REVERSIBLE CHANGES IN THE SUSCEPTIBILITY OF MICE TO BACTERIAL INFECTIONS

I. Changes Brought about by Injection of Pertussis Vaccine or of Bacterial Endotoxins

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The results of studies carried out by several independent groups of investigators during recent years have established that the susceptibility of experimental animals to bacterial infections can be modified at will by a number of non-specific physiological and biochemical disturbances. The findings suggest that the outcome of host-parasite relationships is profoundly affected by a variety of processes which are independent of those involved in specific acquired immunity. Experiments to be described in the present and the following papers illustrate further some of the manifestations of non-specific changes in the response of albino mice to infection with *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis*. In these experiments, susceptibility to infection has been estimated by observing the mortality rates among infected animals, and by enumerating the viable bacteria present in the various organs at different intervals of time after infection. The study of these two manifestations of the infectious process has revealed that, in mice at least, susceptibility and resistance to infection can undergo profound changes which occur rapidly, and which are readily reversible.

As already mentioned, many different types of non-specific stresses can bring about these reversible changes in susceptibility to infection. The effects of injection of bacterial endotoxins will be considered in the present paper. Nutritional disturbances will be dealt with in the second paper of this series (7). The findings in both groups of studies will be discussed together at the end of the second paper.

Among procedures that can be used to alter in a non-specific manner the susceptibility of animals to infection, one of the most effective is the injection of killed microbial cells or certain purified constituents of them. The phenomenon had been clearly recognized by Almroth E. Wright in his studies of antityphoid vaccination. He emphasized the existence of a "negative phase of resistance" that preceded the immunity induced by injection of killed typhoid bacilli. His chapter on the "Law of the ebb, flow and reflow, and subsequent maintained high tide of immunity"

contains much that is directly relevant to the present investigation (10). More recent studies have shown that when a fraction of yeast cell known as "zymosan," is injected into mice, it causes at first a marked depression of resistance to infection with certain bacteria. This negative phase is followed by a state of increased resistance which may last for a few days. Simultaneously, injection of zymosan results in a sudden fall, followed by a rebound, in the blood level of properdin—a serum protein which in association with complement exerts a bactericidal effect on several strains of Gram-negative bacilli (4, 5). Certain fractions of bacterial cells, designated as "cell walls" have been shown to exert a similar biphasic effect both on resistance to infection, and on blood levels of properdin (6). The lipopolysaccharide endotoxins appear also to be very active from both these points of view (1, 2).

The present paper is devoted to a bacteriological study of the effect exerted by suspensions of whole killed cells of Gram-negative bacilli, and by preparations of endotoxins, on the susceptibility of mice to bacterial infections.

EXPERIMENTAL

Bacterial cultures.—Four different strains of bacteria were used for the infection tests:—

1. A virulent bovine strain of tubercle bacillus (MV); this was used in the form of a culture 10 days old in tween-albumin medium.

2. Two coagulase-positive strains of *Staphylococcus aureus*: "Smith" and "Giorgio." These strains are described in reference 8. They were used in the form of 18-hour-old cultures in beef heart infusion–peptone broth.

3. A strain of *Klebsiella pneumoniae* type C which was maintained in a highly virulent state by recovery from mouse heart blood shortly before use. The strain was used in the form of an 18 hour old culture in beef heart infusion–peptone broth.

All cultures were injected into one of the tail veins of the mouse, in the amounts indicated for each experiment.

Animals.—All mice were of the so called Rockefeller Swiss strain. They were weaned at 3 to 4 weeks of age and were used within 1 to 3 weeks after. All animals in any given experiment were of the same age. They were fed pellets and water unless otherwise noted and were housed in metal cages in groups of 2 to 5 during the whole course of experimentation.

Bacterial Enumeration.—The numbers of bacteria present in the organs at any given time was determined by the techniques described elsewhere (3, 8).

Pertussis Vaccine.—Two different preparations were used. One was a preparation released for human use by Lederle Laboratories (Pearl River, New York). It contained approximately 60 billion cells per ml. The other was a preparation prepared for experimental work and kindly supplied to us by Dr. J. Munoz of Sharp and Dohme (Glenolden, Pennsylvania); it contained approximately 1000 billion cells per ml. Proper dilutions of the vaccines were administered by the intraperitoneal route in a final volume of 0.2 ml.

Typhoid Endotoxin.—This was a highly purified preparation of lipopolysaccharide prepared from the typhoid bacillus by Dr. Maurice Landy at the Army Medical School, Washington, D. C. (9). We are much indebted to Dr. Landy for supplying us with two samples of his valuable material. Proper dilutions of the endotoxin were injected by the intraperitoneal route, in a final volume of 0.2 ml.

RESULTS

While studying the effect exerted by certain bacterial products on experimental infections, it was observed that the resistance of mice to staphylococci could be markedly altered by treating the animals with pertussis vaccine before infection. Illustrations of these findings are presented in the following experiments.

TABLE I

Effect of Pretreatment with Pertussis Vaccine on Survival of Mice Infected with Staphylococci

Pertussis vaccine (i.p.)		Cumulative Nos. of deaths* at indicated times after infection*			
Amount	Interval between treatment and infection	3 days	6 days	9 days	12 days
ml.					
0.2	8 days	0	2	2	5
0.05	" "	0	1	1	4
0.2	1 day	0	2	3	6
0.05	" "	0	0	1	5
0.2	5 hrs.	6	9	9	9
0.05	" "	2	5	5	10
0.2	2½ hrs.	7	8	9	10
0.05	" "	1	8	8	10
0.2	1 hr.	3	9	9	10
0.05	" "	5	9	9	10
No vaccine		5	6	6	7

* Out of 10 mice infected intravenously with 0.05 ml. of culture Giorgio.

Eleven groups of 10 mice, 6 weeks old, were infected intravenously with 0.05 ml. of a coagulase-positive culture of *Staphylococcus aureus* (strain Giorgio). Six of the groups had previously received 0.05 ml. of pertussis vaccine (Lederle), administered by the intraperitoneal route, and six 0.2 ml. of the same vaccine. For each amount, the vaccine had been administered either 1 hour, 2½ hours, 5 hours, 1 day, or 8 days before infection. The eleventh group of mice received no vaccine and served as infection control. The rates of deaths in the different groups are presented in Table I.

As appears from the results presented in Table I, administration of pertussis vaccine 1 or 8 days before infection markedly increased the resistance of mice to staphylococci. In contrast, the mice infected 1 hour, 2½ hours, or 5 hours after administration of 0.2 ml. pertussis vaccine died more rapidly than

the controls or than the mice of the other groups. This infection-enhancing effect of the vaccine was less lasting in animals treated with 0.05 ml. than in those receiving 0.2 ml. In this regard it must be pointed out that the minimal dose of pertussis vaccine capable of eliciting a negative phase of resistance

TABLE II

Effect of Pretreatment with Pertussis Vaccine on Fate of Staphylococci in Organs of Mice

Pertussis vaccine (i.p.)			A Staphylococcal colonies* 18 hrs. after infection‡			
Amount	Interval between treatment and infection					
ml.						
0.05	9 days	Blood	4	23		200
"	" "	Liver	2,700	3,900		10,300
"	" "	Kidneys	1,300	240		11,700
"	" "	Lungs	2	23		1,000
"	" "	Spleen	1,200	1,400		5,700
0 (saline)	9 days	Blood	360	480		10,000
"	" "	Liver	6,800	15,000		36,000
"	" "	Kidneys	94,000	250,000		114,000
"	" "	Lungs	700	5,900		1,700
"	" "	Spleen	3,900	7,300		11,000
0.05	17 days	Blood	10	50	70	90
"	" "	Liver	500	10,000	5,600	4,800
"	" "	Kidneys	10	100	500	3,500
"	" "	Lungs	0	40	200	10
"	" "	Spleen	1,200	7,600	10,100	800
0 (Saline)	17 days	Blood	20	40	100	100
"	" "	Liver	9,500	13,200	8,400	4,800
"	" "	Kidneys	500	400	8,300	24,000
"	" "	Lungs	40	40	20	50
"	" "	Spleen	4,700	19,200	6,600	7,600
0.05	25 days	Blood	10	20		40
"	" "	Liver	4,700	1,200		1,700
"	" "	Kidneys	2,500	8,000		75,000
"	" "	Lungs	10	40		10
"	" "	Spleen	600	1,800		800
0 (Saline)	25 days	Blood	20	50		700
"	" "	Liver	3,400	5,000		1,900
"	" "	Kidneys	2,000	5,000		42,000
"	" "	Lungs	10	10		10
"	" "	Spleen	1,400	2,500		5,900

TABLE II—*Concluded*

Pertussis vaccine (i.p.)		B Staphylococcal colonies* 24 hrs. after infection‡					
Amount	Interval between treatment and infection						
ml.							
0.05	6 wks.	Blood	1	5	6	16	164
"	" "	Liver	140	580	200	300	200
"	" "	Kidneys	35	122	35	480	2,200
0 (Saline)	6 wks.	Blood	7	27	41	82	156
"	" "	Liver	1,800	580	1,620	1,100	1,300
"	" "	Kidneys	130	410	140	380	1,440

* To be multiplied by 2000 for whole organ or milliliters of blood.

‡ 0.1 ml. of culture Smith injected i.v.

has varied slightly from experiment to experiment. (Contrast for example the results with 0.05 ml. presented in Tables I and III.) Although these discrepancies have not been systematically analyzed, it is likely that they could be traced to the age of the animals used for the tests. In general, the response to the vaccine of very young mice (4 weeks) seems to be less pronounced than that of older animals (6 to 8 weeks).

The protective effect exerted by pretreatment with pertussis vaccine against staphylococcus infection can be demonstrated also by studying quantitatively the fate of staphylococci in the various organs of infected mice, as shown in the following experiments.

Mice 6 weeks old were injected intraperitoneally with 0.05 ml. of pertussis vaccine (Lederle) diluted to a final volume of 0.2 ml. with saline; the control animals received saline alone. All animals were infected by the intravenous route with 0.1 ml. of culture of *Staphylococcus aureus* (strain Smith). Some of them received this infective dose 9 days, other 17 days, and a third group 25 days after administration of the pertussis vaccine. All mice were sacrificed 18 hours after infection and the numbers of staphylococci in their organs determined by quantitative bacteriological techniques (Table II A).

In another experiment of the same pattern, mice were infected with staphylococci 6 weeks after administration of the pertussis vaccine. In this case the animals were sacrificed 24 hours after infection (Table II B).

The results presented in Table II make clear that pretreatment with pertussis vaccine affected profoundly the fate of staphylococci in the organs of infected mice. Eighteen hours after administration of the infective dose, the numbers of staphylococci in all the organs tested were much higher in the untreated animals than in those having received the vaccine 9 or 17 days before infection. In the first experiment (part A of Table II) the protective effect was no longer apparent in animals infected 25 days after administra-

tion of the vaccine. However, in the second experiment (part B of Table II) evidence of protection was still detectable after a period of 6 weeks.

Further evidence of the protective effect of pertussis vaccine against infection with staphylococci is presented in Table III.

TABLE III

Influence of Dose and Time of Pretreatment with Pertussis Vaccine on Response of Mice to Staphylococcal Infection

Pertussis vaccine* (i.p.)		Cumulative Nos.* of deaths				Staphylococcal colonies‡ recovered 24 hrs. after infection§				
Amount	Interval between treatment and infection	3 days	6 days	9 days	12 days					
ml.										
0.01	8 days	2	6	7	8	Kidneys	1	1	4	40
"	" "					Liver	3	27	38	64
0.05	8 days	0	3	3	4	Kidneys	0	0	0.4	3
"	" "					Liver	2	2	2	10
0.01	1 day	0	1	2	3	Kidneys	0.1	0.2	2	4
"	" "					Liver	52	2	85	52
0.05	1 day	1	3	4	5	Kidneys	0.2	0.2	1	3
"	" "					Liver	7	16	37	46
0.01	2 hrs.	2	4	6	8	Kidneys	2	2	4	29
"	" "					Liver	14	12	4	24
0.05	2 hrs.	1	3	4	7	Kidneys	0.1	2	3	3
"	" "					Liver	9	3	2	13
No vaccine		3	8	—	—	Kidneys	2	16	50	52
						Liver	27	10	48	16

* Out of 8 mice infected i.v. with 0.05 ml. culture Giorgio.

‡ To be multiplied by 2000 for whole organ.

§ Mice infected i.v. with 0.1 ml. culture Smith.

In this experiment both the survival time, and the fate of staphylococci in the organs, were determined on aliquot groups of mice. These had received either 0.05 or 0.01 ml. of pertussis vaccine at different intervals of time prior to infection with either one of two different strains of staphylococcus ("Giorgio" or "Smith").

In confirmation of the results presented in Tables I and II, it is seen in Table III that mice treated with vaccine proved far more resistant than untreated animals when challenged 24 hours later with staphylococci. Resistance

manifested itself by increased survival time (following infection with 0.05 ml. of culture "Giorgio") and by decreased multiplication of the staphylococci in the organs, particularly in the kidneys (following infection with 0.1 ml. of culture "Smith"). The protective effect was obtained with an amount of vaccine as small as 0.01 ml. However, there was evidence that protection lasted longer with 0.05 ml. than with 0.01 ml. of vaccine, as indicated by the results in mice infected 8 days after treatment.

In all experiments recorded so far, the pertussis vaccine employed was a preparation obtained from one commercial manufacturer (Lederle Laboratories) and distributed for use in human beings. It seemed useful to compare

TABLE IV

Effect of Pretreatment with Different Doses of Pertussis Vaccine on Survival of Mice Infected with Staphylococci

Pertussis vaccine*, (i.p.)		Cumulative Nos. of deaths‡				
Amount	Interval between treatment and infection	3 days	6 days	9 days	12 days	15 days
ml.						
0.02	1 wk.	0	3	4	4	5
0.006	" "	1	3	4	7	7
0.002	" "	2	4	4	4	4
0.0006	" "	3	5	7	8	9
0	" "	6	10	—	—	—

* This preparation of vaccine contained 1000 billion cells per ml.

‡ Out of 10 mice infected i.v. with 0.05 ml. culture Giorgio.

its activity with that of a suspension of *Hemophilus pertussis* obtained from another source and prepared under different conditions.

As already mentioned, the pertussis vaccine distributed by Lederle Laboratories contained approximately 60 billion bacilli per ml. The other preparation used for comparative studies was the experimental lot from Sharp and Dohme, containing approximately 1000 billion cells per ml.

The protective effect of this new vaccine preparation was tested in several experiments. Graded amounts of the vaccine (from 0.02 ml. to 0.0006 ml.) were injected into the peritoneal cavity of mice. In one experiment, the mice were infected intravenously with 0.05 ml. of culture Giorgio 1 week after treatment; these animals were allowed to die and the survival time recorded (Table IV). In a second experiment the animals were infected intravenously with 0.1 ml. of staphylococcus culture "Smith" 22 hours after treatment. In a third experiment, they were infected 1 week after treatment with the same dose. In both the second and third experiments, the animals were sacrificed 24 hours after infection and the numbers of living staphylococci present at that time in their kidneys and livers determined by bacteriological techniques (Table V).

As is seen in Table V, the animals treated 22 hours before infection with the two largest amounts of pertussis vaccine proved more susceptible to staphylococci than did the untreated animals. This infection-enhancing effect was no longer noticeable 1 week after treatment. At that time evidence of protective effect could be recognized in all the treated groups, even in those having received only 0.0006 ml. of pertussis vaccine. It expressed itself both in increased survival time of the infected animals (Table IV) and in decrease in the numbers of staphylococci present in their organs 24 hours after infection (Table V).

TABLE V

Effect of Pretreatment with Different Doses of Pertussis Vaccine on Fate of Staphylococci in Organs of Mice

Pertussis vaccine* (i.p.)		Staphylococcus colonies‡ recovered 24 hrs. after infection§								
Amount	Interval between treatment and infection	Kidney					Liver			
ml.										
0.02	22 hrs.	6	600	5000	Dead	Dead	Not done			
0.006	" "	60	60	180	1100	5000	" "			
0.002	" "	1	2	3	22	80	" "			
0.0006	" "	1	2	3	7	30	" "			
0	" "	10	80	180	260	800	" "			
0.02	1 wk.	5	8		8	20	830	520	580	280
0.006	" "	1	6		6	20	690	110	630	140
0.002	" "	4	10		120	9300	140	130	100	960
0.0006	" "	5	50		80	130	500	250	150	530
0	" "	30	50		90	370	120	120	120	100

* This preparation of vaccine contained 1000 billion cells per ml.

‡ To be multiplied by 2000 for whole organ.

§ Mice infected i.v. with 0.1 ml. culture Smith.

Comparison of the results presented in the different tables makes it clear that the level of protection against staphylococci obtained with 0.001 ml. of the second preparation of vaccine was of the same order as that obtained with 0.01 ml. of the first preparation. Since the latter contained only $\frac{1}{10}$ to $\frac{1}{15}$ as many bacilli as the first, it is evident that the bacillary material had approximately the same activity in both cases.

Pretreatment with pertussis vaccine also affected profoundly the response of mice to infection with bacteria other than staphylococci: It is seen in Table VI that increased resistance to an overwhelming dose of Friedländer bacilli was still evident in mice treated 4 weeks before infection with small amounts of vaccine (Sharp and Dohme preparation).

TABLE VI

Effect of Pretreatment with Pertussis Vaccine on Survival of Mice Infected with K. pneumoniae

Pertussis* vaccine (i.p.)		Time of death‡ (days) after infection‡							
Amount	Interval between treatment and infection								
ml.									
0.006	4 wks.	2	5	5	—	—	—	—	—
0.002	" "	2	2	5	5	—	—	—	—
0.0006	" "	1	1	1	5	5	—	—	—
Saline	" "	1	1	1	1	1	1	2	2

* This preparation of vaccine contained 1000 billion cells per ml.

‡ 5 mice per group injected i.v. with 0.2×10^{-2} culture.

TABLE VII

Effect of Pretreatment with Pertussis Vaccine on Survival of Mice Infected with Tubercle Bacilli

Pertussis vaccine (i.p.)		Cumulative nos. of deaths*					
Amount	Interval between treatment and infection	4 wks.	5 wks.	6 wks.	7 wks.	8 wks.	9 wks.
ml.							
0.2	24 days	0	2	2	2	6	7
No vaccine		4	6	8	10	—	—
0.05	14 days	0	2	2	2	4	7
No vaccine		4	6	7	8	8	10
0.2	8 days	1	2	Experiment discontinued			
"	3 "	1	4	"	"		
"	1 "	4	5	"	"		
"	5 hrs.	3	5	"	"		
"	2½ "	1	2	"	"		
"	1 "	8	8	"	"		
No vaccine		7	8	"	"		

* Out of 10 mice infected i.v. with 0.2 ml. of bovine culture MV.

Table VII presents the results of three independent experiments in which mice had received pertussis vaccine (Lederle preparation) at different intervals of time before infection with 0.2 ml. of the bovine culture MV.

Mice 5 weeks old were injected intraperitoneally with 0.2 ml. of pertussis vaccine (Lederle) diluted to a final volume of 0.2 ml. with saline. Other mice of the same age received only saline. Twenty-four days later all animals were infected by the intravenous route with 0.2 ml. of a culture of bovine tubercle bacillus (strain MV).

In a second experiment, the amount of vaccine was 0.05 ml. and the interval between treatment and infection was 2 weeks.

In a third experiment, 0.2 ml. of vaccine was used and the interval between treatment and infection was either 1 hour, 2½ hours, 5 hours, 1 day, 3 days, or 8 days.

It is clear from the results presented in Table VII that the survival time in the groups which had received the vaccine 1 day, 3 days, 8 days, or 24 days before vaccination was longer than in the untreated control groups.

TABLE VIII

Effect of Pretreatment with Klebsiella Endotoxin on Fate of Staphyloccoci in Organs of Mice*

Interval between treatment and infection	Nos. of staphylococci‡ present 18 hrs. after infection§ in					
	Liver			Spleen		
A						
48 hrs. before infection	10	95	200	20	20	15
24 " " "	1	83	119	1	15	123
4 " " "	1,500	2,100	Dead	126	93	Dead
2½ hrs. before infection	700	Dead	Dead	58	Dead	Dead
1 hr. before infection	700	12,500	Dead	1,700	150	Dead
No endotoxin	310	900	1,700	330	440	1,400
B						
5 days before infection	260	390	1,550	1,670	280	200	50	115
54 hrs " "	49	96	510	570	5	14	119	10
6 " " "	60	80	130	140	71	690	59	34
4 " " "	19	170	530	1,420	17	88	14	113
2½" " "	800	800	1,600	Dead	370	680	250	Dead
1 hr. before infection	1,100	3,400	15,000	Dead	64	41	16,400	Dead
No endotoxin	1,000	1,800	2,300	7,900	780	1,700	70	290

* Suspension of heat-killed cells of *Kl. pneumoniae* (type C).

‡ To be multiplied by 2000 for whole organ.

§ 0.1 ml. of culture Smith injected i.v.

Whereas the protective effect was not apparent during the first few hours after treatment, it was very marked in animals infected 24 days later. In another experiment not reported here, protection was still apparent 49 days after administration of the vaccine.

It is unlikely that the profound changes in response to bacterial infection which resulted from treatment with vaccine prepared from cultures of *Hemophilus pertussis* were due to any cellular component peculiar to this microorganism, for similar biphasic effects could be elicited by injection of cells of other species of Gram-negative bacilli. The following experiments illustrate the effect of injection of heat killed cells of *Kl. pneumoniae* on the subsequent response of mice to infection with staphylococci.

The cells of an 18 hours old culture of *Kl. pneumoniae* (Type C) in meat infusion peptone broth were separated by centrifugation, resuspended in saline (1/20 the original volume of culture), and killed by heating at 80°C. for 15 minutes. The potency of this crude endotoxin preparation was tested by injecting various amounts of it intraperitoneally into normal mice 4 weeks old. Under these conditions, half the animals receiving 0.2 ml. of this material died within 1 to 3 days after injection.

Mice 4 to 5 weeks old, fed pellets and water, were infected intravenously with 0.1 ml. of culture of a coagulase-positive staphylococcus (strain Smith). Groups of three of these animals had received intraperitoneally 0.01 ml. of the heat-killed suspension of *Kl. pneumoniae* described above (*i.e.*, 1/20 of the LD_{50}) at the following intervals of time before infection with the staphylococci: 1 hour, 2½ hours, 4 hours, 24 hours, 48 hours. A control group was similarly infected with staphylococci, but had received no toxin. All animals were sacrificed 18

TABLE IX

*Effect of Pretreatment with Typhoid Lipopolysaccharide on the Survival of Mice Infected with Tubercle Bacilli**

Typhoid lipopolysaccharide (i.p.)		Cumulative Nos. of deaths at indicated times after infection					
Amount	Interval between treatment and infection	4 wks.	5 wks.	6 wks.	7 wks.	8 wks.	9 wks.
0	3 wks.	1	4	8	9	10‡	
20γ	" "	0	0	4	5	5	8‡
0	3 wks.	7	10	13§			
3γ	" "	6	10	12	13§		
30γ	" "	3	5	7	12§		

* 0.2 ml. of bovine culture MV injected i.v.

‡ Out of 10 mice.

§ Out of 14 mice.

hours after infection and the numbers of staphylococci present in their livers and spleens at that time were determined by the usual techniques.

Another experiment was carried out according to the same pattern, except that the suspension of heat-killed *Klebsiella* was administered 1 hour, 2½ hours, 6 hours, 54 hours, or 5 days before infection with staphylococci.

The results of these two separate experiments are presented in Table VIII.

In the two experiments presented in Table VIII, intraperitoneal administration of killed cells of *Kl. pneumoniae* modified profoundly the response of mice to infection with staphylococci. The numbers of living staphylococci present in the spleen and liver 18 hours after infection were much smaller in the mice having received the endotoxin 6 hours, 24 hours, 48 hours, or 54 hours before infection than in the untreated animals; indeed, there was still evidence of protection when the animals were infected 5 days after administration of the endotoxin. In contrast, when the staphylococci were injected from 1 to 4 hours after administration of the endotoxin, several of the animals

died, and the staphylococci in the livers of the survivors were more numerous than in the controls. It is clear, therefore, that suspensions of killed Friedländer bacilli exert on the response of mice to staphylococcal infection a biphasic effect similar to that exerted by pertussis vaccine.

It is known that many of the biological effects exerted *in vivo* by Gram-negative bacilli can be traced to lipopolysaccharide components of the bacterial cells. Although endowed with great immunological specificity, these substances are remarkably non-specific in their physiological effects. It was of interest, therefore, to test whether the response of mice to staphylococcal and tuberculous infection could be modified by injection of lipopolysaccharides extracted from Gram-negative species.

One of the most highly purified and best studied preparations of lipopolysaccharide from Gram-negative bacilli is that which has been extracted from *Salmonella typhosa*. In a number of experiments similar in design and execution to those described earlier in this report, it has been found that treatment with minute amounts of this substance exerts a profound effect on the susceptibility of mice to infection with staphylococci or tubercle bacilli. As is seen from the results presented in Table IX, the survival time of mice infected intravenously with virulent bovine tubercle bacilli could be increased by treating the animals with amounts of the typhoid polysaccharide as small as 0.02 or 0.03 mg., administered intraperitoneally 3 weeks before infection. Experiments with other purified lipopolysaccharides are now in progress, and will be reported elsewhere. Some aspects of the significance of these findings are discussed in the following paper (7).

SUMMARY

Mice were injected intraperitoneally with one of the following bacterial products having endotoxin activity: pertussis vaccine, a suspension of heat-killed cells of *Klebsiella pneumoniae* (type C), or a purified lipopolysaccharide prepared from cultures of *Salmonella typhosa*.

Following treatment with either one of these materials, the animals were infected intravenously with virulent cultures of coagulase-positive staphylococci, with bovine tubercle bacilli, or Friedländer bacilli.

The effect of treatment with endotoxin materials on resistance to Friedländer bacilli, staphylococci, or tubercle bacilli was estimated by observing the mortality rates in infected animals, and by determining quantitatively the numbers of living bacteria in the organs at different periods of time after infection.

It was found that mice receiving the infective dose of virulent culture a few hours after treatment with the endotoxin material, were usually more susceptible to infection than were untreated animals. In contrast, mice infected at a later period proved far more resistant to infection than did untreated animals.

The duration of the negative and of the positive phase of resistance was affected by the amount of endotoxin injected. Marked increase in resistance of mice to infection with staphylococci or tubercle bacilli was still evident several weeks after treatment with pertussis vaccine or with purified lipopolysaccharide extracted from typhoid bacilli.

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