses of enterotoxin ranging from 10 to 0.01 micrograms, followed after 18 hours by an intravenous-provoking dose of 100 micrograms of enterotoxin. Shwartzman-like reactions were produced in two of four rabbits when a preparatory dose of 100 micrograms of enterotoxin was given intradermally and a provoking dose of Escherichia coli lipopolysaccharide was given intravenously 18 hours later. With a preparatory dose of ten micrograms of enterotoxin, one of ten rabbits gave positive results. None of four 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 (two micrograms/kilogram) and intradermally with epinephrine (100 μg) at a previously uninjected site. Positive reactions with both toxins were obtained in 11 animals, two rabbits reacted only to enterotoxin, and four rabbits only to endotoxin. These results suggest that the susceptibilities of rabbits to the two toxins are independently determined.

IV. 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. In addition, the relative heat stability of the epinephrine effect suggests association with enterotoxin rather than the other toxins of staphylococcus. 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.

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