THE STIMULATION OF NON-SPECIFIC HOST RESISTANCE TO INFECTION BY CHEMICALLY MODIFIED ENDOTOXIN

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In recent years the use of endotoxins to alter the natural equilibrium between host and parasite has engendered many intriguing questions. One such problem of primary interest is whether the lethal and pyrogenic effects of these materials are necessarily related to their property of increasing resistance of the host to infection. With the development of a relatively detoxified endotoxin, this subject became amenable to direct investigation (1, 2).

The experiments reported in this paper deal with several properties of the detoxified derivative in stimulating host defense mechanisms as directly compared to the original endotoxin. The essentially equivalent capacity to acutely increase resistance to various bacterial infections has been demonstrated, as well as the differences produced by varying the methods of treatment and infection. Studies dealing with the passive transfer of protection and the effects on the level of circulating leucocytes in the mouse are also discussed.

Materials and Methods

Animals.—Male mice of the ICR strain weighing 16 to 18 gm (Bellewood Farm, Englishtown, New Jersey) were housed 10 to a metal cage, fed Purina lab chow and water *ad libitum*. All animals were used routinely within 1 week after receipt.

Bacterial Cultures.—Three representative Gram-negative bacteria were employed, including strains of *Escherichia coli* and *Salmonella typhimurium* previously described (3) and a strain of *Pseudomonas aeruginosa* isolated in this laboratory. A strain of *Staphylococcus aureus* (Smith) known to be virulent for mice was kindly provided by Dr. Ian M. Smith. Each organism was grown in brain heart infusion broth (BHI) (Difco Laboratories, Inc., Detroit) for 18 hours at 35°C prior to each experiment. A standard inoculum was determined for *E. coli*, *Ps. aeruginosa*, and *S. aureus* which produced 70 per cent to 80 per cent mortality within 3 days in those animals injected intraperitoneally. In those mice infected with *S. typhimurium*, half the deaths would occur within 1 day, or, with a smaller inoculum, by 3 days. In certain experiments, an *E. coli* bacteremia was produced by intravenous injection of 0.25 ml of an 18 hour culture into one of the tail veins, a dose which produced 90 per cent to 100 per cent deaths within 3 days. For the Gram-negative cultures, appropriate dilutions were made in sterile BHI broth. The staphylococcal culture was centrifuged, the cells washed once, and resuspended to their original volume in sterile saline. Viable numbers of organisms were determined by triplicate plate counts on BHI agar. All inocula were kept chilled in an ice bath during the challenge procedure and 0.25 ml of the appropriate dilutions was used for intraperitoneal injections.

Endotoxin and Modified Fractions.—The *Salmonella typhosa* 0-901 endotoxin and the fractions obtained by acetylation have been described in the previous report (2). Stock suspensions of each material were made at 1 mg/ml and stored at −20°C. Dilutions were made in non-pyrogenic saline immediately prior to the various pretreatment procedures. All necessary precautions were taken to prevent contamination from extraneous pyrogen and cross-contamination from the parent endotoxin.

Pretreatment Procedures.—The endotoxin and fractions were administered 24 hours prior to challenge with the infecting organism. In each experiment the fractions were tested side by side with the endotoxin from which they were derived. For intraperitoneal pretreatment, the materials were incorporated in 1.0 ml volumes, while 0.2 ml volumes were used for all intravenous, intramuscular, and subcutaneous injections. Appropriate non-pyrogenic saline injected controls were included to measure the virulence of the infecting organism in each test.

Serum and Plasma.—Treated and control mice were anesthetized with ether and exsanguinated from the heart at the time when they ordinarily would be infected. Serum was separated from the clotted blood, pooled, and kept at −20°C. Plasma was collected by using 1 volume of 3.8 per cent sodium citrate for 5 volumes of whole blood. The blood cells were centrifuged off and the pooled plasma stored at −20°C. In all cases, serum and plasma were used within 1 week from the day of collection and injected intraperitoneally 30 minutes prior to the bacterial challenge injected by the same route.

Leucocyte Counts.—Total peripheral white blood cell counts were made in mice pretreated by various routes with either the endotoxin or its acetylated fraction. Blood samples were taken from one of the tail veins. Counts were made just prior to treatment and then at stated hourly intervals described below.

EXPERIMENTAL

Comparative Studies of Endotoxin and Fractions in Stimulating Resistance to Bacterial Infections.—Previous work indicated that a suitable model for measuring the effects of endotoxin and endotoxin-like extracts on the acute modification of resistance to infection was that employed wherein mice were pretreated 24 hours prior to a standardized bacterial challenge (3–6). While no true linear dose response was found to exist in our experiments, effective doses could be determined within the experimental limits of this system.

The degree of enhanced resistance induced by endotoxins, as well as being generally considered dependent on the level of the pretreatment and infective doses, and the time interval between such injections, is also known to be dependent on the route of inoculation (7). Since the results using homologous inoculation routes have been consistent, this approach seemed appropriate for providing a more sensitive system that might distinguish between protective and ineffective materials.

Increased Resistance to S. typhimurium.—*S. typhosa* and *S. typhimurium* share a common somatic antigen 12, according to the Kauffmann-White schema (8). If the antigenic nature of the endotoxins plays a role in the basic mechanism of the so called non-specific resistance to infection, and modifications of the O antigen altered the common determinant groupings, the increased resistance to *S. typhimurium* seen after administration of *S. typhosa* endotoxin

consequently might be altered when using the fractions obtained after acetylation. With this in mind, *S. typhimurium* was the bacterial challenge first chosen to determine whether any of the modified fractions would possess immunogenic activity.

Although specific vaccines have been found to provide only a partial degree of immunity to *S. typhimurium*, nevertheless, increased resistance can be measured in a mouse typhoid infection system by the time to reach 50 per cent mortality (ST_{50}). The various fractions, P, L, and E, obtained after acetylation

TABLE I

Effect of Endotoxin Acetylated Fractions on the Survival of Mice Infected with Salmonella typhimurium

Treatment*, 1.0 ml ip	No. of bacteria per mouse ip	No. of mice	Cumulative deaths, on days													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Experiment 1																
Saline	1.7×10^7	10	8	9	9	10	10	10	10	10	10	10	10	10	10	10
Endotoxin	"	10	0	0	1	6	9	9	9	9	9	9	9	9	9	9
P	"	10	0	1	4	5	6	6	6	7	7	8	8	9	9	9
L	"	10	6	7	9	10	10	10	10	10	10	10	10	10	10	10
E	"	10	0	0	0	1	1	2	5	5	6	6	7	7	7	7
Experiment 2																
Saline	3.3×10^5	20	0	0	10	16	17	19	19	20	20	20	20	20	—	—
Endotoxin	"	20	0	0	1	2	5	9	11	15	16	17	17	17	—	—
P	"	20	0	0	0	3	5	7	9	11	13	13	15	15	—	—
E	"	20	0	0	1	3	4	6	10	10	13	14	15	15	—	—
Saline‡	"	10	0	0	2	5	7	8	9	9	10	10	10	10	—	—
L‡	"	10	0	0	0	2	6	6	7	8	8	9	9	9	—	—

* 10 μg per mouse of endotoxin or fraction.

‡ Tween 80 concentration, 0.05 per cent.

as described (2), were compared at the same dose known to be effective for the original endotoxin. The results are summarized in Table I.

In the first experiment, the endotoxin and detoxified P fraction increased the ST_{50} to 3 to 4 days, while the toxic E fraction provided the same degree of resistance for 7 to 8 days. The L fraction was ineffective; however, it was quite lyophobic, and the possibility remained that actual loss of material left on glassware during dilution procedures may have affected the results. Therefore, a suitable surface-active agent, tween 80, was employed to aid in dispersing this particulate fraction. In a second experiment using a smaller bacterial challenge, the fractions were again compared to the original endotoxin. The results again serve to emphasize the protective properties inherent to the detoxified P fraction, while the L fraction was not significantly effective as compared to its

appropriate control. In two separate trials of 10 mice per group, the endotoxin, P, and E fractions increased the mean ST_{50} approximately 4 days.

Increased Resistance to S. aureus.—Since, of the two detoxified fractions, only the P, henceforth referred to as the acetylated derivative, showed protection against *S. typhimurium*, this derivative was subjected to further study with infections usually considered heterologous to *S. typhosa* O-901.

Two endotoxin preparations and their respective acetylated derivatives were tested at four dosage levels against an intraperitoneal challenge of *S. aureus* (Smith). The results with each endotoxin and its derivative were comparable in

TABLE II

Increased Resistance to Staphylococcus aureus in Mice after Pretreatment with Endotoxin or Derivative

Pretreatment materials	Dose, ip	Deaths within 1 wk. after infection*	Per cent survivors
	μg		
Saline	—	19/30‡	37.0
Endotoxin	0.1	13/20	35.0
"	1.0	13/20	35.0
"	10.0	1/20	95.0
"	50.0	1/20	95.0
Derivative	0.1	12/20	40.0
"	1.0	9/20	55.0
"	10.0	0/20	100.0
"	50.0	0/20	100.0

* Challenge dose given ip.

‡ $\frac{\text{Number of animals dead}}{\text{Number of animals challenged}}$.

all cases and the combined data are presented in Table II. The data clearly indicate levels at which these materials do or do not increase resistance to virulent staphylococci. While a dose of 10 μg is effective for the endotoxin and derivative, 1 μg or less provides no significant protection in either case.

Increased Resistance to E. coli.—A more sensitive system for measuring early resistance was found to exist with an *E. coli* peritonitis. Several individual tests at doses varying from 0.01 μg to 10 μg per mouse were performed. The pooled data given in Table III indicate that while the level of protection was not of a high order at any of the doses used, there were significant responses in all of the treated groups in each test to denote a non-specific increase in resistance. However, in no case was there a discernible pattern of significant differences between the derivative and endotoxin. To further substantiate this observation in this system, an *E. coli* bacteremia was studied, within the experimental design employing homologous injection routes.

While exploratory work revealed that higher doses of endotoxin and derivative were needed by intravenous pretreatment and challenge, resistance could be clearly defined at at least two dosage levels. In this experiment, enhanced resistance was provided in two ways; *i.e.*, by the prolongation of the survival time at one dose and by a significant increase in the number of survivors at a second dose 5-fold greater in magnitude. As shown in Table IV, at the end of 1 week the derivative and endotoxin at 50 μg protected 50 to 60 per cent of the challenged animals, as compared directly to the survivorship in the saline group. A dose of 10 μg of either material provided an ST_{50} of 3 days compared to less than

TABLE III

Increased Resistance to Escherichia coli in Mice after Pretreatment with Endotoxin or Derivative

Pretreatment materials	Dose, ip	Deaths within 1 wk. after infection*	Per cent survivors
	μg		
Saline	—	55/70‡	21.4
Endotoxin	0.01	7/20	65.0
"	0.1	9/20	55.0
"	1.0	14/40	65.0
"	10.0	16/40	60.0
Derivative	0.01	6/20	70.0
"	0.1	9/20	55.0
"	1.0	17/40	57.5
"	10.0	10/30	66.7

* Challenge dose given ip.

‡ $\frac{\text{Number of animals dead}}{\text{Number of animals challenged}}$.

24 hours for the saline controls, although the final survival values in these experimental groups did not significantly differ from the controls. Again, the lack of a significant difference between the endotoxin and its acetylated derivative in their ability to bolster host resistance was evident.

Increased Resistance to Ps. aeruginosa.—Three experimental trials were run with three individual derivative preparations, the first two of which were used in the previously described staphylococcal studies, and the third used exclusively with *Ps. aeruginosa*, to date. The data are summarized in Table V. In the first experiment, no difference appeared between the endotoxin and derivative, although none of the dosage levels failed to show a significant state of enhanced resistance. In the second experiment, a parallelism of behavior was again observed in that protection was provided by both materials at 50 μg but not significantly at 10 μg or below. In the third experiment, dosages were chosen at 2.5- to 5-fold increments in the range of 0.1 μg to 10.0 μg per animal. Individual comparisons were determined with 7 to 10 mice per group. In this case, a difference

between the materials may be observed not apparent with *S. aureus*, *E. coli*, or the aforementioned trials with *Ps. aeruginosa*. Critical doses at which the two materials deviated in their provocative activity occurred at 0.25 μg with the endotoxin, where protection was still significant, but below which no activity was detectable. However, with the derivative significant protection was measured at 2.5 μg, and on the basis of five separate trials, a discrete but comparatively partial level of increased resistance was evident at 1.0 μg. From these data, the materials appeared to differ in potency by at least a factor of 4 but not more than 10-fold. While the first two experiments in this series were not as extensive as the third, it would seem that differences, if they existed in the first

TABLE IV

Increased Resistance to an Intravenous Challenge of Escherichia coli in Mice after Administration of Endotoxin or Derivative

Pretreatment Materials	Dose, iv	Cumulative deaths, on days				Per cent survivors
		1	3	5	7	
	μg					
Saline	—	8/10*	8/10	9/10	9/10	10
Endotoxin	10	4/10	5/10	5/10	7/10	30
"	50	3/10	3/10	3/10	3/10	70
Derivative	10	4/10	5/10	8/10	8/10	20
"	50	3/10	4/10	4/10	4/10	60

* $\frac{\text{Number of animals dead}}{\text{Number of animals challenged}}$.

two preparations, would have been detected. Furthermore, since the tests in these series were run at different times over a period of several months, with consistent results in each case, the variations due to any changes in bacterial culture or the susceptibility of the mice to the effects of the materials under study would appear to be nullified. Thus, the more likely explanation would seem to be in the variation attributable to the acetylation procedure, thereby producing a derivative of somewhat lower potency. Whether the other infection system studies would respond in the same manner to a derivative of this type remains to be determined.

The Effect of the Acetylated Derivative in Stimulating Resistance by Means of Heterologous Pretreatment Routes.—Further confirmation of the observations of Hook and Wagner (7) concerning the greater efficacy in protection when endotoxin and challenge were injected by the same route in mice was obtained in experiments employing heterologous inoculation routes and *E. coli* as the infective challenge. Preliminary work indicated a larger dose of endotoxin was

necessary to provide a significant degree of survival in this type of system than that ordinarily employed for the intraperitoneal homologous route technique.

In order to explore the efficacy of the derivative, a constant dose of both endotoxin and derivative was used, varying independently the injection routes of pretreatment and challenge but maintaining the usual 24 hour interval between such injections. The data in Table VI summarize several trials for each procedure. No difference appeared between the endotoxin and derivative when

TABLE V

Increased Resistance to Pseudomonas aeruginosa in Mice after Administration of Endotoxin or Derivative

Dose, ip	Per cent survivors at 1 wk.*		
	Endotoxin	Saline	Derivative
μg			
Experiment 1		0 (10)‡	
1.0	40 (10)		40 (10)
10.0	60 (10)		70 (10)
50.0	90 (10)		70 (10)
Experiment 2		10 (10)	
1.0	30 (10)		30 (10)
10.0	10 (10)		10 (10)
50.0	60 (10)		60 (10)
Experiment 3		18 (50)	
0.10	30 (20)		35 (20)
0.25	65 (20)		25 (20)
1.0	62 (50)		42 (50)
2.5	90 (20)		80 (20)
10.0	75 (28)		74 (27)

* Challenge dose given ip.

‡ Total number of mice tested in parentheses.

they were given intraperitoneally or intramuscularly and the mice were challenged with an intravenous infection, although for the same dose and time interval, the intramuscular route was less effective for both materials. When the mice were pretreated by the intravenous route, the derivative was found to be about one-half as active as the endotoxin to an *E. coli* peritonitis, although when the animals were pretreated by the intramuscular route no significant difference appeared with the same type of infection.

The Effects of the Transfer of Serum or Plasma from Treated Mice to Normal Mice Infected with E. coli.—Rowley has stated that early immunity can be transferred by the serum of protected mice to normal mice (4, 9). Therefore, to determine whether protective antibody or some other humoral factor may play a

TABLE VI

Increased Resistance to Escherichia coli in Mice after Pretreatment by Heterologous Inoculation Routes with Endotoxin or Derivative

Materials injected	Pretreatment route*	Challenge route	Deaths at 1 wk. after challenge	Per cent survivors
Saline	ip	iv	18/20‡	10.0
Endotoxin	"	"	4/19	78.9
Derivative	"	"	6/20	70.0
Saline	im	iv	23/25	8.0
Endotoxin	"	"	15/25	40.0
Derivative	"	"	14/24	41.7
Saline	iv	ip	18/20	10.0
Endotoxin	"	"	6/20	70.0
Derivative	"	"	13/20	35.0
Saline	im	ip	23/25	8.0
Endotoxin	"	"	13/25	48.0
Derivative	"	"	17/25	32.0

* 50 μg dose, see Materials and Methods for injection volumes.

‡ $\frac{\text{Number of animals dead}}{\text{Number of animals challenged}}$.

TABLE VII

Effects of Transfer of Serum or Plasma from Treated Mice to Normal Mice on Resistance to Escherichia coli Infection

Pretreatment of donor mice*	Intraperitoneal injection of recipient mice	Deaths 1 wk. after challenge‡
	ml	
Experiment 1		
Saline	Serum, 0.1	9/12§
Endotoxin, 10 μg	" "	8/12
Derivative, 10 μg	" "	10/10
Experiment 2		
Saline	Serum, 0.25	10/10
Endotoxin, 10 μg	" "	9/9
Derivative, 10 μg	" "	10/10
Experiment 3		
Saline	Plasma, 0.25	12/14
Endotoxin, 50 μg	" "	14/15
Derivative, 50 μg	" "	12/15

* All injections iv, see Materials and Methods for further details.

‡ Challenge dose given ip 30 minutes after serum or plasma injection of normal recipients.

§ $\frac{\text{Number of animals dead}}{\text{Number of animals challenged}}$.

role in the basic systems described above, somewhat akin to the passive transfer of phagocytosis stimulation and protection against endotoxin pyrogenicity and lethality described by Freedman (10), the transfer of serum or plasma from mice pretreated with endotoxin or derivative was done as described in Materials and Methods.

In order to maintain a rigorously integrated system, the same endotoxin and derivative preparations used in the active immunity studies with *E. coli*, *S. aureus*, and the first trials with *Ps. aeruginosa* were employed here at two doses known to be effective for an *E. coli* peritonitis. The serum or plasma was taken at a time when the treated animals would ordinarily be challenged. The intraperitoneal system was employed for the normal recipients of serum or plasma and the challenge.

TABLE VIII

Total Mean Peripheral Leucocytes in Mice after Treatment with Endotoxin or Derivative

Treatment	Leucocyte count (thousands)/mm³, hrs. postinjection							
	0	1	2	3	4	5	6	24
Endotoxin, 50 μg ip	14.2*	14.6	17.1	18.8	21.0	23.8	24.5	13.8
" 50 μg iv	14.0	14.7	19.7	21.8	24.6	30.1	34.0	34.2
Derivative, 50 μg ip	15.2	15.1	16.2	17.2	18.8	22.3	21.4	15.9
" 50 μg iv	15.0	16.5	21.0	24.4	37.3	37.6	40.8	35.8

* 6 mice per group.

The data of three experimental trials are presented in Table VII. Under these conditions, neither serum nor plasma from treated mice increased resistance to a significant level.

The Effects of Endotoxin and Derivative on the Level of Circulating Leucocytes in the Mouse.—The well documented effect of endotoxin on leucocyte mobilization was next investigated to determine whether the derivative maintained the capacity to cause the characteristic initial leucopenia and subsequent leucocytosis found in rabbits after endotoxin treatment. Using a constant dose of 50 μg per mouse and varying the route of inoculation to coincide with the protection models described above, the numbers of peripheral leucocytes were measured in mice at various time intervals after injection. A serial time study, as depicted in Table VIII, revealed several points of interest.

After an intraperitoneal injection of endotoxin, a gradual increase in the number of white blood cells appeared which became significant at 3 hours. At 24 hours, when such mice would have been infected, the leucocyte counts had returned to the normal level. The response to the derivative was similar, al-

though a significant increase appeared somewhat later at 4 hours. On the other hand, after an intravenous inoculation, both materials produced a sharp rise by the 2nd hour with a continual increase throughout the 6 hour test period. By the next day, the leucocyte levels were still at least 100 per cent higher than the preinjection counts. A 50 μg dose of these same materials given intraperitoneally will protect mice against *E. coli* given intravenously, although the level of circulating leucocytes is unchanged. The same dose given intravenously will also protect against *E. coli* given intravenously or intraperitoneally when the leucocyte count is higher than normal. It would seem the mere quantitative account of the peripheral leucocytes resulting from pretreatment with protective endotoxin or derivative cannot, therefore, independently reflect the increased resistance of the host to infection with *E. coli*. It may be also emphasized that in such young mice of approximately 5 weeks of age, no leucopenia was evident. Other studies to be reported indicate that 3 month old mice do respond with a leucopenia and leucocytosis to endotoxin given intravenously; however, no leucopenia appears when such mice are treated with the same dose of the derivative. Furthermore, 50 μg of either material administered subcutaneously into mice of both ages elicits a definitive leucocyte mobilization at 24 hours equivalent to the intravenous response, although such animals are not protected against an intravenous or intraperitoneal challenge.

DISCUSSION

Where the technique of homologous inoculation routes was employed, a clear separation of the pyrogenic properties of endotoxin from its capacity to provoke increased resistance to infection may be observed. Even if the reduction in pyrogenicity of each of the derivatives is assumed to be 100-fold, the lower limit of the observed decrease (2), no comparable decrease in the resistance-increasing activity of the derivative is evident, for the activities of the endotoxin and its derivative in the experiments with *E. coli* and *S. aureus* infections are indubitably equivalent. This striking dissociation of these two biological effects also appears in those assays involving *Ps. aeruginosa*, although a relatively small difference in activity between one derivative preparation and its parent endotoxin was detected. Furthermore, in those experiments where heterologous routes of injection were used, the similarity of the materials was maintained in all of the combinations, except where the derivative was given intravenously and the host challenged intraperitoneally. However, again the reduction in activity in this case was insignificant compared to the decrease in pyrogenicity.

The relative loss in toxicity of the specific derivatives compared to their parent endotoxins also must be considered in relation to the results of the infection studies. While the LD_{50} for the endotoxin was 0.25 mg intraperitoneally, the derivative which was used in those studies where mice were pretreated intravenously and challenged with *E. coli* by the same route, produced no deaths

at a level of 2 mg per mouse (2). Despite this gross difference in toxicity, a marked parallel capacity of the two materials to differentially alter the resistance to *E. coli* existed at 2 dosage levels. Since the lethality of the derivative is a function of the route of injection and a dichotomy in effects is obtained between the endotoxin and derivative when the intravenous route is employed, *i.e.* the endotoxin is more toxic and the derivative less toxic than when they are given intraperitoneally, the separation of the protective and lethal effects is even more apparent. Assuming the more toxic of the derivatives is typical (approximately an 8-fold reduction in lethality intraperitoneally), still, the only instance where lethality and the protective potency would not be clearly dissociated is the one experiment involving *Ps. aeruginosa*. On the other hand, with this same infection, other derivatives demonstrated parallel protective properties to the parent endotoxin. Thus the weight of the available evidence indicates a distinct dissociation of the lethal and protective properties of endotoxin by the use of the acetylated derivative.

In the experiments concerned with the effects of heterologous injection routes, there became evident the first indication that a significantly detectable difference in the protective capacity existed between the endotoxin and derivative. This difference and the more particulate nature of the derivative suggest the possible alteration of its distribution within the host as compared to the unmodified endotoxin. In turn, this altered distribution may be related directly or otherwise to the primary organs infected by the pathogen. From the standpoint of the infecting agent, the fulminating peritonitis from an intraperitoneal injection (11) contrasts with the initial bacteremia and localization primarily in the liver and spleen when the organisms are introduced by the intravenous route (12). Thus, the fact that the derivative is as effective as endotoxin by the intravenous-intravenous, or intraperitoneal-intraperitoneal method, but less by the intravenous-intraperitoneal method, lends further support to the concept of primarily a localized cellular reaction that so alters resistance to provide a favorable outcome for the host. Furthermore, the finding that the derivative is even less lethal intravenously than intraperitoneally, contrary to the typical behavior of endotoxin in this regard, supports the probable difference in its distribution within the host.

One aspect of a host cellular reaction to endotoxin administration has been the classical response of the number of circulating leucocytes in rabbits. The course of leucopenia and leucocytosis has been repeatedly confirmed; however, the response characteristic for mice used in infection and resistance models has not received the same degree of systematic attention. Smith, *et al.* reported a peripheral granulocytosis in 3 to 4 month old mice 24 hours after 10 μg of *S. typhosa* endotoxin given intraperitoneally correlated with increased resistance to a pseudomonad infection, although repeated endotoxin treatment produced a diminution in white blood cells closely approaching the normal control level

while the increased resistance still persisted (13). In studying factors concerned with innate rather than acquired immunity, Gowen and Calhoun found the degree of resistance to *S. typhimurium* in selected, resistant inbred mice to be correlated with the mean number of circulating leucocytes (14). But Weir, *et al.* found mice bred for low leucocyte counts to be more resistant to the same mouse pathogen (15). In the system used in our experiments, a distinct lack of correlation existed between the level of circulating leucocytes after intraperitoneal or subcutaneous injection of endotoxin or derivative and the degree of resistance to an intravenous infection. The evidence from several viewpoints appears to indicate, then, that the total number of peripheral white blood cells does not contribute as a protective mechanism *per se*; it cannot be considered as an independent factor, and, rather, one must study, as has been done *in vitro*, the qualitative state of those phagocytic elements which are part of the total leucocyte response.

The failure to obtain passive transfer of protection by plasma or serum of resistant mice to normal recipients can, of course, be ascribed to the methods employed, particularly in reference to the dosage schedule of serum or plasma. In the last study, mice were treated with endotoxin or derivative at 500 times that needed to demonstrate active protection, recipient mice received approximately 25 per cent of their total blood fluid volume, and no protection was evident. Nevertheless, the *in vivo* dilution of the known low levels of measurable factors such as specific antibody or opsonins may well have obviated any measurable *in vivo* protective response.

In this connection, some preliminary findings concerning other attributes of the acetylated derivative are worth noting. In rabbits there is a diminution of from 6- to 10-fold in the specific agglutinin response to *S. typhosa* O-901 by the derivative as compared to its endotoxin, although no significant difference existed in the minimal stimulation of agglutinin to the *E. coli* strain used in our infection studies. The total dosages of each material were of the same order of magnitude as those used in the protection studies. Also, where as little as 1 μg of endotoxin given mice 30 minutes prior to an intraperitoneal dose of *S. aureus* increased the number of deaths due to the infection by 50 per cent, no such effect was produced by the derivative at 10 times the endotoxin dose. Thus, the derivative has lost a substantial degree of the capacity of the endotoxin to increase the susceptibility of mice to virulent bacteria. Studies are continuing as to these described effects and their bearing on present humoral and cellular hypotheses for explaining early nonspecific resistance (16–19).

SUMMARY

An acetylated derivative prepared from *Salmonella typhosa* O-901 endotoxin has been found to retain the ability to stimulate non-specific host resistance to a variety of bacterial infections. Relative to the parent endotoxin, the derivative

has been reduced in the gross in its pyrogenicity to rabbits and lethality to mice. With the use of this chemically modified preparation under a variety of conditions, the direct dissociation of the toxic properties of endotoxin from its protective capacity appears evident.

In young male mice, the endotoxin and its derivative produced a leucocytosis but no leucopenia. However, no direct correlation could be found with the level of the peripheral white blood cells and resistance to infection with *Escherichia coli*. Furthermore, under the conditions employed, passive transfer of early resistance to infection by serum or plasma was not detectable.

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