SYMPOSIUM ON BACTERIAL ENDOTOXINS[1]

II. Possible Mechanisms whereby Endotoxins Evoke Increased Nonspecific Resistance to Infection

JAMES L. WHITBY,[2] J. GABRIEL MICHAEL,[3] MARK W. WOODS, AND MAURICE LANDY

National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, U. S. Public Health Service, Bethesda, Maryland

Experiments in which the administration of substances to the host was followed by a rapid change in host resistance to infection have been described many times. The early literature has been well assembled and reviewed by Philipson (19). However, no unifying concepts of either common mechanisms of action or common substances present in administered materials were formulated, so that the phenomena largely remained as isolated and individual examples which had been elicited in special circumstances. On examination of the reported findings, among them those of Pfeiffer and Issaeff (18), Teague and McWilliams, (26) and Philipson (19), it is clear that a number of such phenomena may well have been initiated by endotoxin.

Contemporary work in this field had its beginnings in observations of a similar nature. In the first descriptions, administration of bacterial cell-wall preparations (20) or whole bacteria (8) was found to result in a temporary and rapid increase in the resistance of mice to infection with antigenically unrelated gram-negative bacteria. Shortly thereafter the active component in these preparations was identified as endotoxin (12, 21).

At that time it was thought that the alterations in resistance might be explained as a consequence of the interaction of substrate (endotoxin) with a serum component concerned with the bactericidal reaction, which resulted in an initial depletion followed by a temporary overproduction of the latter substance. The actual pattern of altered resistance fitted this view very well in that, for about 3 hr after receiving large doses of endotoxin, mice are more susceptible to experimental infection with gram-negative bacteria; subsequently the situation is reversed rapidly and is followed by a period of up to 5 days when enhanced resistance is demonstrable. However, it was later found that increased resistance to infection produced by endotoxin was also exerted against mycobacterial and staphylococcal infections (7) and this resistance differed in that it persisted for several weeks. Subsequently, the effects were shown to extend to still further bacterial species and to certain virus infections (25). Moreover, some of the bacterial species were insensitive to the bactericidal action of serum.

As soon as the phenomenon had been adequately characterized, work went forward to elucidate the mechanisms by which enhanced resistance was mediated. Endotoxin produces many changes in the host, which could be significant in altering the state of resistance, but some of these appear to be of special relevance. It has been shown that endotoxin profoundly alters the functional capacity of the reticulo-endothelial system as measured by the clearance of intravenously injected carbon particles (3), P^{32}-labeled endotoxin (10), or similarly labeled bacteria (1). With these tests, an initial period of reduced clearance, lasting for several hours, can be shown. This is followed by enhanced reticulo-endothelial activity characterized by both more rapid clearance and greater phagocytic capacity, which persists for several days before returning to normal. The functional activity of both polymorphonuclear (6) and mononuclear phagocytes (23) has also been shown to be enhanced by endotoxin.

The cellular changes following administration of endotoxin were found to be paralleled by alterations in the serum. An initial depression of the serum properdin level was followed by increased values which persisted for a few days (12). The bactericidal power of serum for *Escherichia coli* was also elevated after bacterial cell wall (21) or endotoxin administration (9). Serum from mice

[1] This symposium was held at the Annual Meeting of the American Society for Microbiology in Chicago, Ill., 24 April 1961, under the sponsorship of the Division of Medical Bacteriology and Immunology, with Maurice Landy as convener.

[2] Present address: Queen Elizabeth Hospital, Birmingham, England.

[3] Present address: House of the Good Samaritan, Boston, Mass.

after endotoxin treatment was found to have enhanced opsonic power for gram-negative bacterial species (11). In addition, increased opsonic power could be measured by the blood clearance of P^{32}-labeled *E. coli* (1) and this capacity could be passively transferred. The accelerated cutaneous reactivity to endotoxins, which followed the intravenous administration of these agents to rabbits, could be passively transferred and displayed some of the characteristics of the Arthus reaction (14). Moreover, the serum from these animals, in the presence of complement, was found to possess enhanced ability to lyse red cells coated with endotoxins serologically unrelated to the administered material.

Hence it is clear that after administration of endotoxin the serum of animals is altered in such a way that it displays increased activity in a number of different tests. Most of these tests employ situations in which antibody is known to be functional, and where an increase in antibody level could account for the improved performance. However, the nature of the host response to endotoxin differs from the classical immune reponse in two important ways. The onset is extremely rapid so that serum changes are readily detectable within hours, and the elicitation of the effect by a single product is followed by activity against a wide range of antigenically unrelated gram-negative organisms. Thus, it has been suggested that endotoxin may stimulate the production by the host of cross-reacting antibodies which have multiple specificity (14); or, alternatively, that gram-negative bacteria possess a common antigen possibly in the lipid component of the endotoxin (2) against which antibodies are produced. No such common antigen has as yet been demonstrated. There are other possibilities. Some nonspecific substance capable of acting like antibody may appear in increased amount following endotoxin administration; this had already been first proposed and later rejected in the case of properdin. Other nonspecific substances have been described (25). Alternatively, greater amounts of some cofactor may appear in the serum after endotoxin, which improve the effectiveness of natural antibodies already present in the serum. Finally, the possibility should be considered that endotoxin stimulates a general increase in the level of serum antibodies which are individually specific; in this case the apparent cross reactivity would not be cross reaction at all but would be a consequence of multiple antibody release.

It is apparent that administration of endotoxin to animals leads both to cellular and to humoral changes in the host. The work to be described relates to both of these parameters. The changes both in cells and serum were different from those which had been described previously, and the objective was to attempt to determine whether the changes, both humoral and cellular, were of such a nature that they might reasonably account for the increased resistance which follows administration of endotoxin.

EFFECTS OF ENDOTOXIN ON MONONUCLEAR PHAGOCYTES

Previous work on the effects of endotoxin on cell metabolism has been concerned with effects elicited on a variety of cells exposed to endotoxin in vitro. The cells, whether mononuclear or granulocytes, originated from rabbits, a species in which nonspecific resistance has not been studied. Our attention was focused on the peritoneal macrophages of mice inasmuch as these are the phagocytic cells directly involved in the resistance of this species to a peritoneally induced experimental infection. These cells can be obtained in adequate numbers essentially free of polymorphonuclear leukocytes without prior stimulation of the host to induce exudate formation (27). Under appropriate conditions, peritoneal macrophages have been shown to function more efficiently as phagocytes in vivo after endotoxin treatment (11) and we were interested in determining whether general changes in metabolic behavior of these cells would also follow administration of endotoxin.[4]

Glycolysis provides a significant index of the metabolism of cells, particularly of phagocytes. We have measured aerobic and anaerobic glycolysis of peritoneal macrophages derived from mice at various times after treatment with endotoxin. For these cells anaerobic glycolysis has shown the effects more clearly than aerobic glycolysis, but similar changes were seen in both parameters. After a dose of endotoxin the rate of aerobic and anaerobic glycolysis showed an early increase, followed by a period of depression which in turn was replaced by a period when glycolytic

[4] The endotoxin preparations employed in this work were prepared by the following investigators: D. A. L. Davies, *Shigella dysenteriae*; O. Westphal, *Escherichia coli* strain O8; E. Ribi, *Salmonella enteritidis*.

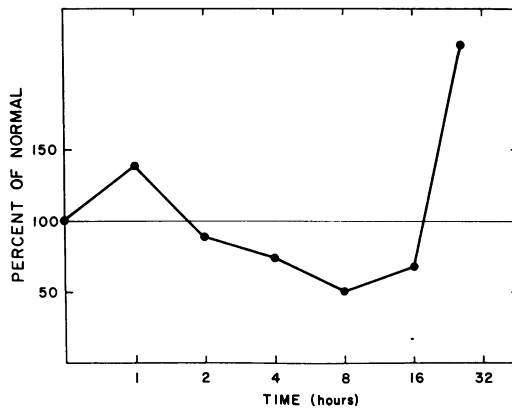


FIG. 1. *Glycolytic activity of mouse peritoneal macrophages during the first 24 hr after administration of 5 μg of Salmonella enteritidis endotoxin. The rate of anaerobic glycolysis for normal macrophages (2 × 10^7 cells per Warburg vessel) averaged 20 μl of CO_2 per 1 hr.*

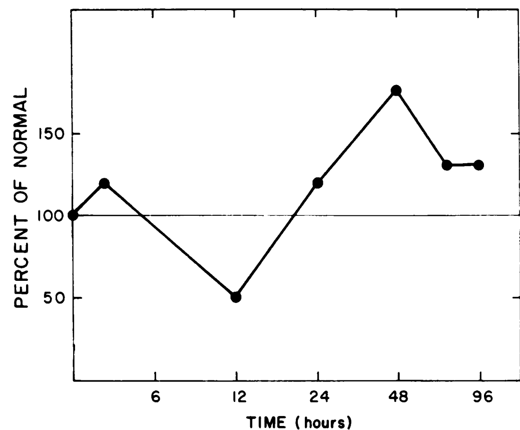


FIG. 2. *Glycolytic activity of mouse peritoneal macrophages after 50 μg of Shigella dysenteriae endotoxin. The rate of anaerobic glycolysis for normal macrophages (2 × 10^7 cells per Warburg vessel) averaged 20 μl of CO_2 per 1 hr.*

activity was elevated. The latter stimulation could persist for many days and did so for at least 8 days in animals we have tested.

Figure 1 outlines the glycolytic activity of peritoneal cells recovered from mice at times up to 24 hr after the animals had received a dose of 5 μg of *Salmonella enteritidis* endotoxin. Cells harvested 1 hr after endotoxin administration show a stimulation of glycolysis, but at all subsequent periods up to 16 hr glycolysis is depressed. At 24 hr and at periods up to 96 hr, as shown in Fig. 2, the glycolytic activity is again elevated above normal. It should be emphasized that the level of anaerobic glycolysis displayed by the cells after harvest continues at a constant rate in the Warburg flasks, and the cells recovered from the host do not pass through the progressive changes in activity which are found at various times in vivo. It is also noteworthy that macrophages exposed to endotoxin in vitro respond only with stimulation and do not show depression (28).

These findings indicate that the depression of glycolysis which is found shortly after in vivo administration of endotoxin is induced by host mechanisms that regulate cell glycolysis and is not a direct effect of endotoxin. With smaller doses of endotoxin, the inhibitory phase is either greatly reduced or absent, and stimulation of glycolysis, although it arises more slowly, is established at levels well above normal, whereas cells from animals receiving larger doses are still in the negative phase. The actual level of stimulation following small doses of endotoxin has varied, but one example, illustrated in Fig. 3, was extremely marked. In this figure, the glycolytic rates 11 hr after various doses of endotoxin are compared.

It is well known that small doses of endotoxin do not induce a state of acute stress in the host, whereas large doses do, and this may well be the cause of the glycolytic depression. After the effects of stress have passed off, the stimulated state returns. Up to now, no data have been obtained to determine whether the long stimulatory effect of high doses of endotoxin is due to the induction of some steady metabolic state in the physiology of these cells, or whether endotoxin as such persists in tissues.

Other workers have shown that phagocytosis of inert particles or bacteria is accompanied by increased glycolytic activity of polymorphonuclear leukocytes (5, 24). Substances which stimulate glycolysis, including endotoxins (6), have been shown to promote phagoyctosis by these same cells, whereas substances which depress glycolysis are accompanied by reduced phagocytic activity. This correlation has not proved so easy for us to demonstrate with macrophages, although a biphasic response of mouse peritoneal macrophages has been shown with respect to their ability to phagocytize bacteria in vivo (11). In flasks, macrophages have been found to display poor phagocytic activity for gram-negative bacteria, unless attached to a surface (22). In contrast

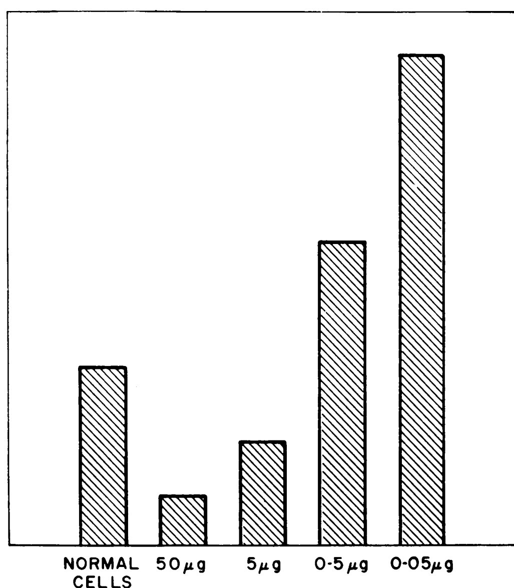
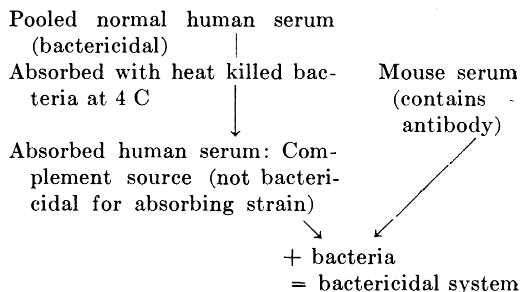


FIG. 3. *Glycolytic activity of mouse peritoneal macrophages 11 hr after various doses of Salmonella enteritidis endotoxin. The rate of anaerobic glycolysis for normal macrophages* (2×10^7 *cells per Warburg vessel) averaged 20* µ*l of* CO_2 *per 1 hr.*

Mackaness (15) found that unattached suspensions of rabbit macrophages readily ingested staphylococci in the presence of serum. In the present experiments, polystyrene particles, included in parallel flasks, were not taken up well, but administration in vivo of polystyrene particles, to animals in parallel with animals in which glycolysis was studied, did induce differences in phagocytic potential. In animals where parallel studies showed depressed glycolysis by macrophages, phagocytosis was less than normal, but increased glycolysis was associated with a larger number of cells containing polystyrene particles. Thus, glycolytic behavior in vitro was reflected in the phagocytic activity of macrophages in vivo.

Effects of Endotoxin Administration on Serum

Endotoxin administration to animals is known to alter a variety of biological activities exerted by serum. We wished to study changes in antibody titers in mice at various times after endotoxin administration to investigate the specificity or lack of it in any alterations that occurred. Under normal conditions, mouse serum appears to have little or no antibody to gram-negative bac-



Mouse serum can be titrated for its antibody content as all other necessary factors are present in excess.

FIG. 4. *Bactericidal reaction with mouse serum*

teria as measured by agglutination tests. However, antibodies to these organisms, together with a suitable complement source, form a bactericidal system, and we have developed a method based on this system (13) which is illustrated in Fig. 4. Under the test conditions outlined, we have found that mouse serum is capable of restoring bactericidal activity to human serum from which antibody has been absorbed. We have further shown by absorption studies that the contribution of normal mouse serum in this test is to supply specific antibody. The method will detect between 0.001 and 0.0005 µg of antibody N as tested against an immunochemically calibrated immune rabbit serum, which makes it among the most sensitive methods of antibody determination at present available (4). Table 1 shows that by adding progressively smaller quantities of mouse serum to a series of reaction mixtures it is possible to titrate the antibody content of the mouse serum as a dose response in relation to the number of bacteria surviving in the reaction mixture after 1 hr of incubation. Here two samples of mouse serum have been compared for activity against *Salmonella typhosa* strain O901, one from normal danimals an the other from animals which had received 50 µg of *Shigella dysenteriae* endotoxin 24 hr previously. At each serum increment, the serum of endotoxin-treated mice shows greater bactericidal activity than that of normal mice.

When the results obtained from such experiments are plotted as probits of percentage survivors against the log serum dose, the dilution of serum at which 50% bacterial survival occurs can be read off the slope. Figure 5 shows the plot of two serum samples, one derived from normal mice and the other from a group of mice 1 hr

TABLE 1. *Bactericidal activity of mouse serum for Salmonella typhosa strain O901*

Serum	Bacterial survival in serum from	
	Normal mice	Endotoxin-treated mice*
ml	%	%
0.01	7	3
0.0066	59	3
0.005	72	23
0.004	87	46
0.0033	95	68

The bacterial inoculum was 10^6 cells.

* Obtained at 24 hr following 50 μg of *Shigella dysenteriae* endotoxin iv. The quantity of serum giving 50% killing was 0.007 ml in the case of normal mice and 0.004 ml for the endotoxin-treated mice.

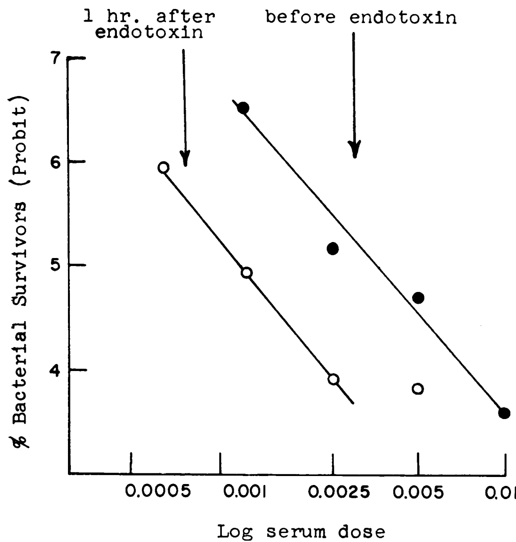


FIG. 5. *Effect of endotoxin on bactericidal activity of mouse serum. Endotoxin, 50 μg of Salmonella enteritidis; inoculum, 10^6 Shigella dysenteriae.*

after the administration of 50 μg of *S. enteritidis* endotoxin. The serum from the endotoxin-treated animals shows greater activity at all levels than the normal mouse serum. The end point values are 0.0013 ml and 0.0037 ml for the sera from the endotoxin-treated and the control animals, respectively.

The differences between individual sera are not of a large order, but by using a method where the end point can be titrated as a dose response, and because the dose response slope is steep, it has been possible to distinguish one serum from another in a way which would not have been possible when measuring agglutination, for instance. After administering 10 to 50 μg of endotoxin to mice we have found that the bactericidal activity of their serum as measured in our assay is consistently raised to an activity of 1.5 to 4.0 times that found in normal mouse serum. Because the phenomenon of nonspecific resistance can be elicited by considerably smaller doses of endotoxin than this, it was important to determine if lower doses would also alter serum bactericidal activity. It was recognized, however, that at low doses an effect might be produced that could not be determined by available methods.

Many factors could influence the duration and magnitude of these changes, as has been shown previously in investigations of increase in natural resistance. Among these are the properties of the endotoxin itself, the size of dose administered, the strain of mice used, the time the samples are taken, and the antigenic relationship of the organism tested in the assay to the endotoxin administered. Tables 2 to 6 illustrate the changes in antibody levels found in mouse serum at various times after administration of endotoxin. Not all of these variables were examined. However, two strains of mice were employed, three endotoxins of different immunological specificities and from different sources were utilized, and bactericidal assays for antibody against three unrelated enterobacteriaceae were carried out. Two of the strains shared common antigens with the endotoxins employed, which enabled a comparison to be made between homologous and heterologous situations. The major variables examined, however, were the dose of endotoxin administered and the interval between injection and bleeding. Each of the endotoxins used was capable of evoking the effect but quantitative comparisons of their potencies were not made. Although there were only two situations when homologous and heterologous responses were compared, the differences were so striking as to be conclusive. The dose effect over an extended range was studied with a single product which provided information on the magnitude and duration of the serum changes in relation to dose.

In the experiment illustrated in Table 2 where 50 μg of *S. enteritidis* endotoxin were adminis-

TABLE 2. *Alterations in level of antibodies for Salmonella typhosa, Shigella dysenteriae, and Escherichia coli in N.I.H. mice receiving Salmonella enteritidis endotoxin*

Test strain	Relative serum antibody levels for test strains								
	Before endotoxin*	Hours after 50 μg of *S. enteritidis* endotoxin							
		1	3	12	24	48	96	144	192
S. typhosa strain O901	1.0	0.3	0.3	0.6	1.2	—	1.5	—	9.0
S. dysenteriae type 1	1.0	3.0	2.9	2.9	2.4	2.5	1.3	—	—
E. coli strain O127	1.0	2.8	2.9	2.6	2.1	1.8	1.3	0.8	1.3

* Quantity of normal serum effecting a 50% reduction in an inoculum of 10^6 bacteria: 0.005 ml of *S. typhosa* strain O901; 0.004 ml of *S. dysenteriae* type 1; and 0.0001 ml of *E. coli* strain O127.

TABLE 3. *Alterations in level of antibodies for Salmonella typhosa, Shigella dysenteriae, and Escherichia coli in* CAF_1 *mice receiving S. dysenteriae endotoxin*

Test strain	Relative serum antibody levels for test strains							
	Before endotoxin*	Hours after 50 μg of *S. dysenteriae* endotoxin						
		1	3	6	12	24	48	96
S. typhosa strain O901	1.0	1.0	1.2	1.9	1.9	1.7	1.7	1.0
S. dysenteriae type 1	1.0	0.4	0.4	0.6	0.6	0.6	1.5	3.8
E. coli strain O127	1.0	1.2	2.6	2.3	2.5	2.5	1.9	1.3

* Quantity of normal serum effecting a 50% reduction in an inoculum of 10^6 bacteria: 0.007 ml of *S. typhosa* strain O901; 0.006 ml of *S. dystenteriae* type 1; and 0.0001 ml of *E. coli* strain O127.

TABLE 4. *Alterations in level of antibodies for Salmonella typhosa, Shigella dysenteriae, and Escherichia coli in* CAF_1 *mice receiving E. coli strain O8 endotoxin*

Test strain	Relative serum antibody levels for test strains							
	Before endotoxin*	Hours after 10 μg of *E. coli* strain O8 endotoxin						
		½	1	2	4	6	12	24
S. typhosa strain O901	1.0	1.1	1.5	1.8	1.7	1.6	1.6	1.6
S. dysenteriae type 1	1.0	1.2	2.5	—	2.3	1.8	—	1.8
E. coli strain O127	1.0	1.1	1.9	2.5	—	3.4	2.6	3.4

* Quantity of normal serum effecting a 50% reduction in an inoculum of 10^6 bacteria: 0.007 ml of *S. typhosa* strain O901; 0.006 ml of *S. dysenteriae* type 1; and 0.0001 ml of *E. coli* strain O127.

tered, antibody levels for the heterologous organisms tested rise sharply within 1 hr and remain elevated for 48 hr but return to normal limits by 96 hr. By contrast, the antibody level for an antigenically homologous strain of *S. typhosa* is markedly reduced in the first 12 hr and does not reach normal levels until 24 hr or elevated levels until 96 hr. By 8 days, antibody to this organism is elevated to a level much higher than that found to heterologous strains at any time. Similar findings are seen in Table 3 where 50 μg of *S. dysenteriae* endotoxin were injected, except that the rise in antibody to heterologous strains occurred somewhat later, appearing 3 to 6 hr after endotoxin had been given. Table 4 illustrates the changes found after injection of 10 μg of *E. coli* strain O8 endotoxin, which is antigenically unrelated to any of the test organisms. Here elevation of antibody for all the test strains had taken place after 1 hr and then persisted throughout the

TABLE 5. *Alterations in level of antibody for Escherichia coli in N.I.H. mice receiving Salmonella enteritidis endotoxin*

S. enteritidis endotoxin	Relative serum antibody levels for *E. coli* strain O127							
	Before endotoxin*	Hours after endotoxin						
		1	3	6	12	24	48	72
μg								
10	1.0	4.2	2.9	2.3	1.8	1.8	1.0	0.9
1	1.0	0.9	1.9	1.8	2.4	1.5	0.9	1.2
0.1	1.0	0.9	1.8	2.2	2.5	1.0	1.1	1.2
0.01	1.0	1.0	0.9	—	0.8	1.1	—	—

* Quantity of normal serum effecting a 50% reduction in an inoculum of 10^6 *E. coli* strain O127: 0.0002 to 0.00015 ml. The values in the table are related to the normal serum value on the day of test.

TABLE 6. *Alterations in level of antibody for Shigella dysenteriae in N.I.H. mice receiving Salmonella enteritidis endotoxin*

S. enteritidis endotoxin	Relative serum antibody levels for *S. dysenteriae* type 1			
	Before endotoxin*	Hours after endotoxin		
		6	24	48
μg				
10	1.0	3.3	2.7	1.6
1	1.0	3.4	2.0	1.0
0.1	1.0	2.3	1.2	1.0

* Quantity of normal serum effecting a 50% reduction in an inoculum of 10^6 *S. dysenteriae* was 0.004 to 0.0035 ml.

test period (24 hr). Illustrations of the effect of different doses of *S. enteritidis* endotoxin on antibody levels are found in Tables 5 and 6, which show that a dose of 0.01 μg fails to produce any change in antibody levels, whereas doses of 0.1 μg or more are followed by elevation of antibody levels. There is evidence that the duration of the effect is related to the dose of endotoxin, lasting only 12 hr after 0.1 μg, 24 hr after 1 μg, and 24 to 48 hr after 10 μg.

These results illustrate that, after endotoxin administration, increased levels of antibody active in a bactericidal system were found. The question whether this antibody showed specific or cross-reactive characterization was next considered. The finding (Tables 2 and 3) that, after a large dose of endotoxin, bactericidal activity for antigenically heterologous strains rises, whereas that for antigenically homologous strains falls, argues for a specific basis for the bactericidal response. The most probable explanation of the difference is that complexing of antigen (endotoxin) with antibody occurs in the host. A nonspecific substance should be equally active against homologous strains; however, evidence of specificity was also provided by other means.

TABLE 7. *Specificity of bactericidal antibody of endotoxin-treated mice*

Treatment of mice	Relative bactericidal activity of serum			
	Unabsorbed		After absorption with *Salmonella typhosa*	
	S. typhosa	*Escherichia coli*	*S. typhosa*	*E. coli*
None	1.0	1.0	0	1.0
Shigella dysenteriae endotoxin (50 μg, iv) 24 hr previously	1.7	2.5	0	2.3

The specific nature of this response has been established by absorption studies (16) and by inhibition tests in the bactericidal reaction with endotoxins of differing antigenic specificity. When sera were absorbed with a bacterial suspension, it was found that antibody activity against the absorbing strain could be removed, whereas activity against other strains was retained. Table 7 shows an example of this finding: 24 hr after 50 μg of *S. dysenteriae* endotoxin had been administered, serum obtained from mice possessed 1.7 times the bactericidal activity against *S. typhosa* and 2.5 times as much activity against *E. coli* strain O127 as normal mouse serum. When the activity against *S. typhosa* was removed by absorption with heat-killed bacteria, the bactericidal activity against *E. coli* was not significantly affected. Other absorption studies have been carried out with similar results.

Further evidence for specificity was obtained by adding antigenically homologous and heterologous endotoxins to reaction mixtures in the bactericidal reaction (17). An example of such an experiment is illustrated in Table 8 where it can

TABLE 8. *Specificity of increased bactericidal activity of serum from endotoxin-treated mice*

Mouse serum	Relative bactericidal activity for *Escherichia coli* strain O127			
	Untreated serum	Somatic antigen added to test serum		
		Salmonella typhosa, 100 μg	*Shigella dysenteriae*, 100 μg	*E. coli* strain O127, 10 μg
Normal	1	1	1	<0.05
At 24 hr after administration of 10 μg of *Salmonella enteritidis* endotoxin	2	2	2	<0.05

be seen that, although 10 μg of homologous endotoxin will abolish bactericidal activity, even 100 μg of antigenically unrelated endotoxins are without effect. Thus it can be concluded that there is a specific basis for the increase in bactericidal antibody which follows administration of endotoxin to animals and no evidence for cross-reacting antibodies was obtained.

Finally, the possibility was investigated that administration of endotoxin leads to the release of some nonspecific substance which will increase the efficiency of antibody in the bactericidal reaction. The findings so far reported would be explicable on such a basis because absorption or blocking studies would behave in a specific manner in such a situation. An example of experiments done to test this hypothesis is now given. Antibody for *S. typhosa* was absorbed from serum obtained from mice which had received 50 μg of *S. dysenteriae* endotoxin. After absorption, this serum was found still to possess increased bactericidal activity for *E. coli*; therefore, if a nonspecific cofactor existed, it still remained in the serum. Furthermore, a portion of this absorbed serum added as a supplement to a bactericidal titration of normal mouse serum against *S. typhosa* did not increase the titer or activity of the normal mouse serum. Such results make it unlikely that a nonspecific cofactor is involved and lead to the conclusion that the increase in bactericidal titer after endotoxin administration to mice was due to a low level, general increase of specific antibodies to gram-negative species.

Summary and Conclusions

Endotoxin administered to mice leads to changes in the metabolic activity of the mononuclear phagocytes. With small doses, only stimulation occurs, but with higher doses there is an initial period of depression followed by a period of stimulation, which with large doses may last for at least 8 days. The metabolic changes may reflect the improved phagocytic performance of these cells but opsonic factors are still necessary for adequate ingestion of enterobacteriaceae. Nonetheless, the metabolic changes alone may explain the longer duration of nonspecific resistance in staphylococcal and mycobacterial infections, where opsonic factors are not an absolute requirement for phagocytosis to occur.

Following endotoxin administration there is a general release of substances active against enterobacteriaceae which show the specific activity characteristic of antibodies. These alterations in the level of antibody were produced with endotoxins in amounts known to modify resistance to experimental infection. Moreover, the serum effect was dose dependent in a manner analogous to changes in resistance. A major activity of specific antibody is the ability to opsonize organisms for phagocytosis. As the antibodies described here prepare bacteria for the lethal action of complement, it is reasonable to assume that they can also function as opsonins. Although no direct correlation has been established, the changes reported here should be intimately involved in determining the outcome of infection, since they affect both the very cells which are concerned in host defense against an intraperitoneal challenge and a humoral factor which is known to increase the efficiency of these cells as phagocytes.

Emphasis has long been laid on the lack of specificity of the phenomenon known as nonspecific resistance. This designation was based on the criterion that administration of any endotoxin led to increased resistance and activity against antigenically unrelated strains. This basis for nonspecificity cannot be questioned, but we have found that, as far as serum changes are concerned, the result of endotoxin administration is to increase the general level of specific antibody in the host. It may be appropriate, therefore, to consider whether "nonspecific resistance" is a suitable designation for this effect or whether a title more

truly descriptive of the immunological changes induced by endotoxin should be sought.

LITERATURE CITED

1. BENACERRAF, B., M. M. SEBESTYEN, AND S. SCHLOSSMAN. 1959. A quantitative study of the kinetics of blood clearance of P^{32}-labelled *Escherichia coli* and staphylococci by the reticulo-endothelial system. J. Exptl. Med. **110**:27–48.
2. BENACERRAF, B., AND P. MIESCHER. 1960. Bacterial phagocytosis by the reticulo-endothelial system *in vivo* under different immune conditions. Ann. N. Y. Acad. Sc. **88**:184–195.
3. BIOZZI, G., B. BENACERRAF, AND B. N. HALPERN. 1955. The effect of *Salmonella typhi* and its endotoxin on the phagocytic activity of the reticulo-endothelial system in mice. Brit. J. Exptl. Pathol. **36**:226–235.
4. BOYD, W. C. 1956. Fundamentals of immunology. 3rd ed. Interscience Publishers, Inc., New York, 776 p.
5. COHN, Z. A., AND S. I. MORSE. 1960. Functional and metabolic properties of polymorphonuclear leucocytes. I. Observations on the requirements and consequences of particle ingestion. J. Exptl. Med. **111**:667–687.
6. COHN, Z. A., AND S. I. MORSE. 1960. Functional and metabolic properties of polymorphonuclear leucocytes. II. The influence of a lipopolysaccharide endotoxin. J. Exptl. Med. **111**:689–704.
7. DUBOS, R. J., AND R. W. SCHAEDLER. 1956. Reversible changes in the susceptibility of mice to bacterial infections. I. Changes brought about by injection of pertussis vaccine or of bacterial endotoxins. J. Exptl. Med. **104**:53–65.
8. FIELD, T. E., J. G. HOWARD, AND J. L. WHITBY. 1955. Studies on the rapid production of a non-specific type of immunity to *Salmonella typhi* infection in mice. J. Roy. Army Med. Corps **101**:324–334.
9. FISCHER, J. 1959. Zur Beeinflussung der Serumbakterizidie. Schweiz. Z. allgem. Pathol. u. Bakteriol. **22**:827–843.
10. HOWARD, J. G., D. ROWLEY, AND A. C. WARDLAW. 1958. Investigations on the mechanism of stimulation of non-specific immunity by bacterial lipopolysaccharides. Immunology **1**:181–203.
11. JENKIN, C., AND D. L. PALMER. 1960. Changes in the titre of serum opsonins and phagocytic properties of mouse peritoneal macrophages following injection of endotoxin. J. Exptl. Med. **112**:419–429.
12. LANDY, M., AND L. PILLEMER. 1956. Increased resistance to infection and accompanying alteration in properdin levels following administration of bacterial lipopolysaccharides. J. Exptl. Med. **104**:383–409.
13. LANDY, M., J. G. MICHAEL, AND J. L. WHITBY. 1962. A bactericidal method for the measurement in normal serum of antibody to gram-negative bacteria. J. Bacteriol. **83** (*in press*).
14. LEE, L., AND C. A. STETSON. 1960. Studies on the mechanism of the Shwartzman phenomenon. Accelerated cutaneous reactivity to bacterial endotoxins. J. Exptl. Med. **111**: 761–770.
15. MACKANESS, G. B. 1960. The phagocytosis and inactivation of staphylococci by macrophages of normal rabbits. J. Exptl. Med. **112**:35–53.
16. MICHAEL, J. G., J. L. WHITBY, AND M. LANDY. 1961. Increase in specific bactericidal antibodies after administration of endotoxin. Nature **191**: 296–297.
17. MICHAEL, J. G., J. L. WHITBY, AND M. LANDY. 1961. Studies on natural antibodies to gram-negative bacteria. J. Exptl. Med. (*in press*).
18. PFEIFFER, R., AND ISSAEFF. 1894. Ueber die spezifische Bedeutung der Choleraimmunität. Z. Hyg. Infektionskrankh. **17**:355–400.
19. PHILIPSON, J. 1937. Experimental studies on enhanced resistance to infection following some non-specific measures. Acta Pathol. Microbiol. Scand. Suppl. No. **32**:1–148.
20. ROWLEY, D. 1955. Stimulation of natural immunity to *Escherichia coli* infections. Observations on mice. Lancet **1**:232–234.
21. ROWLEY, D. 1956. Rapidly induced changes in the level of non-specific immunity in laboratory animals. Brit. J. Exptl. Pathol. **37**: 223–34.
22. ROWLEY, D., AND J. L. WHITBY. 1959. The bactericidal activity of mouse macrophages *in vitro*. Brit. J. Exptl. Pathol. **40**:507–515.
23. ROWLEY, D. 1960. The role of opsonins in non-specific immunity. J. Exptl. Med. **111**:137–144.
24. SBARRA, A. J., AND M. L. KARNOVSKY. 1959. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leucocytes. J. Biol. Chem. **234**:1355–1362.
25. SHILO, M. 1959. Nonspecific resistance to infections. Ann. Rev. Microbiol. **13**:255–278.

26. TEAGUE, O., AND H. I. MCWILLIAMS. 1917. Experiments with a possible bearing upon treatment of typhoid fever with typhoid vaccine administered intravenously. J. Immunol. **2**:185–191.
27. WHITBY, J. L., AND D. ROWLEY. 1959. The role of macrophages in the elimination of bacteria from the mouse peritoneum. Brit. J. Exptl. Pathol. **39**:358–370.
28. WOODS, M. W., M. LANDY, J. L. WHITBY, AND D. BURK. 1961. Symposium on bacterial endotoxins. III. Metabolic effects of endotoxins on mammalian cells. Bacteriol. Rev. **25**:447–456.