INCREASED RESISTANCE TO INFECTION AND ACCOMPANYING ALTERATION IN PROPERDIN LEVELS FOLLOWING ADMINISTRATION OF BACTERIAL LIPOPOLYSACCHARIDES*

By MAURICE LANDY,‡ Ph.D., and LOUIS PILLEMER, Ph.D.

(From the Walter Reed Army Institute of Research, Washington, D.C., and the Institute of Pathology, Western Reserve University, Cleveland)

(Received for publication, June 8, 1956)

The attention of immunologists has long been focused on acquired immunity produced by specific antigens. In the classical sense this type of immunity to infection, which generally is of a high order, depends upon the appearance of protective antibodies. The obvious importance of such artificial immunity, and the relative ease with which these antigens and antibodies could be studied, contributed to the comparative neglect of other components of bacterial cells which may also give rise to protective reactions. Nevertheless, it has been recognized that there is also a type of resistance which transcends the limits of antigenic specificity, and from time to time this kind of protection against infection was observed even though demonstrable antibodies could not be detected. In fact it was noted by a number of workers that the injection of various substances of bacterial and of other origin produced an increase in normal resistance. While the level of resistance thus achieved was lower than that which followed the injection of specific immunizing agents, it was sufficient to indicate that it might nonetheless contribute to immunity.

These observations extend back to some of the early work in microbiology and were first brought together by Kolle and Prigge (1). The more recent literature has been assembled by Brandis (2) who also reported the rapid production of a protective effect against challenge with *Salmonella derby* by the injection into mice of a number of bacterial vaccines or unrelated products. He called this effect "proimmunity" and considered it to be a result of non-specific stimulation of the cellular defenses of the host. Rowley (3) showed that mice injected with cell walls of *Escherichia coli*, *Salmonella typhimurium*, or zymosan (4), within the first few hours were more susceptible than normal mice to challenge with *E. coli*. 24 to 48 hours after the administration of these products, however, the animals became highly resistant to

* This investigation was conducted under the auspices of the Commission on Immunization, Armed Forces Epidemiological Board, and was supported in part by the Office of the Surgeon General, Department of the Army, Washington, D. C.

‡ Present address: Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda.

challenge and remained so for several days. Field, Howard, and Whitby (5) observed that mice immunized with *S. typhosa* or other Gram-negative bacilli rapidly developed resistance to challenge with *S. typhosa.* The protective effect of the immunologically unrelated organisms was of rather short duration, while that produced by the typhoid vaccine persisted. It has been the experience of many investigators that animals injected with a variety of homologous or unrelated organisms develop increased non-specific resistance to experimental infections before the appearance of antibodies.

Certain of these bacteria and bacterial products, as well as other substances such as zymosan, have been shown to interact with the properdin system *in vitro* and to alter properdin levels *in vivo* (6). The properdin system (7), consisting of properdin, complement, and magnesium ions, exerts a marked bactericidal effect on Gram-negative bacteria (8). The wide variety of infectious agents that are killed or inactivated by the properdin system, and its presence in the serum of normal human beings and other mammals, suggests that it may be a factor in natural resistance.

It occurred to us that the somatic antigen (lipopolysaccharide) might be the component in the bacteria or their cell walls responsible for the effects previously observed. Indeed, it was shown that a small dose of any of a variety of bacterial lipopolysaccharides did, in fact, evoke in mice a rapidly developing, non-specific increase in resistance to infection with Gram-negative pathogens (9). The bacterial lipopolysaccharides employed in these studies were also shown to combine with properdin *in vitro* and to alter properdin levels *in vivo* in a manner similar to that observed with cell walls and related bacterial products. Furthermore, it was shown (10) that these lipopolysaccharides not only raised properdin levels, but that on a weight basis they possessed greater activity than other substances previously studied. A quantity as small as 1 μg. was effective in producing in mice a significant rise in properdin.

The present study is concerned with a more complete characterization of this increased resistance. Particular attention is directed toward the influence of the nature of the bacterial product, the dose, and the timing of its administration on the rate, magnitude, and duration of the ensuing increased resistance. More definitive information is presented on the relationship of properdin titers to the transitory ability of the host to resist infection caused by Gram-negative pathogens.

Materials and Methods

Bacterial Lipopolysaccharides.[1]—The product used in most of the experimental work was derived from viable *S. typhosa* 0901 by Webster *et al.* An array of other lipopolysaccharide

[1] It should be pointed out that the terms "bacterial lipopolysaccharide," endotoxin," and "somatic antigen," are equally applicable to the products derived from smooth "O" strains of Gram-negative bacteria. However, the products obtained from rough variants differ in that they do not possess characteristic somatic antigenic properties and they do not appear to be located on the bacterial surface. Consequently, the term "somatic antigen" is not appropriate for these substances. Westphal and Lüderitz isolated toxic lipopolysaccharides

preparations, generously donated by various investigators, were also examined for their effect on resistance. The source of the products which are referred to in Table II is indicated by the following code letters.

Code	Source	Reference to preparation and/or properties
A	Dr. M. Usdin, Baxter Laboratories, Morton Grove, Illinois	(11)
B	Mr. H. W. Schoenlein, Difco Laboratories, Detroit, Michigan	
C	Dr. M. Raynaud, Institut Pasteur, Garches, France	(12)
D	Dr. M. J. Shear, National Cancer Institute, Bethesda, Maryland	(13)
E	Dr. O. Westphal, Dr. A. Wander Forschungsinstitut, Sackingen, Germany	(14, 24)
F	Dr. M. E. Webster, Walter Reed Army Institute of Research, Washington, D. C.	(15)

Cultures.—The cultures employed to induce experimental infections in mice; and their source, cultivation and infectivity are as follows:

Organism	Strain	Supplied by	Cultivation	LD_{50}*
Salmonella typhosa	Ty 2	WRAIR cult. coll.	6 hrs. on veal infusion agar	8×10^8
Escherichia coli	2380	Dr. D. Rowley		2×10^7
Proteus vulgaris	From post-irradiation bacteriemia of mice	Dr. W. W. Smith		5×10^7
Pseudomonas aeruginosa				2.5×10^7
Klebsiella pneumoniae	Type 1	Dr. O. A. Ross	16 hrs. in brain-heart infusion broth	<25
Staphylococcus aureus	Giorgio	Dr. R. J. Dubos		25×10^6
Streptococcus pyogenes	C-203	WRAIR cult. coll.		25
Diplococcus pneumoniae	Type 1	Dr. O. Stark		10

* By the i.p. route, with the exception of *S. aureus*, which was injected i.v.

Tests for Production of Resistance to Infection.—Female white albino mice of the Bagg strain, weighing 14 to 16 gm. were employed throughout. Assignment of animals to jars and the order of administration of lipopolysaccharide and challenge injection, by groups,

from rough strains of Gram-negative organisms by phenol-water extraction (24), while Raynaud *et al.* (12) obtained this type of product from the R-2 strain of *S. typhosa* by extraction of acetone-treated cells with hypertonic saline and subsequent fractionation with trichloroacetic acid. Other procedures suitable for the extraction of lipopolysaccharides from smooth strains have proved to be ineffective for rough strains. Therefore, for a long time, it was considered that rough strains did not contain any endotoxic material. However, the work of the aforementioned investigators has clearly shown that by appropriate means, toxic lipopolysaccharides may be obtained from rough, as well as smooth, Gram-negative organisms.

were all determined by randomization procedures. Unless otherwise indicated, each group consisted of 10 or more mice. Injection of lipopolysaccharide was made intraperitoneally in a standard volume of 0.5 ml. saline at intervals ranging from 120 hours to 1 hour prior to challenge. In the experimental infection of mice with *S. typhosa* or other Gram-negative pathogens, the use of mucin as a host resistance depressant was avoided, since it has been shown that this material is anticomplementary (16) and that it combines with properdin *in vivo* (6). Its use might, therefore, introduce still another factor into an already complex system. Instead, 6 hour agar-grown cultures of the challenge organisms, suspended in saline, served as the challenge inocula. The number of organisms administered generally represented 3 to 6 LD_{50}. Unless otherwise indicated, mice were challenged intraperitoneally with 5×10^7 *S. typhosa* Ty 2. This represents approximately 6 LD_{50}.

For a number of years Bagg mice and the Ty 2 strain of *S. typhosa* have been used by one of us for typhoid studies. There was thus provided a well standardized experimental infection in which the major immunological components involved have been identified (17).

TABLE I

Increased Resistance to Infection Following Administration of Bacterial Lipopolysaccharide Non-Specificity of the Effect as Regards Product and Route

Treatment			Challenge	Hrs. elapsed between injection of lipopolysaccharide and challenge				
Product derived from	Dose	Route		1	3	6	12	24
				Per cent Survivors				
E. coli 08	10 μg.	I.p.	2.4×10^7 *S. typhosa* Ty 2	10	15	80	100	100
		I.v.		5	20	90	100	95
S. typhosa 0901		I.p.		0	10	85	100	90
		I.v.		15	30	95	95	100

Consequently, in addition to the use of this system in the study of non-specific resistance, it was also possible to make certain comparisons with specific immunity.

Properdin Titration.—Mice were exsanguinated by bleeding directly from the heart under chloroform anesthesia. Blood from groups of 10 mice was pooled to provide a single serum specimen for properdin assay. Serum samples were frozen promptly, held at −55°C., and submitted to one of us coded, for properdin assay.

Serum samples were thawed at room temperature, centrifuged at 35,000 *g* at 2°C. for 2 hours (10) and the supernatants tested for properdin by the zymosan assay (18). All serum samples of any given experiment were titrated for properdin content on the same day and with the same reagents.

EXPERIMENTAL

Within a few hours after the injection of bacterial lipopolysaccharides the resistance of mice to infection with *S. typhosa* rose markedly. Thus, in a typical experiment, groups of mice were injected intravenously or intraperitoneally with 10μg. of coli or typhoid lipopolysaccharide at varying intervals prior to challenge with *S. typhosa*. The results of this experiment, given in Table I,

show that the rise in resistance produced by immunologically unrelated, as well as homologous lipopolysaccharides, was similar. Furthermore, a similar effect was evoked by these products administered by either i.p. (the same route as the challenge) or i.v. routes. Since the lipopolysaccharide administered by either route produced the same rise in resistance, the more convenient i.p. route was used in all subsequent experiments.

Screening of Bacterial Lipopolysaccharides.—The bacterial lipopolysaccharides employed in this study were the somatic antigenic components of Gram-negative organisms. In addition to their specific immunological attri-

TABLE II

Biological Activity Shown by Various Bacterial Lipopolysaccharides

Lipopolysaccharides derived from		Preparations tested		Response elicted in mice by 1 or 10 μg. administered 24 hrs. earlier			
Organism	Colonial type	Source*	No. examined	Resistance to challenge with *S. typhosa*		Elevation in properdin level	
				1 μg.	10 μg.	1 μg.	10 μg.
Escherichia coli (5 strains)	S	B, E, F	5	+(3)	+(5)	+(2)	+(4)
Hemophilus pertussis	S	F	1	+	+	−	+
Pseudomonas aeruginosa	S	A, F	2	+	+	−	+
Salmonella enteritidis	R	E	1	+	+	+	+
" *schottmuelleri*	R	E	1	+	+	−	+
" *typhosa*	S	B, E, F	3	+(2)	+(3)	+(2)	+(2)
" "	R	C	1	+	+	−	+
Serratia marcescens	S	B, D, F	3	+(1)	+(3)	−(1)	−(1)
Shigella flexneri	S	F	1	+	+	−	−

+, significant increase in resistance or elevation in properdin level; − = lack of effect. Nos. in parentheses, number of specimens of total tested which provided effect indicated.

* Code given under Materials and Methods.

butes, they evoke in certain experimental animals and in man a characteristic array of physiological alterations including fever, cellular changes, local skin reactivity, toxicity and even lethal effects (19).

A total of 18 lipopolysaccharide preparations were examined for their ability to increase resistance of mice to infection and to elevate properdin levels. As is shown in Table II, these products were derived from a total of 6 genera, from a number of serotypes, and from smooth and rough colonial variants. It is noteworthy that they were prepared in 6 different laboratories by varied methods of fractionation and purification. Nonetheless, all were active in producing in mice an increase in resistance to challenge with *S. typhosa*. Many, but not all, caused an elevation in properdin titer of 50 per cent or more above

normal. The screening of this collection of preparations for these biological activities was limited to two dose levels and one time interval. A more extensive study might well reveal further quantitative or perhaps even qualitative differences between these preparations. However, this survey is sufficient to indicate that the components responsible for these effects are common to Gram-negative organisms and that they are obtained by a variety of preparative techniques.

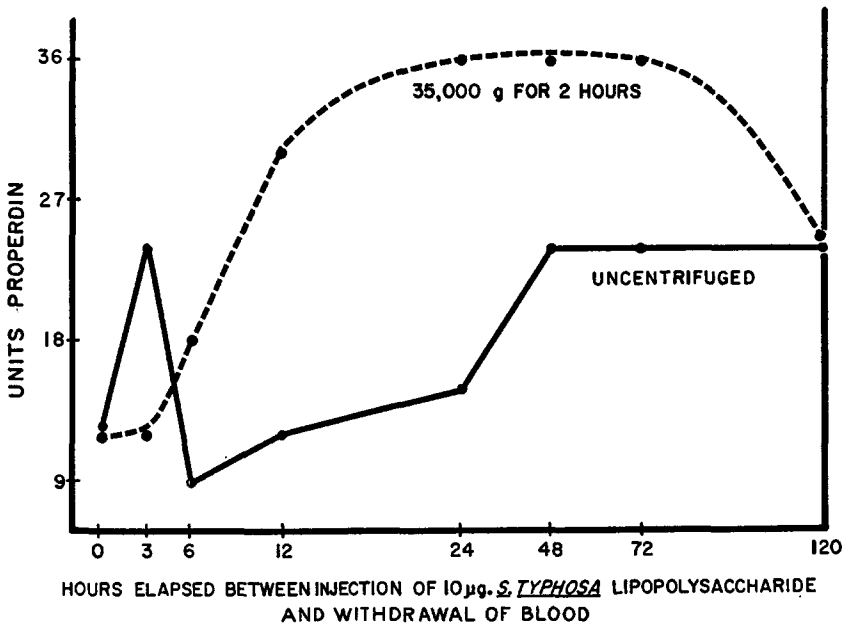

FIG. 1. Properdin levels in mice following injection of lipopolysaccharide. Effect of centrifugation on properdin values.

Production of Host Products.—It was reported by Landy and Pillemer (10) that following the administration of small amounts of bacterial lipopolysaccharides, high molecular weight substances appeared in the blood of mice, which interfered with the assay of properdin. These substances could be removed from serum by centrifugation at 35,000 *g*. This effect is demonstrated in the following experiment which is typical of a number conducted.

Seven groups of 100 mice each were injected i.p. with 10 μg. of lipopolysaccharide at intervals ranging from 120 to 3 hours prior to exsanguination. The serum pools from these seven groups and a control group of mice were assayed for properdin before and after centrifugation at 35,000 *g* for 2 hours at 2°C. The resultant properdin values, plotted against time elapsed between injection of lipopolysaccharide and exsanguination are shown in Fig. 1.

It is apparent that the centrifuged serums present a smooth pattern of early rise in properdin values, reaching a maximum of approximately 3 times normal, which is maintained for several days. In contrast the same serums, uncentrifuged, yield much lower and rather erratic properdin values. The difference in these two sets of values is believed to be due to the production or release by the host of high molecular weight substances which appear in the blood and interfere with the interaction of properdin and complement with

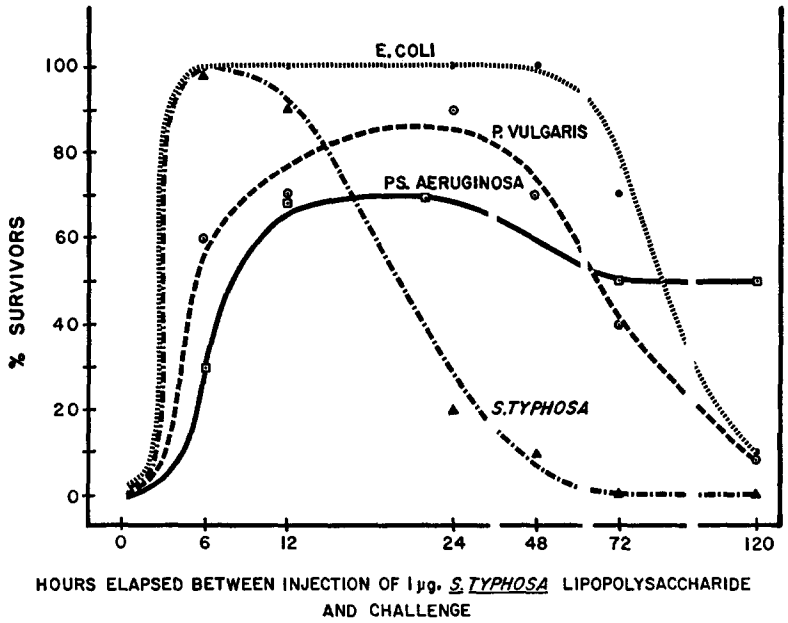

FIG. 2. Increase in resistance to various Gram-negative pathogens following administration of lipopolysaccharide.

zymosan. For this reason the properdin titrations here reported were conducted on serum samples centrifuged at 35,000 *g*.

Resistance to Various Experimental Infections.—For reasons previously stated, in most of the present work experimental infection was produced in mice by the intraperitoneal inoculation of *S. typhosa*. It was of interest, however, to determine whether the observed increased resistance to infection produced in mice by lipopolysaccharide extended to other experimental infections. These fell into two major categories—infections produced by Gram-negative pathogens and by Gram-positive bacteria.

Among the former, the organisms tested in addition to *S. typhosa* were *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*. The LD_{50} for these cultures ranged from 10 to 50 million organisms. All organisms were administered in saline at a level of approximately

1 to 3 lethal doses. These comparisons were made in the form of time-dose experiments in which groups of mice received either 10, 1, or 0.1 μg. of *S. typhosa* lipopolysaccharide at various intervals prior to infection with one of the four challenge organisms. The mortality data obtained are too voluminous and too complex to be presented in full but that portion shown in Fig. 2 indicates the differences in patterns of resistance observed for each challenge strain. The mice received 1 μg. of lipopolysaccharide at the indicated intervals prior to infection with the four challenge organisms.

It is apparent that the effect of this treatment of mice with a given dose of lipopolysaccharide varies considerably for the four experimental infections, particularly the rate of development of resistance and its duration. Not shown, but also exerting an important effect on onset and duration, is the quantity of lipopolysaccharide administered. The reasons for these differences remain to be determined.

Treatment of mice with bacterial lipopolysaccharides in a manner highly effective in protecting against experimental infection with the aforementioned Gram-negative bacterial species, failed to produce a demonstrable protective effect in animals challenged with *Staphylococcus aureus*, *Streptococcus pyogenes*, or *Diplococcus pneumoniae*. In each instance the challenge employed was minimal in amount in order to provide the most favorable conditions for demonstrating even a slight increase in resistance following injection of lipopolysaccharide. However, these animals succumbed to each of these experimental Gram-positive infections in a manner indistinguishable from the controls.

It should be pointed out that these Gram-positive cocci are insensitive to the properdin system as measured by the *in vitro* bactericidal test (8) while the four Gram-negative organisms studied are killed by the properdin system *in vitro*.

Infections with Highly Virulent Gram-Negative Species.—The foregoing experiments clearly showed that a single injection of lipopolysaccharide increased the resistance of mice to infection with any of 4 different Gram-negative bacterial species. Since relatively large numbers of these organisms are required to produce a fatal infection in mice, it was of interest to extend the study to a bacterial species pathogenic for mice to a degree exceeding that of the organisms previously examined. *Klebsiella pneumoniae*, Type 1, was employed because it is capable of establishing a fatal infection with few cells. Preliminary tests indicated that lipopolysaccharide also exerted a protective effect in mice challenged with this organism. Accordingly, a number of dose-time experiments were conducted and the results of one of these tests are given in Table III. Inspection of this table shows that the increased resistance to this highly pathogenic organism induced by lipopolysaccharide was generally similar to that described above for a number of Gram-negative organisms of much lower virulence. The administration of a large amount of lipopolysaccharide (100 μg.) results in a delay in the development of resist-

ance, while smaller amounts evoke a prompt rise. The duration of increased resistance is related to dose in that the smallest amount of lipopolysaccharide provided the shortest period of protection and this resistance increased stepwise as the dose was raised by 10-fold increments. In this experiment, the challenge dose of 250 organisms represented more than 10 LD_{50}. In other experiments made with challenges of 100 to 1000 LD_{50} or more, only a small proportion of the mice were protected 6 hours after receiving 10 μg., or 24 hours after injection of 100 μg. However, in tests employing these larger challenges, it was noted that the animals receiving lipopolysaccharide survived 24 to 36 hours longer than did the controls. Results somewhat similar to these have been obtained by our colleague, Dr. L. Baron, in mice experimentally infected with *S. typhimurium*. He observed (20) that mice treated with 10

TABLE III

Development of Resistance to Infection with Klebsiella pneumoniae Following Administration of a Bacterial Lipopolysaccharide

Quantity of *S. typhosa* lipopolysaccharide injected i.p.	Challenge*	Hrs. elapsed between injection of lipopolysaccharide and challenge					
		3	6	12	24	48	72
		Per cent survivors					
μg.							
100	*Klebsiella*	20	30	70	100	80	100
10	*pneumoniae*	70	80	100	100	50	20
1	Type 1	90	80	100	0	10	0
0.1	250 organisms	70	90	10	10	0	0

* LD_{50} <25 organisms.

of 100 μg. of the typhoid lipopolysaccharide, survived longer than control mice, and that the period of survival was related to the quantity of lipopolysaccharide administered. However, with this natural pathogen of mice, the protection observed was not comparable to that here reported for a number of other Gram-negative infections.

Time and Dose Relationships.—Early in these studies it was observed that increased resistance developed in mice within hours after injection of a bacterial lipopolysaccharide. However, it soon became apparent that the induction of this early rise in resistance and the accompanying increase in properdin titer were affected by a number of factors. It was not possible to examine systematically all these factors. However it was established that, with an effective lipopolysaccharide and an appropriate experimental infection, the major variables affecting properdin titers and survival were the dose of lipopolysaccharide administered and the interval between its injection and chal-

lenge or bleeding. Other important variables were the strain of mice employed[2] and a number of factors pertaining to the challenge inoculum, such as quantity, age of the culture, menstruum used for suspending the challenge, etc.

These observations, made in a series of pilot experiments, led to the use of "dose-time" experiments as a means of obtaining more complete information on the effect of lipopolysaccharide on properdin titers and on resistance or susceptibility to infection. The results of a typical experiment are depicted graphically in Figs. 3, 4, and 5. In Fig. 3 it will be seen that the administration of a *large* dose (100 μg.) of typhosa lipopolysaccharide was attended by a considerable delay of 12 hours or more, in the development of resistance. During this time the animals were *more* susceptible to challenge than were normal mice in that smaller number of organisms proved fatal. With smaller quantities (10, 1, or 0.1 μg.) delay in the development of resistance was reduced to a few hours and during this induction phase there was no evidence of increased susceptibility.[3] With respect to the duration of the effect, the converse occurred, the 100 μg. quantity provided the most prolonged period of increased resistance, while 10, 1, and 0.1 μg. gave proportionately shorter periods of protection.

Fig. 4 shows the serum properdin titers at the time the aforementioned groups were challenged. The injection of 100 μg. caused a fall in properdin titer to approximately half of normal between 12 and 24 hours, which was succeeded by a marked rise to 2 to 3 times the normal which persisted for 5 days. The injection of 10 μg. resulted in an increase as early as 6 hours, which then rose to levels of 2 to 3 times the normal at 24 hours and persisted for an

[2] In a preliminary screening of 7 inbred mouse strains, groups of 10 mice each were given 10, 1, or 0.1 μg. of bacterial lipopolysaccharide at intervals of 48, 24, 12, or 6 hours prior to challenge with *S. typhosa*. Control mice of all strains succumbed to the challenge; however, marked differences were observed in the pattern of resistance to infection developed in these strains as a result of the lipopolysaccharide treatment. Thus Bagg (BALB), BALB/c, and NIH mice exhibited the type of response here described, with relatively minor variations. CFW mice failed to respond to 0.1 μg. and with 1 or 10 μg. developed resistance of shorter duration than the above mentioned strains. A/LN and C57BL mice showed practically no evidence of response to 0.1 or 1 μg., while 10 μg. produced an effect lasting only 24 hours. Rockefeller (28) mice were relatively unaffected by the treatment.

[3] To examine these early changes more closely, particularly as they might be related to the amount of lipopolysaccharide administered, quantities of lipopolysaccharide ranging from 0.1 to 0.001 μg. were given to groups of mice at 1 hour intervals from 12 hours to 1 hour prior to challenge with *S. typhosa*. These experiments showed that, when the quantity of lipopolysaccharide was reduced to less than 0.1 μg., the induction period preceding increased resistance was minimal. On the other hand, the duration of the increased protection was shortened correspondingly until with 0.001 μg. it lasted but 4 hours. Quantities of lipopolysaccharide less than 0.001 μg. failed to produce any consistent rise in resistance. It probably is significant that no product or dose tested provided any substantial increased resistance occurring earlier than 3 to 4 hours after its administration. It was observed repeatedly that when the rise in resistance occurred it was sudden and rapid, rather than gradual.

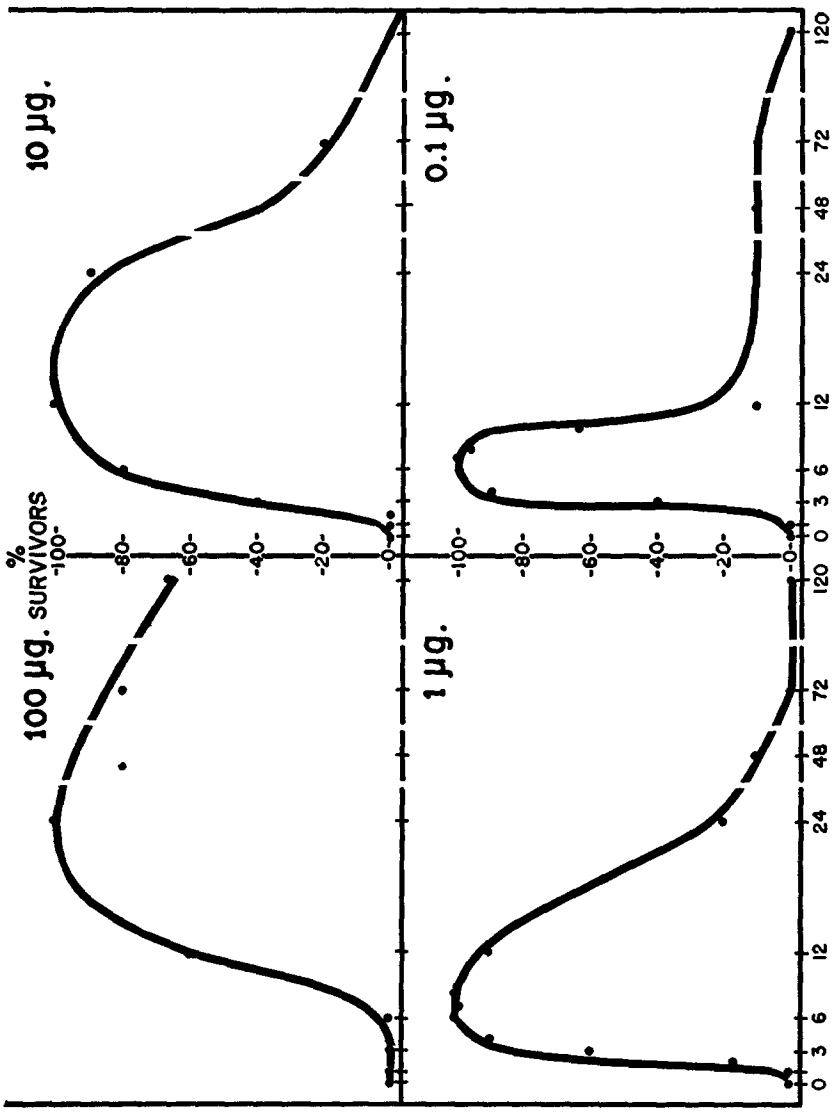

FIG. 3. Development of resistance to *S. typhosa* infection following administration of lipopolysaccharide. Dose-time relationships.

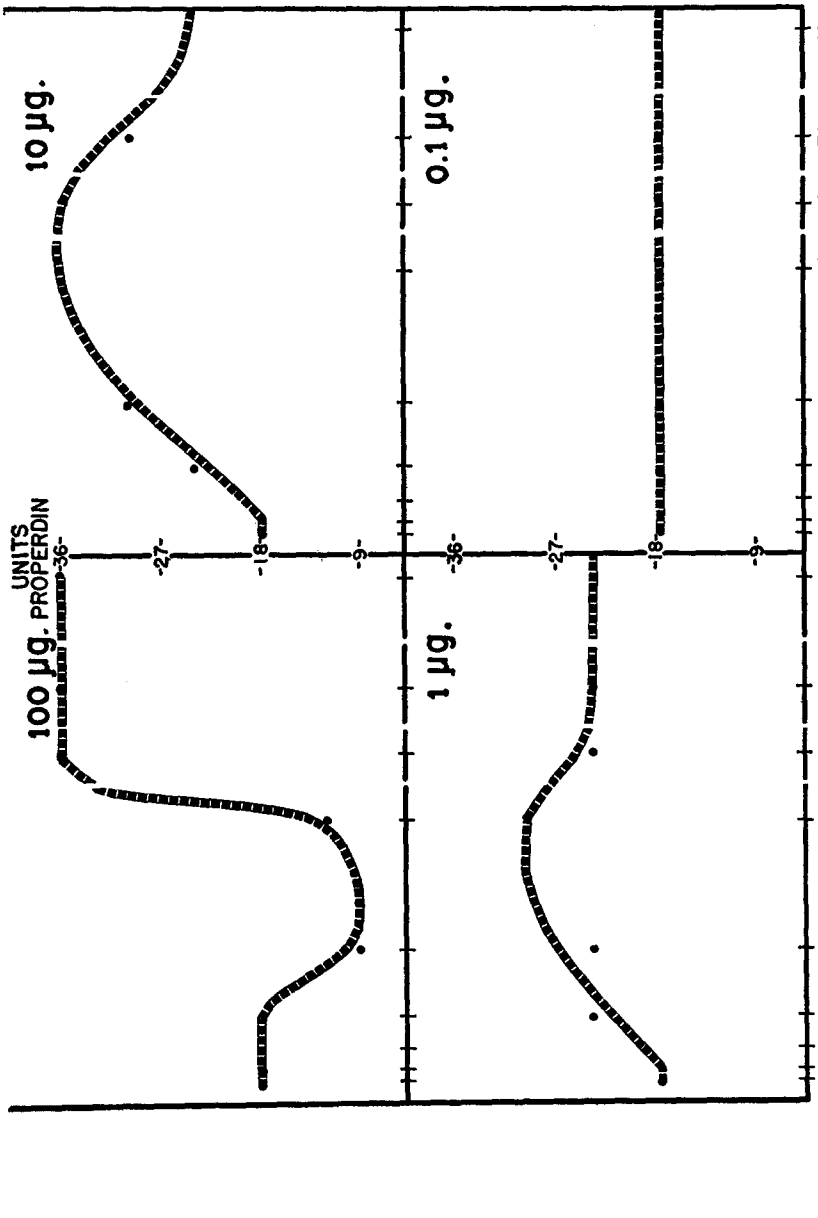

FIG. 4. Elevated properdin levels following administration of lipopolysaccharide. Dose-time relationships.

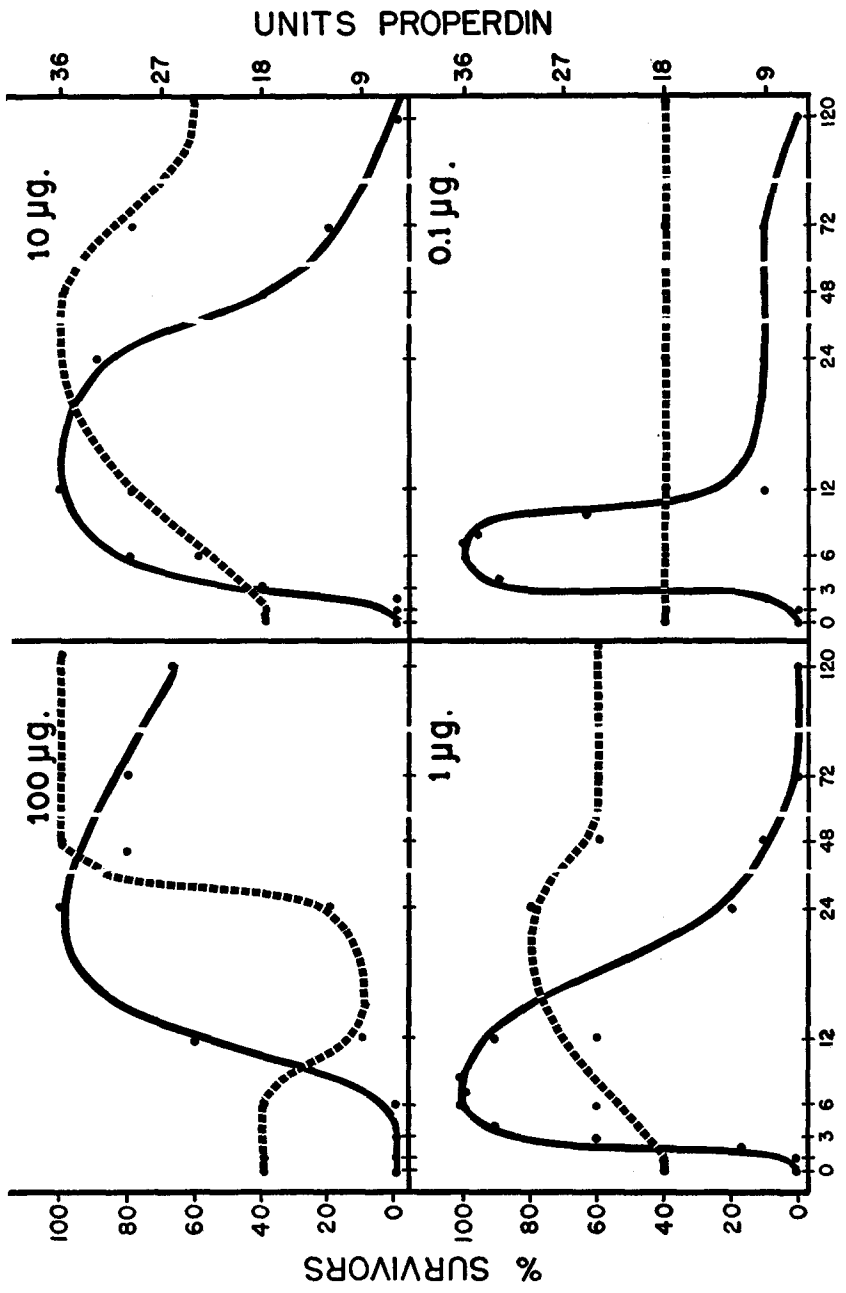

FIG. 5. Relationship of elevated properdin levels to increased resistance following administration of lipopolysaccharide. ——, per cent survivors; ....., units properdin/ml. of serum.

additional 48 hours. Attention should be called to the fact that this elevation occurred without the prior fall in properdin titers as observed previously when large doses of zymosan (6) were employed. This effect may, therefore, be due to a direct stimulation of the synthesis or release of properdin rather than to a mechanism which represents over-compensation on the part of the host. The effect of 1 μg. was qualitatively similar to that obtained with 10 μg. except that the increase in properdin titer was somewhat less. No alteration in properdin levels occurred following either 0.1 μg. of lipopolysaccharide or 0.5 ml. of saline. Indeed, the constancy of the values in control animals and those receiving 0.1 μg. was noteworthy. Thus, in the development of resistance and the rise in properdin levels, the rate, magnitude, and duration of these effects were related to the dose of lipopolysaccharide employed.

Although Fig. 5 shows that some sort of relationship exists between the properdin titers and the degree of protection developed in response to the injection of lipopolysaccharide, several inconsistencies will be noted. For example, (*a*) properdin titers generally rose after resistance had developed; (*b*) in mice receiving 100 μg. of lipopolysaccharide the rise in resistance occurred 12 hours after injection, a time when properdin levels were at their lowest; (*c*) elevated properdin levels generally persisted well after resistance had waned; and (*d*) increased resistance for a period of 4 to 8 hours was afforded by as little as 0.1 μg. in the absence of any demonstrable increase in properdin titer.

Influence of Infection on Properdin Response.—From the results presented above, it was apparent that, following the administration of lipopolysaccharide, the ensuing pattern of elevated properdin levels of the animal prior to infection did not uniformly reflect the ability of the host to maintain that titer during infection. It was therefore visualized that following the initiation of infection, host-parasite interactions could become extremely complex and that during this important period properdin levels may differ considerably from those of the prechallenge pattern. In order to obtain information on the nature of these changes, the bacteriologic sequence of events and the serum properdin alterations were followed post challenge in specifically and non-specifically protected mice, as well as in controls. These are shown graphically in Fig. 6. For specific immunization, mice were given 1 μg. of purified Vi antigen 5 days prior to challenge. This treatment affords protection accounted for by the specific antibody produced in response to this antigenic stimulus (21). Non-specific protection was provided by injection of 10 μg. of a coli lipopolysaccharide. This effect is independent of the production of specific antibody. Previous tests have shown that both types of treatment provide complete protection against challenge with *S. typhosa.*

In control mice, properdin titers remained constant during the period of 3 to 6 hours post challenge. However, by 12 hours, when deaths began to

occur, there was a drop in properdin which, in time, became even more pronounced as an increasing proportion of the animals became moribund. These changes were correlated with increasing bacterial populations (viable count of peritoneal contents) in these animals. On the other hand, mice treated with 10 μg. of a coli lipopolysaccharide 24 hours earlier, and exhibiting peak properdin levels at the time of challenge, showed a contrasting pattern of bacteriologic and properdin responses. During the first 6 hours there was moderate

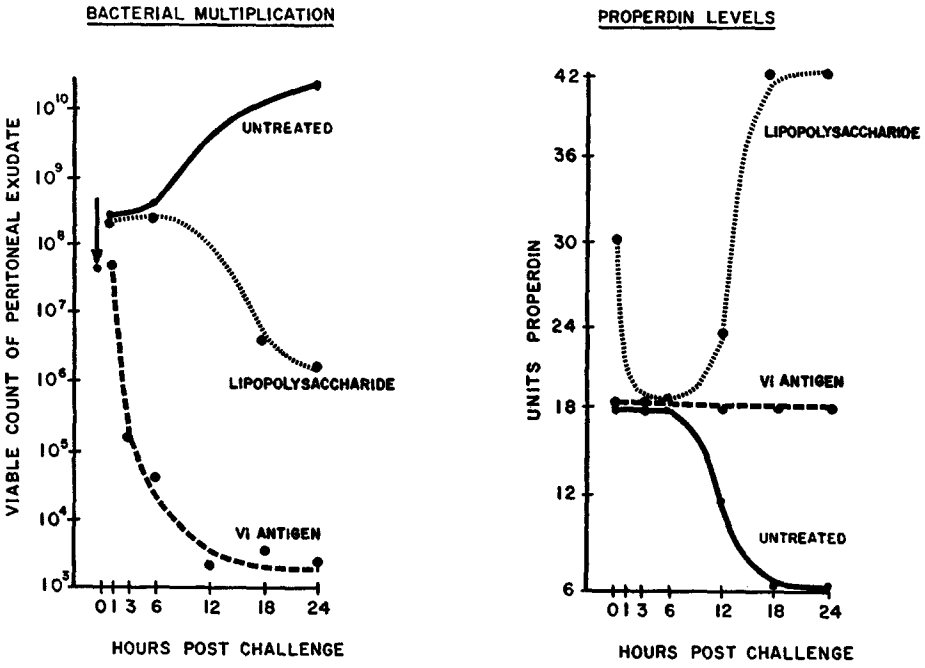

FIG. 6. Comparison of non-specific resistance and specific immunity in mice experimentally infected with *S. typhosa*.

multiplication of organisms and a drop in properdin titer from the initial high value (of approximately twice normal) to normal. At 12 to 18 hours, post challenge, the very time when bacterial populations in the controls were rapidly rising to lethal levels and properdin reserves were being depleted, the lipopolysacchride-treated mice not only were maintaining normal properdin values but these were, in fact, increased within 12 hours to 3-½ times normal, a value extremely high for this species. At the same time, further bacterial multiplication was suppressed and the number of organisms declined from 102 million at 12 hours, to 4 million at 18 hours and 0.4 million at 24 hours. These are well below lethal levels. Thus, at the time the control mice were

dying rapidly, the properdin titer of the lipopolysaccharide-treated animals was 7 times higher and these animals survived.

The Vi immunized mice present an entirely different type of response. In the presence of specific antibody, the multiplication of organisms ceased promptly and the numbers of viable bacilli fell precipitously from 110 million at 1 hour post challenge, to approximately 40 thousand at 6 hours and all animals survived. It is espically noteworthy that in these mice the properdin levels remained quite unchanged.

Relationship of Timing and Dose of Lipopolysaccharide to Postchallenge Properdin Alterations.—The preceding experiment provided some insight into the nature of the properdin alterations following the initiation of infection. Consequently, it was felt that this experimental approach might help to resolve the discrepancies evident in Fig. 5, in the apparent lack of correlation between properdin levels in normal mice and their subsequent resistance or susceptibility to infection. Of these, the three most divergent situations were reexamined as follows:

(*a*) In mice receiving 100 μg. of lipopolysaccharide there occurred a rise in resistance at 12 hours becoming complete at 24 hours, the interval when properdin levels are at their lowest. Accordingly, post challenge properdin levels were followed in groups of mice injected with 100 μg. of lipopolysaccharide at intervals of 24, 12, and 6 hours prior to challenge; mortality was determined in mice similarly treated. The results of this experiment are given in Table IV and show that in mice injected 6 hours prior to challenge, there occurred a continuous decline in properdin titers and these animals were not protected. Most of the mice injected 12 hours prior to challenge were protected. In such animals the properdin level was very low at the time of challenge, remained so for some time and then gradually rose to levels above the normal. The mice injected 24 hours before challenge all survived. At the time of challenge their properdin level was below normal, but shortly thereafter progressively rose to titers far above the normal.

(*b*) The injection of 10 μg. of lipopolysaccharide, 6, 12, or 24 hours prior to challenge, resulted in essentially complete protection, but provided little or no protection when the interval was increased to 48 or 72 hours. Therefore, groups of mice were injected with 10 μg. of lipopolysaccharide at intervals of 72, 48, 24, 12, and 6 hours prior to challenge. Their properdin levels, before and after challenge, are shown in Fig. 7. Even though high properdin levels were manifest at the time of challenge in mice injected 48 or 72 hours prechallenge, these animals were quite unprotected. Properdin levels in such mice declined precipitously to extremely low levels and these animals died. On the other hand, mice treated at the 24 hour interval were just approaching peak properdin titer at the time of challenge. In animals treated in this manner the challenge caused a rapid drop from the elevated properdin values

TABLE IV

Properdin Levels in Lipopolysaccharide-Treated Mice before and after Infection with S. typhosa

Treatment	Properdin levels at time of challenge	Properdin levels following initiation of infection							Survivors 72 hrs. following challenge
		Hrs. elapsed between challenge and exsanguination							
		3	6	12	18	24	48	72	
	(units/ml.)								*per cent*
100 μg. lipopolysaccharide 24 hrs. prior to challenge or bleeding	12	12	12	12	18	24	36	30	100
12 hrs. prior to challenge or bleeding	6	6	6	12	12	12	24	24	100
6 hrs. prior to challenge or bleeding	18	12	6	6	6	6	No survivors		10
0.1 μg. lipopolysaccharide 12 hrs. prior to challenge or bleeding	18	24	18	12	6	6	No survivors		20
6 hrs. prior to challenge or bleeding	18	12	24	12	6	12	18	18	100
0.5 ml. saline 12 hrs. prior to challenge or bleeding	18	18	18	6	6	3	No survivors		0

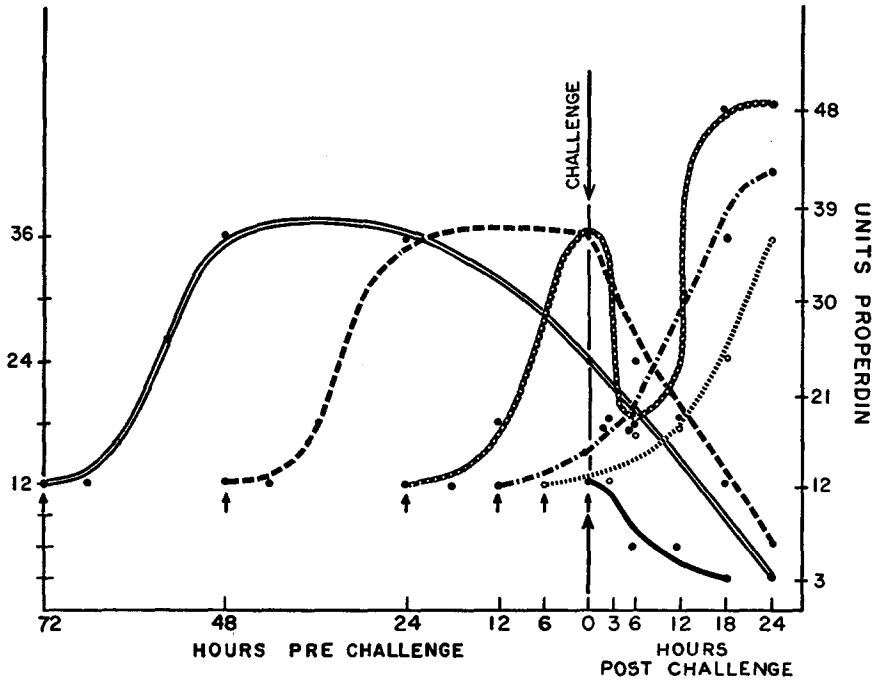

FIG. 7. Properdin levels in mice treated with 10 μg. of lipopolysaccharide. Effect of time of treatment on reaction to infection with *S. typhosa*. Intervals prior to challenge, at which lipopolysaccharide was administered; ═══, 72 hours; ▬ ▬ ▬, 48 hours; ××××××, 24 hours; ▬ ▪ ▬ ▪, 12 hours, ..., 6 hours. ▬▬▬▬, controls.

down to the normal range, and this was then succeeded by a swift and steep elevation in properdin to very high titers. These animals survived.[4] Treatment at 12 hours prechallenge, produced a modest rise in properdin, while at the 6 hour interval, in this experiment, there was no discernible increase in the level of properdin. Following challenge there was a continued and progressive rise in properdin titers which, at 24 hours post challenge, were approximately 3 times as high as those of normal untreated mice.

(*c*) In mice receiving 0.1 μg. there was observed a rise in resistance to challenge between 4 and 8 hours after injection, despite the absence of any demonstrable rise in properdin level. Consequently, groups of mice were injected with 0.1 μg. of lipopolysaccharide 12 and 6 hours prior to challenge and their properdin levels followed after challenge. The results of this test are given in Table IV. The mice receiving lipopolysaccharide 12 hours before challenge succumbed, while those treated 6 hours before challenge survived. In the former, the normal properdin value at the time of challenge remained at this level during the first 6 hours and then progressively declined until all animals succumbed. In mice injected 6 hours before challenge properdin levels at first declined and then slowly rose to normal.

In each of these experiments, control mice exhibited a consistent pattern of little or no alteration in properdin levels during the first 6 hours post challenge and thereafter a decline in properdin titer to extremely low levels and death.

Comparison of Non-Specific and Specific Resistance.—While the level of antibacterial immunity conferred by specific immunization is much higher than the increased resistance produced by a variety of non-specific agents, relatively little information of a quantitative nature is available. It was therefore desirable to make a comparison of the effectiveness of the non-specific resistance to infection evoked in mice by lipopolysaccharide with that developed in response to specific immunization.

The experimental infection of mice with *S. typhosa* provides a convenient model for such a comparison, inasmuch as it had been previously established that the Vi antigen is the specific immunogenic component highly protective against the infection of mice with typical virulent strains of *S. typhosa*. The level of antibody produced by the mouse in response to this purified antigen suffices to fully account for the observed protection in active immunization tests.

Experiments were conducted with groups of mice injected with 10 μg. of lipopolysaccharide 24 hours prior to challenge or with 10 μg. of Vi antigen 5 days prior to challenge (an interval sufficient for production of demonstrable levels of antibody). These groups

[4] It is noteworthy that the results obtained with animals injected 24 hours prior to challenge practically duplicate those of an earlier experiment involving the use of the same dose and time interval but another lipopolysaccharide (see Fig. 6).

and control mice were challenged with serial dilutions of *S. typhosa* suspended in mucin or in saline. In effect these were virulence titrations in specifically and non-specifically protected mice. The data thus obtained were plotted as probits mortality *vs.* the log of the challenge dose, and the results of these experiments summarized in Table V and expressed in terms of the quantity of challenge agent required to kill 50 per cent of the mice receiving the treatment indicated.

It will be seen that in unprotected mice the LD_{50} for *S. typhosa* in mucin was 10 organisms, while 11,000 organisms were required to kill 50 per cent of the (*E. coli* 08) lipopolysaccharide-treated mice. To produce this degree of mortality in Vi-immunized mice required 9 million organisms.

Without the use of a host resistance depressant, the number of organisms required to fatally infect mice was, of course, much greater. Nevertheless,

TABLE V

Protection Against Challenge Afforded by Non-Specific and Specific Immunization

Immunization	Quantity of challenge agent required to kill 50 per cent of mice		
	No. of *S. typhosa* Ty 2		μg. typhoid endotoxin
	In 5 per cent mucin	In saline	
Controls	10	14×10^6	250
Non-specific 10 μg. *E. coli* 08 lipopolysaccharide	11×10^3	90×10^6	850
Specific 10 μg. Vi antigen	9×10^6	200×10^6	220

the increase in resistance produced by lipopolysaccharide and the even higher level of protection afforded by immunization with the Vi antigen were again manifested. Although both types of typhoid challenge gave similar results, in that specific immunization gave superior protection in each, it is apparent that when this protection is expressed in terms of the number of LD_{50}'s, the results differ considerably with these two types of challenge inocula. However, in terms of the number of organisms in this comparison, the results would have another meaning. In part, this may be a reflection of the fact that mucin, a well known host resistance depressant, considerably reduces the number of bacilli which constitute an infective dose. As a consequence it greatly magnifies the differences in the effect of raising the resistance of mice by either specific or non-specific means. In any case, these results clearly show that the non-specific resistance evoked by lipopolysaccharide is effective, but of more limited proportions than the level of immunity conferred by specific immunization.

The increased resistance of mice to experimental infection is accompanied by the development of resistance or "tolerance" to lethal levels of lipopolysaccharide. Thus, mice which had received 10 μg. of coli lipopolysaccharide 24 hours earlier withstood approximately 3 times as great a challenge of typhoid lipopolysaccharide as did control mice. As would be expected, specific immunization, directed against the Vi "capsular" antigen, was quite without effect in raising resistance to this type of challenge (Table V).

Resistance to Challenge with Endotoxin.—The development of "tolerance" to certain of the physiological effects of endotoxin (particularly fever), in animals previously treated with endotoxin, has been observed by many workers. To obtain information on the extent to which "tolerance" was involved in

TABLE VI

Development of "Tolerance" to Lethal Action of Endotoxin Following Administration of Bacterial Lipopolysaccharide

Treatment		Challenge	Hrs. elapsed between injection of lipopolysaccharide and challenge							
Product derived from	Dose		1	6	16	24	48	72	120	240
			Per cent survivors							
S. schottmuelleri (rough)	10 μg.	8×10^7 *S. typhosa* Ty 2	0	50	90	90	40	50	20	0
E. coli 0127			10	70	70	90	20	10	10	0
S. schottmuelleri (rough)		500 μg. *S. typhosa* endotoxin*	10	0	70	100	100	70	80	70
E. coli 0127			20	10	90	90	70	80	80	60

* Crude trichloroacetic acid extract of *S. typhosa* 0901.

the development of resistance to infection, experiments were conducted in which groups of mice were challenged with endotoxin at the same time that other groups were challenged with viable bacteria.

Groups of mice were given 10 μg. of certain lipopolysaccharides at varying intervals prior to challenge with a lethal dose of endotoxin. Mice given the same treatment were also challenged with *S. typhosa.*

The results of this experiment are summarized in Table VI and show that, in addition to increased resistance to infection, mice also became resistant to a quantity of lipopolysaccharide normally lethal. However, it should be noted that, compared with the increase of resistance to infection, resistance to endotoxin develops more slowly, persists for a much longer period and, in this respect, appears to follow the properdin levels more closely.

In order to further examine these relationships, still another experiment was carried out in which parallel groups of mice were given graded doses of *E. coli* 08 lipopolysaccharide at varying intervals. One set of animals was challenged with viable *S. typhosa* and the other with a lethal amount of a coli endotoxin. It was shown that the smallest amount effective in eliciting protection against the lethal action of endotoxin was 10 μg., an amount much more than that which suffices to produce an increase in resistance to infection. Thus, the two effects differ not only with respect to onset and duration, as indicated in the previous experiment, but also with respect to the effective dose of lipopolysaccharide.

DISCUSSION

These studies have shown that a general attribute of bacterial lipopolysaccharides is the ability to increase resistance to infection. The increased resistance thus developed is associated with either elevated properdin levels or the maintenance of normal levels during the infectious process, in contrast to a progressive fall in properdin levels in control animals which succumb to infection.

The eighteen lipopolysaccharides studied, which were derived from six genera, from colonially smooth and rough strains, and from numerous serotypes, all evoked the development of early resistance to infection. Thus, to the already extensive list of physiological effects ascribed to these substances, may now be added still another. However, no relationship has been apparent between the induction of increased resistance in mice and other, well-known physiological attributes of lipopolysaccharides; *i.e.*, their ability to produce fever, cellular alterations, Shwartzman activity, and lethality.

During the course of this work it became evident that the activity of these lipopolysaccharides in inducing increased resistance was not directly related to their pyrogenic and toxic activity, the properties of endotoxins most frequently studied. Moreover, the amount of bound lipide or protein in the products did not materially increase or decrease the characteristic effect of lipopolysaccharides here described. The product derived from *S. typhosa* by Webster *et al.*, which was used in many of these experiments, had already been shown to be free of protein. The preparation from *E. coli* 08 had also been shown by Westphal *et al.* (14) to be protein-free. In an experiment carried out with a number of characterized components of *Shigella dysenteriae* (22) kindly provided by Dr. D. A. L. Davies, the lipoprotein derived from the whole endotoxic complex did not evoke increased resistance to any significant degree, while the undegraded polysaccharide, from the same source, which is reported to contain little if any lipide, was as active as the lipopolysaccharide (23). The qualitative and quantitative differences in the individual sugars, which make up the polysaccharide moiety, obviously do not influence the degree of activ-

ity of lipopolysaccharides, inasmuch as sugars differ widely among the various Gram-negative species (24). Moreover, certain highly branched dextrans, which have been completely charcterized and shown to be free of nitrogen, phosphorous or lipide, are nonetheless able to stimulate early resistance when given in high doses (6). It would appear, therefore, that the salient features of these substances, indeed the only features they possess in common, are those of being carbohydrates of high molecular weight and of high degree of branching. It is likely that their spatial configuration of sugar residues is also important. Those features of the bacterial polysaccharides which are essential for the induction of early rise in resistance appear also to be required for their interaction in the properdin system, both *in vitro* and *in vivo* (6).

The parenteral administration of bacterial lipopolysaccharide initiates in the host a complex series of reactions, of which probably only a few have thus far been identified. Certain of these alterations produced in the host by lipopolysaccharide, such as granulocytosis, activation of the reticulo-endothelial system, stimulation of the adrenal cortex, etc., are among the known physiological activities which, there is reason to believe, may influence immunity. However, the extent to which these changes affect the phenomenon described in this report is unknown and must await further study.

In the present work it was shown that the injection of lipopolysaccharide in mice increased the amount of properdin, a naturally occurring protein, which participates in the destruction or inactivation of many infectious agents *in vitro*. As far as we are aware, this is the first naturally occurring substance, with antibacterial properties, that has been shown to vary during infection and to parallel the observed changes in natural resistance. In contrast, complement, which participates in many immune reactions, and is also an important constituent of the properdin system, remains relatively unaffected in titer during the physiological response of the host to either lipopolysaccharide or infection. While properdin titer falls in shock and in radiation illness, there is no change in complement activity (7, 25).

The present study shows that experimental infections produced in mice by Gram-negative pathogens, which are susceptible to the *in vitro* bactericidal effect of the properdin system are also controlled by the prior administration of lipopolysaccharide. Since this control is accompanied by maintenance or increase of the properdin level in the host, this system may prove to be important in resistance to infection with many Gram-negative pathogens.

Study of the properdin alterations in experimentally infected mice has shown that when these animals are made resistant to infection by treatment with either high or low doses of lipopolysaccharide, at certain intervals prior to challenge, they may have initially normal or even subnormal properdin titers. In such animals, there occurs little or no rise in properdin titers following challenge. Instead, properdin titers are maintained at either normal or only

moderately reduced levels, in contrast to a progressive decline to exceedingly low levels and death in control mice. These observations suggest that, in themselves, properdin levels determined prior to challenge may not be a reliable guide or index to the animal's subsequent reaction to infection. Rather, the critical measure of the effectiveness of the treatment employed is its effect in enabling the animal to *maintain* either normal or elevated properdin levels during the early phases of infection. *Obviously, individual properdin values do not provide this information.* Properdin levels, particularly those obtained in postchallenge experiments, and protection against the lethal effects of Gram-negative infections are well correlated; that is to say, normal or elevated properdin titer was associated with survival, while markedly reduced properdin titer was exhibited by those animals who later succumbed to the infection.

These observations may be interpreted in at least two ways. Properdin titers may be maintained at normal levels following administration of lipopolysaccharide because this agent stimulates other systems which in turn prevent the multiplication of bacteria otherwise capable of combining with and depleting the supply of properdin available to the host.[5] On the other hand, the elevation or maintenance of properdin titers may be caused by the direct action of lipopolysaccharide on the synthesis or release of available properdin. Present evidence appears to support the latter explanation. However, it still remains to be determined whether there is a causal relationship between increased or maintained properdin levels and increased resistance to infection.

The possibility was considered that lipopolysaccharide might increase resistance to infection by producing an increase in resistance or "tolerance" of the host to the lethal action of endotoxin since there is reason to believe that endotoxin probably is the toxic component in the challenge which contributes, in large part, to the demise of the infected host. This type of resistance to the lethal action of endotoxin has been observed previously by Creech *et al.* (26, 27). In the present study it was shown that resistance to lethal amounts of endotoxin does develop after a single injection of lipopolysaccharide, thus confirming and supplementing the earlier observations of Creech. The finding that 0.1 or 1 μg. of lipopolysaccharide which is sufficient to produce resistance to infection fails to evoke tolerance to a lethal dose of endotoxin, and the fact that such tolerance persists well beyond the period of immunity to bacterial challenge, raises a doubt whether this "tolerance" to endotoxin is in itself sufficient to account for the observed resistance to infection. The tolerance thus developed may be nonetheless an ancillary factor in the over-all effect on resistance.

As mentioned previously, it has been observed by a number of workers that the administration of either viable or killed Gram-negative organisms, and

[5] It has already been shown that Gram-negative bacteria are able to bring about these changes both *in vitro* and *in vivo*.

more recently of bacterial cell walls from certain of these species, induce in mice an early non-specific rise in resistance. Since extremely small quantities of lipopolysaccharide derived by different isolation procedures, from various bacterial genera of the family Enterobacteriaceae, from many serotypes, and from smooth and rough strains, are capable of eliciting in mice a similar protective effect, the data here presented support the concept that the various expressions of non-specific immunity previously reported for Gram-negative bacteria are ascribable to the lipopolysaccharides which they contain.

It is significant that the increase in natural resistance induced in mice by administration of lipopolysaccharide is directed principally against a broad group of Gram-negative bacilli and is apparently ineffective against Gram-positive cocci. The cellular constituent common to the organisms thus affected is the very same kind of lipopolysaccharide shown to produce this protective action. This indicates that, despite the lack of specificity of this effect in the accepted immunological sense, it may nonetheless be chemically specific, depending upon the size of the molecule and spatial arrangements of the sugar residues.

It cannot be too strongly emphasized that the effects of lipopolysaccharides on properdin levels and on resistance are influenced by many factors of which dose, timing of administration, and the products employed have been shown to be important. Furthermore, even the strain of mice employed has a major effect, because depending on the strain used, injection of lipopolysaccharide may result in no apparent increase in resistance or varying degrees of resistance ranging to 100 per cent protection during the test period. It was not feasible to examine systematically the array of variables which appear to be implicated in these reactions, but it is apparent that seemingly minor deviations in experimental design can result in major changes in the type of effect produced.

The present study supplies data which abundantly demonstrate that appropriate treatment of the host with lipopolysaccharide produces a characteristic rise in resistance to infection with Gram-negative pathogens which is accompanied by an increase or maintenance of properdin level. Inasmuch as a number of important physiological changes in the host are also known to occur as a result of this treatment, further investigation will be required to determine the exact host defense mechanisms responsible for this effect. Regardless of the exact nature, mode of action, and interaction of these various defense systems, the evidence presented in this report shows that their effectiveness in controlling bacterial multiplication is greatly increased by appropriate treatment of the host with a structural component of the bacterial cell identified as lipopolysaccharide.

SUMMARY

It has been shown that injection of lipopolysaccharides, derived from a variety of Gram-negative bacterial species, evokes in mice a rapidly developing rise in resistance to infection with Gram-negative pathogens. This is accompanied by an elevation in properdin titer, at times to levels 2 to 3 times the normal. The rate, magnitude, and duration of these responses are dependent on many factors, the most important of which are the quantity and timing of the lipopolysaccharide administered.

The increased resistance to infection evoked in mice by lipopolysaccharides was effective against infections produced by endotoxin-bearing organisms–bacterial species highly susceptible *in vitro* to the bactericidal action of the properdin system.

Properdin titers of mice *prior* to infection provide an incomplete picture of the subsequent reaction of the host to the infective agent. Following infection with Gram-negative organisms, properdin levels accurately reflect the bacteriologic course and outcome of the infection. Thus, in control animals, properdin titers progressively declined and the animals died, while in mice appropriately treated with lipopolysaccharide, properdin levels were either maintained in the normal range or increased, depending on the dose and time of administration of lipopolysaccharide; this was always accompanied by successful management of the infection.

The complex nature of the alterations produced in the host by lipopolysaccharides is stressed. It is pointed out that the increase in the ability of the host to cope with Gram-negative infections may be the result of stimulation of other defense mechanisms, in addition to the properdin system.

We are indebted to Captain A. Mandel, MSC, Mr. R. J. Trapani, and Mr. O. Washington for assistance in conducting the tests in experimentally infected mice and to Miss L. Wurz and Mr. E. W. Todd for aid in carrying out properdin titrations.

BIBLIOGRAPHY

1. Kolle, W. and Prigge, R., Die grundlagen der Lehre von der erworbenen aktiven (allgemeinen und lokalen) und passiven immunitat, In *Handbuch Pathogen. Mikroorg.*, 1929, **1,** 623.
2. Brandis, H., Pt. III Über die Promunität (Depressionsimmunität), *Ergebn. Hyg. Bakt., Immunitätsforsch. u. exp. Therap.*, 1954, **28,** 141.
3. Rowley, D., Stimulation of natural immunity to *Escherichia coli* infections. Observations on mice, *Lancet*, 1955, **1,** 232.
4. Pillemer, L. and Ecker, E. E., Anticomplementary factor in fresh yeast, *J. Biol. Chem.*, 1941, **137,** 139.
5. Field, T. E., Howard, J. G., and Whitby, J. L., Studies on the rapid production of a non-specific type of immunity to *Salmonella typhi* infection in mice, *J. Roy. Army Med. Corps*, London, 1955, **101,** 324.

6. Pillemer, L., Schoenberg, M. D., Blum, L. and Wurz, L., Properdin system and immunity. II. Interaction of the properdin system with polysaccharides, *Science*, 1955, **122,** 545.
7a. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., and Wardlaw, A. C., The properdin system and immunity: I. Demonstration and isolation of a new serum protein, properdin and its role in immune phenomena, *Science*, 1954, **120,** 279.
7b. Pillemer, L., The properdin system, *Ann. New York Acad. Sc.*, 1955, **17,** 526.
8. Wardlaw, A. C., and Pillemer, L., The properdin system and immunity V. The bactericidal activity of the properdin system, *J. Exp. Med.*, 1956, **103,** 553.
9. Landy, M., Increased resistance to infection developed rapidly after administration of bacterial lipopolysaccharides, *Fed. Proc.*, 1956, **15,** 598.
10. Landy, M. and Pillemer, L., Elevation of properdin levels in mice following administration of bacterial lipopolysaccharides, *J. Exp. Med.*, 1956, **103,** 823.
11. Nesset, N. M., McLallen, J., Anthony, P. Z., and Ginger, L. G., Bacterial pyrogens. I. Pyrogenic preparation from a *Pseudomonas* species, *J. Am. Pharmaceut. Assn.*, 1950, **39,** 456.
12. Digeon, M., Raynaud, M., and Turpin, A., Etude de la toxine R_2 du Bacille typhique (*Eberthella typhosa*), *Ann. Inst. Pasteur*, 1952, **82,** 206.
13. Shear, M. J., and Turner, F. C., Chemical treatment of tumors. V. Isolation of the hemorrhage-producing fraction from *Serratia marcescens* (*Bacillus prodigiosus*) culture filtrate, *J. Nat. Cancer Inst.*, 1943, **4,** 81.
14. Westphal, O., Lüderitz, O., Eichenberger, E., and Keiderling, W., Über bakterielle reizstoffe I. Mitt: Reindarstellung eines Polysaccharid-pyrogens aus *Bacterium coli*, *Z. Naturforsch.*, 1952, **7 b,** 536.
15. Webster, M. E., Sagin, J. F., Landy, M., and Johnson, A. G., Studies on the O antigen of *Salmonella typhosa* I. Purification of the antigen, *J. Immunol.*, 1955, **74,** 455.
16. Lambert, H. P., and Richley, J., The action of mucin in promoting infections: The anticomplementary effect of mucin extracts and certain other substances, *Brit. J. Exp. Path.*, 1952, **33,** 327.
17. Landy, M., The immunological properties of the isolated Vi and O antigens of *Salmonella typhosa*, *Bull. New York Acad. Med.*, 1955, **31,** 774.
18. Pillemer, L., Blum, L., Lepow, I. H., Wurz, L., and Todd, E. W., The properdin system and immunity. III. The zymosan assay of properdin, *J. Exp. Med.*, 1956, **103,** 1.
19. Thomas, L., The physiological disturbances produced by endotoxins, *Ann. Rev. Physiol.*, 1954, **16,** 467.
20. Baron, L., unpublished data.
21. Landy, M. and Webster, M. E., Studies on Vi antigen III. Immunological properties of purified Vi antigen derived from *Escherichia coli* 5396/38, *J. Immunol.*, 1952, **69,** 143.
22. Davies, D. A. L., Morgan, W. T. J., and Record, B. R., Studies in immunochemistry 15. The specific polysaccharide of the dominant "O" somatic antigen of *Shigella dysenteriae*, *Biochem. J.*, 1955, **60,** 290.
23. Pillemer, L. and Davies, D. A. L., data to be published.

24. Westphal, O. and Lüderitz, O., Chemische erforschung von lipopolysacchariden gramnegativer bakterien, *Z. Angew. Chem.*, 1954, **66,** 407.
25. Frank, E., Fine, J., and Pillemer, L., Serum properdin levels in hemorrhagic shock, *Proc. Soc. Exp. Biol. and Med.*, 1955, **89,** 223.
26. Creech, H. J., Hankwitz, R. F., and Wharton, D. R. A., Further studies of the immunological properties of polysaccharides from *Serratia marcescens* (*Bacillus prodigiosus*) I. Effects of passive and active immunization on lethal activity of polysaccharides, *Cancer Research*, 1949, **9,** 150.
27. Wharton, D. R. A. and Creech, H. J., Further studies of the immunological properties of polysaccharides from *Serratia marcescens* (*Bacillus prodigiosus*) II. Nature of the antigenic action and the antibody response in mice, *J. Immunol.*, 1949, **62,** 135.
28. Barrett, M. K., The influence of genetic constitution upon the induction of resistance to transplantable mouse tumors, *J. Nat. Cancer Inst.*, 1940, **26,** 387.