Effects of Lipoid A Component of *Escherichia coli* Endotoxin on Dermal Reactivity of Rabbits to Epinephrine.* (25669)

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The endotoxic complex of Gram-negative bacilli is composed of polysaccharide, lipoid, and protein. Two lipoid components, A and B, have been isolated(1). The latter is easily extractable from the endotoxin by fat solvents. In contrast, lipoid A is firmly bound to polysaccharide (lipopolysaccharide). In experimental animals the endotoxic complexes and the purified lipopolysaccharides derived therefrom produce the well known nonspecific effects, such as fever, Shwartzman reaction etc.(2-6). It is the polysaccharide moiety of the complex which is responsible for serologic specificity. Recent studies(7) strongly suggest that toxicity and pyrogenicity of the endotoxins are due to the lipoid A component. Evidence for this conclusion was obtained by demonstration that artificial complexes made up of lipoid A and an inert carrier, such as casein, exert these biologic effects. The inert carrier merely renders water-soluble the otherwise poorly soluble lipoid A, a function which in the natural endotoxic lipopolysaccharide complex is performed by the polysaccharide moiety. Westphal *et al.*(7) isolated from *E. coli* endotoxin the lipoid A component in aqueous solution of varying degrees of dispersion. The acute endotoxic activity of lipoid A depends largely upon degree of dispersion, being maximal in the original endotoxic complex. Lipoid A also markedly enhances nonspecific resistance of mice to experimental infection(7). The present experiments revealed that purified lipoid A, like the lipopolysaccharide complex, strikingly alters dermal reactivity of rabbits to epinephrine.

Materials and methods. Highly purified lipopolysaccharide from *E. coli* 0111:B4 was prepared according to the method described previously(8). Lipoid A was isolated from the lipopolysaccharide by hydrolysis and further purified by fractionation with organic solvents. The purified product, a white powder with a melting point of 180-82°C, was free of polysaccharide constituents. It contained 19% of glucosamine(1) and gave the following analytical figures: C 61.1, H 9.5, N 2.7, P 1.9, (C)-CH_3 4.1%, respectively. A solution of lipoid A in pyridine was added to an equal volume of 0.5% low molecular dextran. The mixture was concentrated under reduced pressure until completely free of pyridine and enough water was added to give a final concentration of 1000 μg/ml of lipoid A in 0.5% dextran. This solution was kept at 4°C. Immediately prior to injection into rabbits, the appropriate dilution was prepared aseptically in phosphate buffer (pH 7.3; Difco). The dextran diluent and the lipopolysaccharide, from which lipoid A was prepared, were used in parallel experiments. Lipoid A, lipopolysaccharide, or dextran diluent, in appropriate dilutions, was injected intravenously into white female rabbits, weighing between 1.7 and 2.3 kg. Within a few minutes thereafter epinephrine (in aqueous solution or peanut oil) as well as saline solution, in amounts of 0.1 ml, were injected into the shaved abdominal skin at different sites. At intervals for several days, the dermal lesions were measured and graded according to intensity as strongly positive, weakly positive, or negative; presence or absence of hemorrhages was recorded.

Results. The alteration of dermal reactivity to epinephrine effected by intravenously injected lipoid A, lipopolysaccharide, and 0.5% dextran is shown in Table I.

Of 71 rabbits which received from 2 to 50 μg/kg of lipoid A intravenously, 26 gave strong reactions at site of epinephrine in water (100 μg) and 40 animals at that of epinephrine in oil (200 μg). Lipoid A in effective doses caused strongly positive reactions to both aqueous epinephrine and epinephrine in

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TABLE I. Effects of *E. coli* Lipoid A and Lipopolysaccharide on Dermal Reactivity of Rabbits to Epinephrine.

Materials and dosage	Epinephrine (100 μg) Strongly positive	Weakly positive	Negative	Epinephrine in oil (200 μg) Strongly positive	Weakly positive	Negative	Total
Lipoid A							
50 μg/kg	2	2	4	6	1	1	8
20	19	10	8	22	10	5	37
5	1	3	10	5	4	5	14
2	4	8	0	7	5	0	12
.5	0	0	4	0	1	3	4
.2	0	0	8	1	2	5	8
Lipopolysaccharide							
2 μg/kg	3	1	0	3	0	1	4
.2	3	2	0	5	0	0	5
.05	0	0	2	1	1	0	2
.02	0	0	6	0	0	6	6
Diluent for Lipoid A							
1:20*	0	2	2	0	0	4	4
1:50	0	3	12	0	4	11	15
1:200	0	0	5	0	0	5	5
1:200	†	†	†	0	0	3	3

* Equivalent to 50 μg/kg of Lipoid A. † Not done.

oil in many animals. A few rabbits, however, gave such reactions to only one or the other. None of the 27 animals injected with dextran had strongly positive reactions. Numerous other rabbits injected intravenously with phosphate buffer, used as diluent, (not shown in Table) failed to react to epinephrine, indicating that endotoxic lipopolysaccharide was not encountered as a contaminant. Weakly positive reactions were seen in rabbits injected with the dextran diluent in amounts equivalent to 20 and 50 μg/kg of lipoid A, but not in amounts equivalent to 5 μg/kg. Therefore, weakly positive reactions obtained in 11 rabbits that were given 5 μg/kg of lipoid A or less, appear to be significant. Epinephrine in amounts of 10 and 1 μg failed to elicit the reaction in rabbits injected with lipoid A in effective doses. It is evident, therefore, that lipoid A obtained from *E. coli* 0111:B4 markedly alters dermal reactivity of rabbits to epinephrine.

The original lipopolysaccharide, from which the lipoid A fraction was obtained, similarly alters reactivity to epinephrine. Statistical analysis (courtesy of Miss V. Pessin) revealed that the lipopolysaccharide is approximately 10 times more effective than lipoid A, a finding that agrees with the observation on relative pyrogenicity of these compounds(7).

On gross examination the epinephrine lesions following intravenous injection of lipoid A (20 and 50 μg/kg) were red or brown in approximately 25% of the animals and hemorrhagic in 75%. Typical reactions are shown in Fig. 1. The mean size was 16 x 53 mm, with a range of 10 to 30 x 20 to 90 mm. No difference was noted in appearance of reactions between aqueous epinephrine and epinephrine in oil. Hemorrhages were seen less frequently in animals receiving smaller amounts of lipoid A.

The question arises as to whether the epinephrine lesion is due to localization of intravenously administered lipoid A in the skin injected with epinephrine. To test this hypothesis, 5 rabbits were injected intravenously with lipoid A (20 μg/kg) and intracutaneously at different sites with epinephrine, lipoid A (2 μg/kg), and saline. Lesions did not develop at site of intradermally administered lipoid A.

The possibility was considered that shaving may contribute to development of the epinephrine reaction. Several rabbits were injected with lipoid A intravenously and with epinephrine into the unshaved abdominal skin. On the following day, when the results were determined, the skin was shaved. Marked reactions were present. These findings indicate that shaving is not the determinant factor.

Five rabbits were injected intravenously

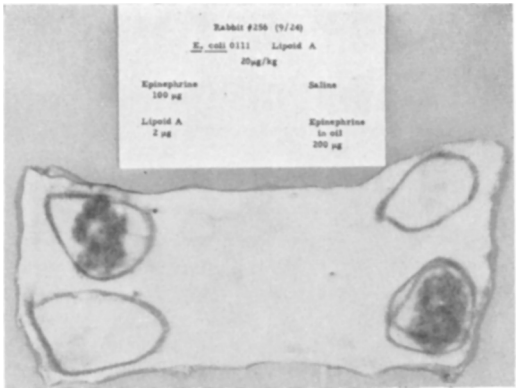

FIG. 1. Effect of lipoid A on dermal reactivity to epinephrine.

with 0.5 mg/kg of the adrenergic blocking agent phenoxybenzamine hydrochloride (Dibenzyline), and 5 control rabbits with saline; 6 hours later all animals received lipoid A (20 μg/kg) intravenously and epinephrine intradermally. No reaction whatever occurred in the former animals, whereas 4 out of 5 control rabbits showed marked lesions. It is concluded, therefore, that phenoxybenzamine hydrochloride inhibits dermal reactivity to epinephrine in rabbits injected with lipoid A.

Discussion. The present investigation has revealed that the lipoid A component of endotoxin obtained from *E. coli* 0111:B4, upon intravenous injection, alters dermal reactivity of rabbits to epinephrine. The lipoid A component is active in amounts as small as 2 to 5 μg/kg. Its potency appears to be approximately one-tenth of that of the original lipopolysaccharide. A similar 10-fold difference in pyrogenic potency in horses was observed with the same preparations(7). The observation on alteration of dermal reactivity of rabbits to epinephrine by intravenously injected endotoxin confirms the findings of Thomas (9). The question remains as to whether chemical compounds of the endotoxic complex other than lipoid A are responsible for some of the numerous nonspecific biologic effects, and/or whether the other components of the complex, such as the polysaccharide, merely affect physico-chemical characteristics of lipoid A. The difference in activity between lipoid A and lipopolysaccharide may be due to differences in degree of dispersion of lipoid A component or chemical alterations (hydrolysis etc.) taking place during preparation of the latter. It is conceivable that in the natural lipopolysaccharide complex lipoid A is better dispersed than in the preparation dissolved in dextran. The findings reported here further support the thesis that at least some of the toxic effects of endotoxins from Gram-negative bacilli are mainly due to lipoid A component. Recently, Ribi and co-workers isolated an aqueous ether extract from *Salmonella enteritidis* which had the biologic properties of a Boivin-type antigen, yet had an exceptionally low lipoid content(10).

The pyrogenic effect of lipoid A and of lipopolysaccharide is abolished by horse serum (11,12). This inactivation is not due to antibody. It remains to be ascertained whether serum also inhibits the effects of lipoid A on reactivity of rabbits to epinephrine. Zweifach and associates(13) observed that endotoxin alters reactivity to epinephrine of vessels of the isolated rabbit ear and of the mesoappendix of the rat. On the other hand, Meyer and Ballin(14) found only slight potentiation of epinephrine activity after small doses of endotoxin and then only if barbiturate, but not ether anesthesia, was employed. The question arises as to whether endotoxin and lipoid A affect different vascular systems differently. For example, in our own laboratories we have failed to elicit the epinephrine reaction in the ear of the rabbit following intravenous injection of a potent staphylococcal toxin or of *E. coli* lipopolysaccharide, although these preparations were highly effective in producing the epinephrine lesion in the skin of the abdomen and back.

Of great interest are the observations of Gatling(15), that epinephrine elicits a striking local reaction in hypersensitive rabbits, provided both antigen and antibody are present in the circulation. It will be of interest to determine whether pre-treatment of animals with lipoid A alters this reactivity. The possibility may be considered that the remarkable reactivity of animals and man to minute amounts of endotoxins may be related to previous exposure, under natural conditions, to materials containing lipoid A, resulting in altered reactivity of certain tissues to endotoxins and their lipoid A component.

Summary. The purified and polysaccharide-free lipoid A component of *E. coli* 0111: B4 endotoxin, upon intravenous injection into rabbits, strikingly alters dermal reactivity to epinephrine. Grossly, the lesions thus produced are red or brownish in appearance or show marked hemorrhages. Lipoid A is active in amounts as small as 2 μg/kg. The original lipopolysaccharide is approximately 10 times more potent than the lipoid A fraction obtained therefrom. Phenoxybenzamine hydrochloride inhibits the epinephrine reaction in rabbits injected with lipoid A.

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Accumulation of Dermal Mucopolysaccharides in Animals Following Injection of Estradiol Benzoate.* (25670)

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It has been demonstrated that following administration of estrogenic hormones the amount of ground substance mucopolysaccharides can be increased in immature monkeys of both sexes(1,2). Similar effects were obtained by estradiol following local application of the hormone on skin of rhino and hairless mice(3), and in mice injected subcutaneously with this hormone(4). That part of this increase in dermal ground substance is due to hyaluronic acid or chondroitin sulfate was demonstrated by Schmidt(5). This paper presents the effect of a single subcutaneous inoculation of estradiol benzoate on dermal mucopolysaccharides of mice, rats, guinea pigs and hamsters.

Materials and methods. Male and female white Swiss mice weighing 12 to 15 g and 20 to 22 g respectively, female albino rats weighing 60 to 75 g, female Hartley line guinea pigs weighing 225 to 275 g and female Golden hamsters weighing 60 to 75 g were studied. Animals were obtained from local dealers. Mice, rats and hamsters were fed Purina lab chow while guinea pigs were fed Purina rabbit chow and lettuce *ad lib.* Animals had free access to water. Test animals received a single subcutaneous injection of 0.1 ml of a suspension of estradiol benzoate in sesame oil in inguinal region. Control animals received 0.1 ml of sesame oil. At 2 day intervals beginning with zero days and usually extending through the 21st, duplicate animals were sacrificed; mice by severing cervical cord, other animals by ether. Fur was initially removed

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