response occurs. In human infection which is usually inapparent, the introduction of virus into the skin is followed by proliferation of virus outside of the CNS but is rarely followed by CNS disease(10). More than half of those who do develop encephalitis recover. A significant factor in resistance to CNS invasion and in recovery after encephalitis is thought to be specific AB response. If so, administration of AB followed by exposure to CO_2 would augment the natural immune response and might be more effective in the human than in the experimental disease. That some AB treated mice did recover after development of CNS symptoms indicates that the precarious balance between host and parasite can be influenced favorably even when the CNS is invaded. Further experimental study of combined CO_2 AB therapy with an experimental disease more like human arborvirus encephalitis is desirable.

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Effect of *Salmonella enteritidis* Endotoxin Preparations and Lipid A on Dermal Reactivity in Rabbits to Epinephrine.* (28013)

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Although the biologic effects of bacterial endotoxins have been studied extensively, information on chemical and/or physical determinants of the endotoxin complex remains limited. Westphal and associates(1) have suggested that the lipid A portion of the complex may be responsible for some or all of the biologic effects (*e.g.,* fever, enhancement of resistance to experimental infection) other than antigenicity. The lipid A component of phenol-extracted *Escherichia coli* endotoxin also produces necrosis of sarcoma 180 in mice and alters dermal reactivity in rabbits to epinephrine(2,3). However, the lipid A was only approximately 1/10 as active as the original lipopolysaccharide from which it was isolated. The aqueous ether extraction method to prepare endotoxin from *Salmonella enteritidis* has yielded products of high activity and it has been shown that the biologic activity of these products does not bear a direct relationship to their lipid A content(4-6). It was suggested that the configuration of the entire complex plays an important role in the elicitation of host reactivity. The present study was undertaken to determine on a comparative basis the po-

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TABLE I. Effects of Endotoxin and Lipid A from *S. Enteritidis* on Dermal Reactivity of Rabbits to Epinephrine.

Dose, μg/kg	Preparations: No. 2 (Se 155A) Aqueous ether extract (25°C) Lipid A: 17.6% Total FA: 10.8% N: 2.3%	No. 1/10 (Se 154A) Aqueous ether extract (12°C) Lipid A: 4.9% Total FA: 8.3% N: 2.6%	No. 13 (Se 197C/203Ae) Aqueous ether extract phenol-water treated FA: 3.45% N: .50%	No. 15 (Se 232/241P) Aqueous ether extract phenol-water treated FA: 4.65% N: .58%	No. 16 (Se 232/241 P$_2$A1) A1-citrate complex of No. 15 FA: 2.6 % N: .35%	No. 6 (Se 162/168 IIIB) Lipid A prepared from *S. ent.* aqueous ether extract	Number of positive rabbits/Total
10	16/16	13/14	2/2	–	–	–	6/12
2	4/4	8/8	3/3	–	–	–	1/4
.4	4/4	4/4	4/4	11/11	6/6	–	
.08	4/4	7/8	3/6	3/6	3/6	–	
.016	4/4	2/4	2/4	5/9	2/10	–	
.0032	4/8	4/8	1/4	0/8	1/8	–	
.0016	2/4	2/4	–	–	–	–	
.0008	0/4	2/4	–	–	–	–	
.0006	–	0/6	–	0/4	0/4	–	
.0004	–	0/6	–	–	–	–	
ED_{50} (μg)*	.003	.003	.03	.03	.04	.04	8

* Determined by the method of Reed and Muench(9).

tency of lipid A and endotoxins from *S. enteritidis* with varying content of lipid A to alter the dermal reactivity in rabbits to epinephrine.

Materials and methods. The methods used for isolation and characterization of various endotoxic preparations have been described (4-7). Albino rabbits, weighing approximately 2 kg, were employed. Endotoxins in suitable dilution were injected intravenously, and immediately thereafter aqueous epinephrine (100 μg in 0.1 ml) and buffer as controls were injected intradermally at different sites into the shaved abdominal skin. Skin reactions, present 18 hours after injections, were considered to be positive if they were larger than 25 mm^2. In all experiments pyrogen-free phosphate buffer (pH ~7.3; Difco) was used as diluent, and pyrogen-free needles and syringes were employed.

Results. Results of tests to determine the ability of several endotoxins and of a lipid A preparation from *S. enteritidis* to alter dermal reactivity to epinephrine are recorded

TABLE II. Alteration of Dermal Reactivity to Epinephrine of Rabbit and Tumor Damage in Mice by Endotoxins and Lipid A from *S. Enteritidis* and *E. Coli.*

Preparations	Epinephrine reaction (ED_{50}) (μg)	Tumor damage (TD_{50}) (μg)
E. coli		
Phenol-water endotoxin	.2*	20†
Lipid A	2*	50†
S. enteritidis		
Aqueous ether endotoxin (No. 2)	.003‡	–
" " " (No. 1)	.003‡	.24§
" " " after extraction with phenol-water (No. 13)	.03‡	.30§
" " " " " " " " (No. 15)	.03‡	.15§
" " " " " " " " (No. 16)	.04‡	.23§
Lipid A (No. 6)	8‡	50§

* Data from (2).
† " " (3).
‡ " " Table I.
§ Median effective doses for producing hemorrhagic necrosis in Sarcoma 37 implants in mice determined by Dr. Maurice Landy by the method of Leiter *et al.*(8).

in Table I. Aqueous ether extracts were highly active and more potent than similar preparations which had been treated with phenol-water. The lipid A was of low potency; its activity was approximately 1/200 that of phenol-water treated aqueous ether extracts and 1/2000 that of the original aqueous ether extracts.

Discussion. The present investigation has revealed that endotoxin from *S. enteritidis* obtained by aqueous ether extraction is highly effective in altering dermal reactivity in rabbits to epinephrine. The ED_{50} dose of intravenously injected endotoxin is as low as 0.003 μg. Phenol-water treatment of these preparations results in decreased activity, and lipid A itself was of low potency.

From previous investigations information is available on the tumor damaging activity in mice of identical *S. enteritidis* preparations and of the biologic activities of *E. coli* endotoxin obtained by phenol-water extraction and its lipid A component. These data, together with the findings recorded here, are summarized in Table II.

It is evident that aqueous-ether extracted endotoxin from *S. enteritidis* is considerably more active in producing epinephrine reaction and tumor damage than the phenol-water extracted *E. coli* endotoxin tested. In addition, phenol-water treatment of the aqueous-ether endotoxin reduces its ability to alter dermal reactivity to epinephrine but not its tumor damaging effect. There can be little doubt that lipid A from either *E. coli* or *S. enteritidis* causes both epinephrine reaction and tumor damage as do the parent endotoxins; however, the 2 types of endotoxins differed significantly in potency in these tests whereas the lipids possessed the same order of activity. So far as aqueous-ether extracted endotoxin from *S. enteritidis* is concerned, it is clear that it is approximately 2,000 times more effective than its lipid A component in altering epinephrine reactivity and approximately 200 times more potent in producing tumor damage. The difference in potency of the phenol-water extracted *E. coli* endotoxin and its lipid A component is of considerably smaller order of magnitude. Clarification of the role of lipid A in endotoxic activity must await future investigation but, so far as ether extracted *S. enteritidis* endotoxin is concerned, lipid A accounts for only a minor part in altering dermal reactivity in rabbits to epinephrine or in producing tumor damage in mice.

Summary. Minute amounts of aqueous-ether extracted *S. enteritidis* endotoxin, upon intravenous injection into rabbits, alter dermal reactivity to intradermally injected epinephrine. The lipid A component is only

approximately 1/2000 as active as the endotoxin when potent endotoxins are tested. Phenol-water treatment of aqueous-ether extracted endotoxin reduces this activity.

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