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Epinephrine Induced Dermal Necrosis in Endotoxemia: Attempts to Define Response on an Immunological Basis.* (31939)

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Rabbits given endotoxin intravenously develop hemorrhagic necrosis at the site of a subsequent intradermal injection of epinephrine. Resistance to the local effect of epinephrine has followed an 8-day course of endotoxin administered intravenously(1). Because the nature of acquired resistance to endotoxin is poorly understood, the present study involved attempts to explain along immunological lines the hemorrhagic dermal necrosis resulting from endotoxin and epinephrine. Since active immunization of rabbits with endotoxin prevents dermal necrosis by epinephrine it appeared feasible to attempt a similar resistant state in susceptible rabbits by the transfer of whole serum or serum fractions from endotoxin-resistant rabbits. It has been reported that the pyrogenic response to endotoxin could be prevented in susceptible rabbits by the transfer of serum fractions from resistant animals(2). In addition, it was desirable to reexamine the reproducibility of the hemorrhagic response to epinephrine in rabbits given endotoxin, and the duration of resistance to epinephrine in animals given repeated doses of endotoxin.

Materials and methods. One kg female New Zealand white rabbits, 6-8 weeks old, were used throughout. A single lot of *Escherichia coli* endotoxin prepared in our laboratory, as previously described, was used in all experiments(3). For challenge, the desired quantity of endotoxin was injected into a marginal ear vein. Immediately thereafter, 0.1 ml epinephrine 1:1000 (aqueous) was injected intradermally into the rabbit's shaved flank. The site of epinephrine injection was examined at 24 hours for hemorrhagic necrosis. Negative reactions consisted of a 3 × 3 mm white or pink spot. Positive (+) reactions appeared as larger patches of purple discoloration; reactions measuring 6 cm^2 or greater were considered strongly positive (++). *Active desensitization.* Rabbits were given daily injections of endotoxin into the marginal ear vein as previously described(1): 1 μg days 1-4, 10 μg days 5-8. All animals were challenged 24 hours following the final injection. Serum was obtained by heart puncture just prior to challenge and frozen. Control animals were given either no injections prior to challenge or injections containing equivalent volumes of sterile, non-pyrogenic

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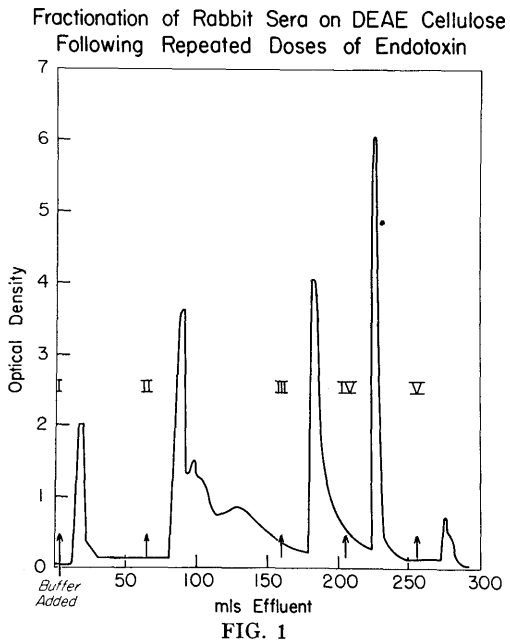

FIG. 1

saline. *Preparation of DEAE fractions.* Serum from animals given repeated doses of endotoxin was fractionated on columns containing 100 g DEAE cellulose and run at 4°C. One hundred ml serum were added to each column and eluted stepwise with the following phosphate buffers, each run until protein-free: (I) 0.015 M, pH 6.3; (II) 0.04 M, pH 6.0; (III) 0.1 M, pH 5.8; (IV) 0.3 M, pH 5.5; (V) 0.4 M + 2 M NaCl, pH 4.3. Eluates were collected at 60 ml per hour in 20 ml aliquots and assayed for protein concentration in a Beckman spectrophotometer at 280 mμ. Each buffer yielded a discrete protein peak. Aliquots within each peak were pooled and concentrated by pervaporation while being dialyzed against 0.15 M NaCl. Each buffer thus yielded a corresponding protein fraction for injection (Fig. 1). Material from each of these fractions was analyzed by micro-immunoelectrophoresis by the method of Scheidegger(4), using both goat anti-rabbit-gamma-globulin and goat anti-rabbit-whole serum (Hyland).

Results. Normal rabbits were challenged intravenously with varying doses of endotoxin followed by 100 μg epinephrine intradermally (Table I). A positive response was consistently obtained with 10 μg endotoxin or greater. Response to 1-5 μg varied from animal to animal and from day to day of testing. Ten μg endotoxin and 100 μg epinephrine were chosen in subsequent experiments as the standard challenge. Although less consistent, the magnitude of response was also noted to correlate with the amount of endotoxin given. No rabbit given epinephrine intradermally alone developed a positive reaction.

Active desensitization. Nineteen of 22 control animals challenged with endotoxin and epinephrine developed lesions, as opposed to 2 of 25 desensitized animals. To study the duration of the desensitized state, 9 rabbits were desensitized. All were negative to challenge 24 hours after the last desensitizing dose. Six days later, 4 animals were rechallenged. All developed positive reactions, as did all of 4 controls. Challenge with a lower dose of endotoxin (1 μg) produced lesions in neither the remaining 5 desensitized animals nor a group of 4 controls. No residual resistance was thus demonstrated 6 days after desensitization. In an attempt to demonstrate an accelerated secondary return of the desensitized state 13 rabbits were desensitized as above, and proved negative to standard challenge 24 hours later. Twenty days following desensitization, 8 of these animals were begun on a second course of intravenous endotoxin, receiving 10, 10, 30, and 50 μg on successive days. Twenty-four hours following this final booster dose, all 13 animals were challenged as before. Of 5 animals receiving only the primary course of desensitization, all gave positive skin tests. Of those 8 receiving the secondary course as well, 3 gave negative tests. But this is no more than would have become negative after a primary course of 4 injections, as shown by earlier

TABLE I. Titration of Endotoxin in Relationship to Subsequent Dermal Response to 100 μg Epinephrine.

Endotoxin dose (μg)	Total No. rabbits	Dermal response		
		0	+	++
0	3	3	0	0
1	14	5	6	3
10	12	0	6	6
>10	13	1	1	11

TABLE II. Dermal Response to Endotoxin and Epinephrine in Rabbits Given Serum Fractions from Endotoxin-Resistant Animals.

Serum fraction transferred	Total No. rabbits	Dermal response		
		0	+	++
Control	8	1	0	7
I	6	3	3	0
II	6	3	2	1
III	6	4	2	0
IV	6	3	2	1
V	5	3	1	1

experiments. Thus no accelerated secondary immune response to endotoxin could be demonstrated.

Passive desensitization. Seven normal rabbits were each injected intravenously with 10 ml serum from desensitized rabbits. These animals as well as 6 injected with 10 ml saline and 3 given 10 ml normal rabbit serum, received the standard challenge of epinephrine and 10 μg of endotoxin in 24 hours. All animals gave positive reactions, thus showing no transfer of resistance. When a similar experiment was performed using only 1 μg endotoxin for challenge, all 5 animals receiving serum from resistant rabbits as well as all 5 controls gave positive reactions. By preparing serum fractions, transfer of a greater quantity of each class of immune globulin was obtained than when whole serum was used. Two hundred ml serum from resistant rabbits were thus subjected to DEAE fractionation as described above. Each fraction obtained was divided into 6 portions, each portion injected intravenously into a normal rabbit, and the rabbit challenged as usual in 24 hours, using 10 μg/kg as the endotoxin dose. No consistent pattern of protection could be detected (Table II). To determine its immunoglobulin content, each serum fraction was subjected to immunoelectrophoresis. Detectable IgG was limited to fraction I; IgA to fraction II; and IgM to fraction III, with perhaps a trace still present in fraction IV.

Discussion. In the original description of hemorrhagic necrosis produced by endotoxin and epinephrine, resistance to the reaction was shown to follow an 8-day course of intravenous endotoxin(1). Tolerance to the pyrogenic effect of endotoxin could similarly be induced by a course of desensitization(5). This febrile tolerance was abolished by blocking the reticuloendothelial system with thorotrast(6). Such tolerance correlated poorly with detectable levels of circulating O-agglutinins(7) or antibodies bactericidal for the parent Gram-negative organism(8). Resistance to endotoxin appeared to represent an alteration in the reticuloendothelial system rather than the presence of a circulating antibody. However, recent studies on the febrile response to endotoxin in rabbits revealed that tolerance could be transferred with a fraction of serum from tolerant animals rich in 19S gamma globulin(2). Further purification of this fraction yielded a product conferring resistance to the lethal effect of endotoxin as well. The status of endotoxin resistance as an immunological phenomenon was thus again brought into question.

The present findings confirm the reproducibility of the skin reaction in response to epinephrine and endotoxin, and failure to induce the response by intradermal epinephrine alone. While this would appear to be a reliable method for detecting amounts of endotoxin in concentrations greater than 10 μg, as previously observed(9), evidence has been brought forth stating that serotonin can be substituted for endotoxin in the production of hemorrhagic necrosis(10). The pharmacologic specificity of the reaction clearly demands further study. This type of assay for detecting the presence of endotoxemia in patients with suspected endotoxin shock has been questioned(11).

The efficacy of active desensitization by successive daily endotoxin injections is also confirmed. The nature of the desensitized state, however, remains in doubt. The present study demonstrates both the brief duration of the desensitized state and the absence of an accelerated response to a secondary course of desensitization at 24 days. The former observation is consistent with an earlier finding(5) that tolerance to the pyrogenic effect of endotoxin had diminished by 7 days and disappeared by 21 following a course of desensitization. The latter, however, differs from a more recent study(12) in which, 35 days following a course of active desensitiza-

tion, administration of one further desensitizing dose of endotoxin brought about the return of "nearly complete tolerance" to its febrile effect at subsequent challenge. This discrepancy may be due to a fundamental difference in the reactions employed in the two studies, or to a difference in the sensitivity with which they can be observed. The present observations suggest that at least one parameter of endotoxin tolerance may differ from classical immune systems in both its duration and its susceptibility to recall.

While the pyrogenic effect of endotoxin can be prevented by the passive transfer of serum or serum fractions from rabbits given repeated doses of endotoxin it is difficult to explain why epinephrine induced dermal necrosis could not be abolished in a similar way. If protection against the pyrogenic response represents the action of antibody, it is possible that larger amounts of antibody are needed at the local site to protect against hemorrhagic necrosis. The present study points out that resistance to dermal necrosis differs in an unknown manner from resistance to endotoxin-induced fever or the lethal effect of endotoxin, both of which can be prevented by passive transfer of serum.

Summary. Several aspects of hemorrhagic necrosis of rabbit skin following intravenous injection of endotoxin and intradermal administration of epinephrine have been studied. The reaction was consistently reproduced using 100 μg epinephrine and 10 μg endotoxin. It could be abolished by an 8-day course of intravenous endotoxin as previously demonstrated. No resistance to the reaction could be shown to persist 6 days following immunization. No accelerated response to a booster course of endotoxin injections could be demonstrated during the fourth week following the primary injections. Hemorrhagic necrosis could not be prevented by the passive transfer of 10 ml of whole serum, or with serum fractions separated by DEAE cellulose chromatography and rich in the individual classes of immune globulins. The specificity of dermal necrosis induced by epinephrine in endotoxemia remains unclear. It is not possible at this time to define the nature of the temporary protection against epinephrine on an immunological basis.

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Intensification of Experimental Atherosclerosis by Semi-Starvation Diet.* (31940)

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It is well known that addition of 1% cholesterol to the diet of the rabbit produces extensive deposits of atherosclerotic plaques in thoracic aorta within about 12 weeks. It has been shown previously(1,2), that there is little or no regression of the preformed plaques when the animals are returned to normal diets. It was of interest therefore to test the influence of a semi-starvation regime on regression of the plaques. It was found

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