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Detoxification of Bacterial Endotoxin with Retention of Ability to Stimulate Non-Specific Resistance to Infection. (26766)

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The highly pyrogenic and toxic endotoxins elicit, at microgram doses, a wide spectrum of biological effects including stimulation of non-specific host resistance to infection(1-3). Attempts at dissociation of *in vivo* properties of endotoxin by chemical modification, largely by hydrolytic measures, have been unsuccessful. This preliminary report of our studies on chemical modification of endotoxin, utilizing reactions resulting in substitution at functional groups known to be present in the molecule, describes a fraction isolated after acylation which is grossly detoxified but retains quantitatively the ability to stimulate resistance to infection with heterologous microorganisms. Recovery of original pyrogenicity and acute toxicity by mild saponification, failure to confer tolerance to the original endotoxin, degree of immunogenicity, retention of tumor-necrotizing activity, as well as the properties of other fractions isolated, will be reported separately. While our manuscripts were in preparation Noll and Braude(4) reported detoxification of endotoxin by reductive cleavage of ester bonds, yielding a product of high immunogenic potency.

Materials and methods. In all procedures, preparative and testing, usual precautions to avoid pyrogen contamination were observed. Preparation was accomplished by adding 25 ml acetic anhydride to 100 mg *S. typhosa* 0-901 endotoxin (Difco) and 10 mg anhydrous sodium acetate, and heating in a boiling water bath for 2 hours. After cooling, the anhydride was removed as a clear solution containing approximately 30% of the starting material, by centrifugation. The residue was dried under N_2 to remove all anhydride, washed 3 times with pyrogen-free saline and finally with water, the washes removing a fraction (20%) yielding a fine suspension like the original endotoxin. The washed residue (50% yield), which is the subject of this report, was dried under N_2 and ground to a fine powder in a mortar. This lyophobic fraction was triturated with small amounts of pyrogen-free saline, then diluted to desired concentrations. Acetyl content, by alkaline hydrolysis, was 12% compared to 1.6% for the original endotoxin.

Tests for acute toxicity and protection against infection were done with ICR mice (Bellewood) weighing 16-18 g. For pyrogen tests 2-2.5 kg male rabbits of mixed breed were used. Dosage, routes of administration, and time schedules are given below. Strains of *Pseudomonas aeruginosa* and *Escherichia coli* from our culture collection were employed in the resistance studies described below. Both organisms were grown in brain heart infusion broth (Difco) for 18 hours at 35°C and appropriately diluted in sterile broth prior to injection of 0.25 ml *i.p.* The *E. coli* inoculum consisted of approximately 2×10^8 viable cells per ml while the *Ps. aeruginosa* viable count amounted to about 6×10^8 cells per ml.

Results. The data in Table I demonstrate the reduction in acute toxicity: the acetyl-

TABLE I. Acute Toxicity of Endotoxin and Acetylated Fraction in Mice.

Dose, mg	Dead/Total Endotoxin	Dead/Total Acetylated fraction
.3	13/20	1/10
.6	17/24	0/9
.9	10/10	0/10

All doses given *i.p.* in saline with deaths recorded for 72 hr after inj.

ated fraction was non-lethal at a dose which for the original endotoxin killed all animals. Another preparation, available in larger amount, was also non-lethal at 1 mg but produced deaths at 2 mg. Reduction in pyrogenicity is illustrated in Fig. 1. The data in-

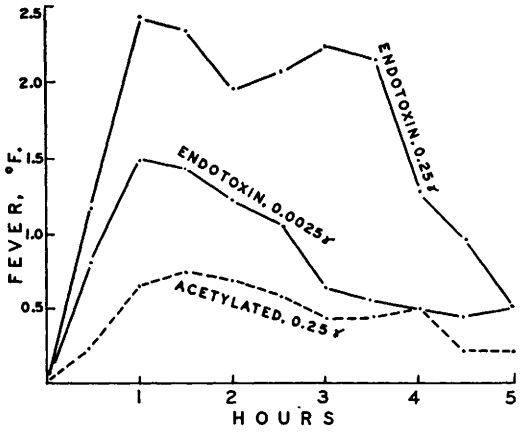

FIG. 1. Mean febrile responses of groups of 6-8 rabbits each, given original endotoxin or acetylated fraction *i.v.*

dicate a reduction by one-thousand fold; individual preparations, by this method, have ranged from 1/100 to 1/10,000 the original pyrogenicity.

The resistance to a lethal challenge of *Ps. aeruginosa* or *E. coli* in mice pretreated with endotoxin or the acetylated fraction was significantly increased compared to the saline controls (Table II). In this system the degree of resistance elicited by microgram doses of either endotoxin or its detoxified derivative was essentially the same.

Discussion. The acetylated fraction of bacterial endotoxin described herein represents a clear dissociation of *in vivo* properties, the toxic and pyrogenic effects being grossly reduced without alteration of stimulation of non-specific resistance to infection. The latter, it should be emphasized, is demonstrated with doses of the same order of magnitude as those shown for determining pyrogenicity. Additional data also indicate equivalence between the 2 materials in promoting resistance to infection with various other gram-negative and gram-positive microorganisms. That this selective reduction in toxic properties is attributable to the acetyl groups introduced is supported by the finding, to be reported, that the original pyrogenicity and acute toxicity are recovered by de-acetylation with alkali. The detoxified immunogenic preparation recently reported by Noll and Braude(4) confers tolerance to both the pyrogenicity and lethality of the original endotoxin; it is of interest that the acetylated fraction described above does not confer such tolerance, measured by the same parameters.

Summary. The dissociation of biological properties of bacterial endotoxin by chemical modification is described. A fraction iso-

TABLE II. Increased Resistance to Infection in Mice Following Administration of Endotoxin and Acetylated Fraction.

Group	Dose, μg*	Challenge organism	Dead/Total†	% survivors
Saline	—	*Ps. aeruginosa*	10/10	0
Endotoxin	1	" "	6/10	40
"	10	" "	4/10	60
Acetylated fraction	1	" "	6/10	40
" "	10	" "	3/10	70
Saline	—	*E. coli*	7/10	30
Endotoxin	0.1	" "	4/10	60
"	1	" "	2/10	80
Acetylated fraction	0.1	" "	4/10	60
" "	1	" "	2/10	80

* All doses given *i.p.* in 1.0 ml of saline 24 hr prior to challenging infection.
† Deaths recorded after 72 hr.

lated after acetylation of endotoxin exhibits grossly reduced pyrogenicity and acute toxicity but retains the original potency in stimulating non-specific host resistance to infection.

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Suppression of the Foreign Bone Marrow Reaction by Preirradiation of Donor Mice. (26767)

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The bone marrow of normal mice is composed of a mixed population of cells differing in morphology and function. On transplantation of viable marrow cells into lethally irradiated homologous recipients, various blood-forming elements repopulate the marrow, the red pulp of the spleen [see review article of Smith and Congdon(1)] and the antibody-forming lymphoid tissues of the host(2,3). Probably only a small percentage of the marrow cells or their precursors are antibody-forming, since as few as 1×10^6 transplanted marrow cells protect against radiation lethality(4,5), whereas as many as 12×10^6 transplanted marrow cells do not confer on lethally irradiated recipients the capacity to synthesize antibodies(6). The radiation dose required to reduce cell survival by a factor of 0.37 is estimated at about 115 rads of Co^{60} rays for blood-forming cells(7) and 130 r of 250 kvp X-rays for spleen cells producing humoral antibodies(8). It seemed possible, therefore—the radiosensitivity of the 2 cell types being comparable—that the antibody-forming cells in mouse bone marrow could be largely eliminated by exposing the animal to an X-ray dose lethal to all but 2-6% of its total marrow population. Marrow thus deprived of immunologically active cells and transplanted into a lethally irradiated homologous recipient would presumably repopulate the recipient's hemopoietic sites but lack usual graft-versus-host immunological reactivity. Data presented here do, in fact, indicate that preirradiation of marrow donors reduces the incidence and severity of secondary disease in irradiated homologous recipients.

Materials and methods. Adult hybrid mice (5-8 months old) of the following strains were used as recipients and donors: (101/Cum × C3H/Anf Cum)F_1 (C57BL/Cum × 101/Cum)F_1, (C57L/Cum × A/He Cum)F_1, (C57BL/6 Cum × DBA/2 Cum)F_1, and (BALB/c Cum × A/He Cum) F_1. These strains are hereafter designated as $1C3F_1$, $B1F_1$, LAF_1, $B6D2F_1$, and CAF_1, respectively. In every case, donor and recipient were of the same sex. Recipients were exposed to 950 r whole-body X radiation; irradiated donors were exposed to 400 r or 500 r 30 minutes before sacrifice. In some instances, the donor mice were given a radioprotective compound, S, β-aminoethylisothiuronium · Br · HBr (AET), 20-30 minutes before irradiation. Nine milligrams of AET in 0.2 M phosphate buffer (pH = 7.4) were injected intraperitoneally per mouse. The X-ray dose rate was approximately 85 r/min; TSD, 93.5 cm; 300 kvp; 20 mA; 4.78 mm of Be inherent filtration and 3 mm of Al added filtration, and hvl, 0.55 mm of Cu.

* Operated by Union Carbide Corp. for U. S. Atomic Energy Comm.