THE SUSCEPTIBILITY OF MICE TO BACTERIAL ENDOTOXINS

By RUSSELL W. SCHAEDLER, M.D., AND RENE J. DUBOS, PH.D.

(From The Rockefeller Institute)

(Received for publication, November 4, 1960)

We have reported in a preceding paper some of the unusual characteristics of a new colony of albino mice raised and maintained at The Rockefeller Institute under conditions which render them free of ordinary mouse pathogens, as well as intestinal *Escherichia coli* and of *Proteus* bacilli. This new colony (designated NCS) was originally derived from 12 young animals obtained by cesarean section on 3 animals of the so called "standard Swiss" colony (SS) of albino mice which has been maintained at The Institute for the past 35 years (1).

As already reported, the NCS mice differ in many respects from the SS animals of the same age and sex, one of their differential characteristics being their much greater resistance to the lethal effect of bacterial endotoxins. The present study was designed to test the effect of prior experience with living or dead bacteria on the response of animals to endotoxins.

Materials and Methods

Mice.—

The origin and maintenance of the SS and NCS colonies have been described in reference 1. Mice of the Bagg SPF colony were obtained from Darrow Laboratories, Brooklyn. This colony, originally derived from the Walter Reed Hospital, is maintained free of ordinary mouse pathogens—hence its designation "specific pathogen-free" (SPF). However, bacteriological tests made in our laboratory have revealed that the stools of the Bagg SPF animals consistently yield large numbers of *E. coli* and *Proteus* bacilli.

The animals were fed *ad lib.* Dietrich & Gambrill, Inc., Frederick, Maryland, diet. This diet is essentially bacteria-free, at least as determined by culture tests on nutrient agar incubated aerobically. The drinking water was autoclaved and was provided *ad lib.* in sterilized containers. Despite these precautions, it was found that the water rapidly became contaminated with large numbers of various bacterial species. Several of the types of organisms present in the water could be cultivated in large numbers from the stools of animals drinking it. An attempt was therefore made to prevent bacterial multiplication in the water by the addition of antiseptics. To this end, HCl in a final concentration of 0.001 N was added to the drinking water in all recent experiments.

Bacterial Cultures.—

Escherichia coli 17 was isolated from the intestinal contents of SS mice (1). Fresh isolates of this strain are capable of becoming established in the intestinal wall of NCS mice when the culture is given to these animals either in the drinking water or more effectively with

the food. There is suggestive evidence that cultures of *E. coli* 17 lose this colonizing property when maintained for several weeks *in vitro*.

Klebsiella pneumoniae type C was maintained in the virulent state by repeated passages in mice and isolation from the heart blood.

Mycobacterium tuberculosis BCG was the substrain P described in reference 2.

Staphylococcus aureus strain Giorgio was described in reference 3. The cultures used for challenge infection were grown for 18 hours at 37°C. in meat infusion peptone broth.

Vaccines of E. *coli* 17 and *K. pneumoniae* were made by cultivating these organisms in pan-assay broth, enriched with 0.5 per cent proteose peptone (Difco Laboratories, Detroit). The bacterial cells were separated by centrifugation from 8-hour-old cultures, and were resuspended in saline. The BCG suspension was prepared as described in reference 4. All bacterial suspensions used as vaccines were heated at 80°C. for 1 hour.

Endotoxins.—

Endotoxins from *E. coli* 17 and *K. pneumoniae* type C were prepared in our laboratory as described in reference 5. The endotoxin from *Serratia marscescens* was obtained from Difco Laboratories, Detroit.

Cultures of Stools and Intestinal Tract.—

Fresh stools were collected on clean paper, and emulsified by agitation in sterile water containing 0.01 per cent activated charcoal; the charcoal was used because of its ability to counteract the antibacterial effect of certain chemical contaminants of the water. In other tests, the animals were sacrificed and the intestinal contents, as well as the intestinal wall, were emulsified or homogenized in charcoal water. In all cases dilutions of the samples were streaked on the surface of various types of nutrient agar. These were incubated at 37°C. under aerobic conditions. Anaerobic cultures were not made.

The most notable fact revealed by these aerobic cultures was that no *E. coli* or *Proteus* bacilli could be recovered from NCS animals (unless these had been contaminated deliberately or by accident). In contrast, rapid lactose fermenters and *Proteus* bacilli were commonly found in the stools and in the intestinal wall of SS and Bagg SPF mice.

EXPERIMENTAL FINDINGS

A. Intestinal Flora and the Lethal Effects of Endotoxins.—When tested within 4 weeks after weaning, NCS mice uniformly survived the injection of endotoxins in amounts up to 0.8 mg. administered intravenously, intraperitoneally, or subcutaneously. However, deaths occurred erratically following injection of endotoxins as the animals became older. Mice of the Bagg SPF strain (tested between March, 1960 and September, 1960) proved at least as susceptible as the SS mice tested in preceding years (1), and more susceptible than SS mice tested during recent months.

A decrease in susceptibility of SS mice to the lethal effect of endotoxins took place concomitantly with a change in the breeding program of these animals. Early in 1960 it was decided to substitute the NCS colony for the SS colony at The Rockefeller Institute. As a consequence, breeding of large numbers of SS animals was discontinued and the few that were produced were maintained in less crowded quarters, and received greater individual care. It has been

observed in our laboratory that SS animals produced under these changed conditions were larger at weaning time, and contained fewer coliform and *Proteus* bacilli in their stools than did those produced in very large numbers the preceding years.

It was noticed that when NCS animals were transferred immediately after birth to contaminated foster mothers (either SS mice, or NCS mice deliberately contaminated with cultures of *E. coli* 17), they gained weight less rapidly than did uncontaminated NCS animals of the same sex and age (Table I). Approximately half of them became carriers of *E. coli* and of *Proteus* bacilli. A certain number of the animals thus contaminated proved susceptible to endotoxins

TABLE I

Effect of Early Contamination on Susceptibility of Mice to Lethal Effects of Endotoxins

Mouse colony	Newborn nursed on	*Proteus* sp. and *E. coli* in stools	Weight at 4 wks.‡	Deaths following injection of endotoxin* prepared from	
				E. coli (0.4 mg.)	*K. pneumoniae* (0.5 mg.)
			gm.		
NCS	NCS	−	25.7	0/7	0/5
NCS	SS§	+	19.8	2/7	4/5
SS	SS	+	22.3	Approx. 60 per cent‖	Approx. 60 per cent‖
Bagg SPF	Bagg SPF	+	?	Approx. 80 per cent‖	Approx. 80 per cent‖

* Endotoxin injected intravenously in 0.2 ml. saline into 7-week-old animals.

‡ These weights are averages for groups of 20 mice, all born within a period of 2 days. The Bagg SPF mice were obtained from a commercial firm and were not weighed at the time of weaning.

§ The NCS mice nursed on SS animals were transferred to the foster mothers when 1 day old and were weaned 3 weeks later.

‖ These figures correspond to approximate averages from large numbers of experiments carried out over the past 2 years, (6 months for the Bagg SPF mice).

at an age when uncontaminated NCS animals were uniformly resistant (Table I). It must be acknowledged, however, that the results were erratic, with regard to both composition of the intestinal flora and susceptibility to endotoxin. In contrast, the Bagg SPF mice were consistently susceptible and they also yielded the largest numbers of coliform and *Proteus* bacilli in their stools.

B. Effect of Vaccination with Killed Bacterial Cells on Susceptibility to the Lethal Effect of Endotoxin.—In five separate experiments, it has been found that injection of heat-killed bacterial suspensions into NCS mice rendered them susceptible to the lethal effect of endotoxins. The most effective sensitization has been achieved by two vaccinating doses, at 2-week intervals. Many of the animals so sensitized died when challenged with endotoxin several weeks later (Table II). As seen in Table II, the lethal effect was most marked with endotoxin prepared from the strain of the bacteria used for vaccination. On

TABLE II

Effect of Injection of Heat-Killed Bacteria on Susceptibility of NCS Mice to Lethal Effect of Endotoxins

Heat-killed bacteria* intraperitoneally injected	Interval between 2nd vaccination and challenge	Deaths after injection of endotoxin‡ prepared from	
		E. coli (0.4 mg.)	*K. pneumoniae* (0.4 mg.)
	wks.		
E. coli	3	4/6	0/6
K. pneumoniae	3	0/6	4/6
BCG	3	0/6	1/6
Saline	3	0/7	0/7
		(0.5 mg.)	(0.5 mg.)
E. coli	4	7/7	0/6
K. pneumoniae	4	2/6	6/6
BCG	4	1/6	2/6
Saline	4	0/6	0/6

Most deaths occurred within 18 hours after injection of endotoxin, none later than 48 hours after injection.

* Male NCS mice received by the intraperitoneal route a first dose of heat-killed bacteria when 1 month old, and a second dose 2 weeks later. Each dose consisted of approximately 0.1 mg. of *E. coli* 17, or *K. pneumoniae* type C, or 0.3 mg. of BCG (dry weight). The bacterial suspensions were heated at 80°C. for 30 minutes. They were injected intraperitoneally in a volume of 0.2 ml.

‡ The endotoxins were injected intravenously in a volume of 0.2 ml. of saline.

TABLE III

Effect of Endotoxin on Weight of NCS Mice

Endotoxin intraperitoneally injected (μg./mouse)	Weight change in 24 hr. (gm. per 5 mice)	
	E. coli	*K. pneumoniae*
400	−12.5	?
30	−5.0	−5.5
10	−5.0	−2.0
3	−0.5	0.0
1	−0.5	+2.0
0.3	+1.5	+2.0
0	+4.0	+3.5

the other hand, there was evidence of overlapping, and it can be seen furthermore that some sensitization to endotoxin prepared from Gram-negative bacilli was achieved by vaccination with heat-killed mycobacteria (BCG).

C. Physiological Toxicity of Endotoxins.—The LD_{50} of endotoxin is rarely

TABLE IV

Enhancing Effect of Endotoxin on Staphylococcal Infection in NCS Mice‡*

Endotoxin§ intraperitoneally injected	Time of death (hrs. after infection)				
μg.					
10	6	6	6	6	7
3	6	6	6	6	7
1	6	7	17	17	S
0.3	6	7	7	17	S
0.1	17	S	S	S	S
0	S	S	S	S	S

S, survival.

Similar results were obtained with endotoxins prepared from other species of Gram-negative bacilli, with other cultures of coagulase positive *Staph. aureus*, and with mice from other colonies. Depending upon the conditions of the experiment, and the materials used, the minimal effective dose of endotoxin has been found to vary from 0.2 to 2 μg.

‡ NCS male mice 5 weeks old.

* 0.1 ml. of overnight culture of *Staph. aureus* in meat infusion peptone broth; injected intraperitoneally with endotoxin.

§ Prepared from *E. coli* 17; the proper dose of endotoxin was added to the staphylococcus culture, and the mixture injected intraperitoneally in a final volume of 0.2 ml.

TABLE V

Effect of Endotoxin on Multiplication of Staphylococci in NCS Mice

Time between infection and sacrifice	Numbers of Staphylococcal colonies recovered from mice receiving 0.1 ml. of *Staph. aureus* mixed with indicated amounts of endotoxin*			
	0	1 μg.	3 μg.	10 μg.
20 min.	7.0			6.5
45 "	6.0			15
2 hrs.	4			32
3 "	2.5	38	38	46
4 "	3.0			75
5 "	4.0			70

Animals sacrificed with chloroform. Skin, tail, and intestines removed. Remainder of carcass homogenized in 150 ml. of sterile water in a Lourdes blender.

The figures are averages of 4 mice and must be multiplied by 5×10^7 to give the total number of staphylococci per mouse. The table represents 4 separate experiments.

* 0.1 ml. of overnight culture of *Staph. aureus* injected intraperitoneally in admixture with 0.1 ml. of saline or with endotoxin in 0.1 ml. of saline.

less than 150 μg., even in the most sensitive mice. However, evidence of other manifestations of toxicity resulting from the injection of much smaller doses has been obtained with two different techniques. As seen in Table III, injection of 1 μg. of endotoxin into NCS mice was sufficient to cause a loss of weight

which lasted for approximately 24 to 48 hours; this weight loss was more prolonged when the dose of endotoxin was larger.

TABLE VI

Effect of Prior Treatment of NCS Mice with Heat-Killed Bacteria on Enhancement of Staphylococcus Infection by Endotoxin

Exp.‡	Vaccination (heat-killed bacteria intraperitoneally injected)§	Deaths of mice infected with 0.1 ml. *Staph. aureus* mixed with endotoxin*			
		Amount of endotoxin*	Source of endotoxin		
			E. coli	*K. pneumoniae*	*S. marcescens*
		μg.			
18	*E. coli*	20	0/6		2/6
	K. pneumoniae	20	5/7		2/5
	BCG	20	3/7		2/5
	Saline	20	6/6		6/6
	E. coli	5	0/6		
	K. pneumoniae	5	0/6		
	BCG	5	0/6		
	Saline	5	7/7		
16 *a*	*E. coli*	20	0/6	3/5	
	K. pneumoniae	20	2/6	0/4	
	BCG	20	4/6	1/4	
	Saline	20	6/6	3/4	
16 *b*	*E. coli*	6	2/6	4/7	
	K. pneumoniae	6	3/6	2/5	
	BCG	6	4/7	0/5	
	Saline	6	6/6	5/6	
28	*E. coli*	30	1/6	2/6	
	K. pneumoniae	30	6/6	1/6	
	BCG	30	2/6	2/6	
	Saline	30	5/5	7/7	

* The endotoxin and the staphylococci were injected in admixture intraperitoneally.

‡ The interval of time between the second vaccinating dose and the challenge was 3 weeks for Experiments 16 *a* and 18, 4 weeks for Experiment 16 *b*, and 5 weeks for Experiment 28.

§ The doses of bacterial vaccines were the same as in Table II.

Table IV illustrates another toxic manifestation of non-lethal doses of endotoxins. Whereas injection of 0.1 ml. of culture of staphylococci by the intraperitoneal route into normal mice never resulted in death, most animals died within 6 to 24 hours if 1 μg. or less of endotoxin was added to the staphylococcal inoculum. This rapid death was clearly caused by a complete in-

activation of the defense mechanisms against staphylococci; these organisms apparently multiplied in mice which had received endotoxin at the same rate that they would have in a tube of broth (Table V).

Attempts to quantitate enhancement of infection by endotoxin have provided indication that NCS mice are more susceptible to this particular aspect of toxicity than are SS or Bagg SPF mice. The possibility that the greater resistance of the latter was the result of prior immunological contact with Gram-negative bacteria is suggested by the following experiments.

D. Effect of Vaccination with Heat-Killed Bacteria on the Resistance of NCS Mice to Infection with Staphylococci-Endotoxins Mixtures.—Mice were vaccinated with suspensions of heat–killed bacteria as described under *B.* At various periods of time after vaccination they were infected by the intraperitoneal route with mixtures of staphylococci (0.1 ml. of culture) and of various amounts of different endotoxins. Representative findings, summarized in Table VI, make clear that vaccination increased strikingly the resistance of NCS mice to this type of infection. In some cases indeed, all vaccinated animals survived whereas all non-vaccinated died.

It will be noticed in Table VI that there were many irregularities in the level of protection induced by the vaccines, and this has been observed in other experiments not reported here. Granted the somewhat erratic character of the results, it is clear nevertheless that *E. coli* and *K. pneumoniae* vaccines afforded the most effective protection against the homologous endotoxin. On the other hand, there was evidence of cross-protection, and in fact, animals having received BCG vaccine exhibited greater resistance than the controls to enhancement of infection by endotoxin. In other words, the results display both specificity and cross-reactivity among the various endotoxins, as had been found in the experiment described under *B.*

DISCUSSION

Toxicity tests with endotoxins, similar to the ones reported in the preceding and present papers, have been conducted every month in our laboratory between September, 1959 and September, 1960; they have failed to reveal any change in the characteristics of NCS mice raised throughout this period. The fact that seasonal factors cannot account for the resistance of NCS mice to the lethal effect of endotoxins is of some importance because there have been claims that rabbits respond to some of the toxic effects of endotoxins less violently during the summer season than they do during the rest of the year.

As reported in the present paper, comparative studies on the lethal effect of endotoxins have been extended to another breed of mice, commercially known as the Bagg SPF colony. This colony is designated SPF (specific pathogen–free), because it is claimed to be free of ordinary mouse pathogens. In our

experience, however, the Bagg SPF animals proved extremely susceptible to the lethal effect of endotoxins, indeed more susceptible than SS animals.

The fact that various colonies of mice differ so consistently in their susceptibility to endotoxins appears at first sight to support the commonly held view that this characteristic is genetically determined (6–8). Some of our findings, however, point to the role of the bacterial flora in conditioning the response of animals to endotoxins.

Bacteriological studies have revealed that *E. coli* and *Proteus* bacilli are consistently present in the stools of SS and Bagg SPF mice, whereas they are never recovered from NCS animals unless these have become contaminated as a result of contact with other animals, or by food or water. Similar results have been obtained by culturing homogenates of the intestinal tract. In these tests, the intestine was removed, flushed with saline before homogenization of the various animal groups. It has been found in particular that when NCS animals are maintained under bacteriologically clean conditions, aerobic cultures of their stools and of their intestinal tract yield only lactobacilli, enterococci, and non-lactose fermenting Gram-negative bacilli. It must be emphasized that this statement is valid only for animals given food and water essentially free of living bacteria, and that the intestinal bacterial flora is naturally much more complex when the animals are allowed to ingest contaminated materials.

Evidence illustrating the role of prior contact with bacteria in the response of the animals to endotoxins has been obtained from two independent lines of experimentation: (*a*) Newborn NCS mice were transferred to foster SS mothers and were kept in contact with the latter animals until the time of weaning. Under these conditions, they acquired the intestinal flora characteristic of the SS colony, they gained weight less rapidly than uncontaminated NCS mice and they became susceptible to the lethal effect of endotoxin (Table I). (*b*) NCS mice nursed on NCS mothers and, therefore, free of intestinal *E. coli* and of *Proteus* bacilli were pretreated with suspensions of heat-killed bacteria; this vaccination rendered them susceptible to the lethal effect of endotoxin as shown by challenge several weeks later with endotoxins prepared from various cultures of Gram-negative bacilli (Table II).

These findings can probably be related to the recent reports of other investigators working with mice and rabbits. Thus, evidence has been presented that mice treated for 2 weeks with terramycin lost the ability to develop hemorrhagic skin lesions (of the Schwartzman type) following a single intradermal injection of endotoxin (7, 8). It has been postulated that this loss of reactivity was due to the inhibitory effect of the drug on Gram-negative bacilli *in vivo*. Likewise, it has been found in our laboratory (unpublished experiments) that prolonged treatment with oxytetracycline decreases the susceptibility of SS and Bagg SPF mice to the lethal effect of endotoxin.

The observations on rabbits deal with the production of shock by bleeding

(9). Whereas hemorrhagic shock readily becomes irreversible in ordinary rabbits, this phenomenon did not occur in animals that had been freed of intestinal *E. coli* by chemotherapy. On the other hand, susceptibility to irreversible shock could be induced again in resistant rabbits by feeding them cultures of *E. coli* for several weeks—a finding which suggests that the change in response was caused by the bacterial endotoxin absorbed from the intestine during hemorrhagic shock (9).

Taken together, the facts mentioned above indicate that many of the differences in susceptibility to the toxic effect of endotoxins which might have been thought to be genetically determined, in reality are the consequence of prior contact of the animals with bacteria, particularly with some of the Gram-negative species present in the intestinal tract.

Although NCS mice are resistant to the *lethal* effect of large amounts of endotoxin (500 μg.), they suffer a transient but marked loss of weight following injection of even very small amounts (1 μg.) of these same materials (Table III). Further evidence of their great susceptibility to this *physiological* type of toxicity is provided by the enhancing effect of endotoxins on infection with unrelated bacterial pathogens. For example, injection of 1 μg. or less of endotoxin is sufficient to convert a nonlethal staphylococcal infection into a fulminating septicemia which kills the animals within a few hours (Tables IV and V). As seen in Table V, staphylococci injected intraperitoneally into normal NCS mice were progressively destroyed *in vivo*, whereas they started to multiply immediately after infection when administered in admixture with small amounts of endotoxin.

It is of interest that the minimal dose of endotoxin which can be detected by weight loss of the animal or by enhancement of staphylococcal infection is of the same order as that determined by measurement of the effect of endotoxin on the excretion of urinary nitrogen following administration of ACTH (10).

NCS mice are at least as susceptible as SS or Bagg SPF mice to the enhancement of infection by endotoxin. However, their resistance to this toxic effect of endotoxin could be greatly increased by vaccination with killed bacteria, especially when the vaccine consisted of organisms of species from which the endotoxin used in the infection-enhancing test had been prepared (Table VI).

Thus, pretreatment of NCS mice with heat-killed bacteria can have two opposite effects: (*a*) it can increase susceptibility to the lethal effect of large amounts of endotoxin (Table II); (*b*) it can increase resistance to the infection enhancing effect of small amounts of these same materials (Table VI).

The data presented in Tables II and VI reveal a marked degree of specificity in these two aspects of the conditioning of the animals by endotoxins—the influence in either case being more pronounced with the endotoxin prepared from the type of bacteria used to pretreat the mice. On the other hand, it is also apparent that the various endotoxins exhibited a certain amount of over-

lapping in their effect. Indeed, pretreatment with mycobacteria (killed BCG) was found to increase susceptibility to the lethal effect of large doses of endotoxins, and also to increase resistance to their infection-enhancing effect (Tables II and VI). This finding is compatible with the fact that mycobacterial cells contain a fraction which has chemical and biological similarities with the lipopolysaccharide endotoxins of Gram-negative bacilli (11–17). As can be seen in Table II and VI, control of the concentration of the materials used for the various tests makes it possible to emphasize either the cross-reactions between the materials derived from various bacterial species, or the specific aspects of their biological activities.

While the facts observed in the present study and those disclosed in the literature appear to present several incompatibilities, it would seem that they can be reconciled in terms of the following assumptions:

(*a*) Endotoxins possess a primary toxicity which causes profound physiological disturbances in all normal mice tested even when the dose injected is very small (1 μg. or less). These disturbances result in a transient but marked weight loss and in increased susceptibility to certain bacterial infections.

(*b*) The lethal effect of large amounts of endotoxins differs in nature from this physiological toxicity and is more indirect. In some cases death may be the outcome of activation of a latent infectious process occuring during the phase of negative resistance caused by the endotoxin. More often, probably, death results from an allergic-like reaction which is superimposed upon the physiological disturbance mentioned above. This allergic-like state depends upon prior contact of the animal with certain bacterial antigens.

(*c*) These reactions possess a certain degree of immunological specificity but their specific character is usually masked, completely or in part, by the overlapping of antigenic structure among the various endotoxins.

(*d*) The conditioning of mice (and probably of other animals) to endotoxins is determined in large part by the bacterial components of their intestinal flora; the lack of uniformity of this flora from one animal to another accounts for the vagaries of individual responses to the administration of endotoxin.

Although the preceding discussion has been focussed on the immunological aspects of susceptibility to the lethal effect of endotoxin, other mechanisms probably participate in this phenomenon. It is of interest in this regard that zymosan, like BCG infection, has been found to increase the susceptibility of mice to endotoxin; moreover this sensitization occurs concomitantly with an increase in activity of the reticuloendothetial system, and it can be prevented to a large extent by administration of cortisone (18). These facts are compatible with the hypothesis that chronic infections or granulomatous inflammation contribute in some way to the lethal effects of endotoxins (14, 18).

SUMMARY

Albino mice (Rockefeller NCS strain) raised and maintained free of ordinary bacterial pathogens, as well as of intestinal *Escherichia coli* and of *Proteus* bacilli, were found to be highly resistant to the lethal effect of bacterial endotoxins.

When newborn mice of this NCS colony were nursed by foster mothers from another colony raised under ordinary conditions (SS colony from which the NCS colony was derived), they acquired the intestinal flora of the latter animals and became susceptible to the lethal effects of endotoxins.

NCS adult mice could be rendered susceptible to the lethal effect of endotoxins by vaccination with heat killed Gram-negative bacilli. The susceptibility thus induced exhibited a certain degree of specificity for the bacterial strain used in vaccination.

Although untreated NCS mice were resistant to the lethal effect of endotoxins, they proved exquisitively susceptible to the infection-enhancing effect of these materials. For example, 1 μg. or less of endotoxin was found sufficient to help establish a rapidly fatal septicemia with *Staphylococcus aureus*. Small amounts of endotoxin (1 μg. or less), administered alone, caused a marked but transient loss of weight.

Vaccination with heat-killed Gram-negative bacilli or with killed BCG increased the resistance of NCS mice to the infection-enhancing effect of small amounts of endotoxin. This protective effect exhibited a certain degree of specificity for the bacterial strain from which the toxin used in the infection-enhancing test was derived.

These various findings can be explained by assuming that the pathological effects of endotoxins involve at least two unrelated mechanisms; (*a*) a primary toxicity illustrated in this study by the loss of weight and enhancement of infection resulting from the injection of small doses of toxin; (*b*) an immunological reaction with lethal consequences which became manifest only in animals sensitized to the endotoxin by prior exposure to Gram-negative bacilli.

BIBLIOGRAPHY

1. Dubos, R. J., and Schaedler, R. W., The effect of the intestinal flora on the growth rate of mice and on their susceptibility to experimental infections, *J. Exp. Med.*, 1960, **111**, 407.
2. Dubos, R. J., and Pierce, C. H., Differential characteristics *in vitro* and *in vivo* of several substrains of BCG. I. Multiplication and survival *in vitro*, *Am. Rev. Tuberc. and Pulmon. Dis.*, 1956, **74**, 655.
3. Smith, J. M., and Dubos, R. J., The behavior of virulent and avirulent staphylococci in the tissues of normal mice, *J. Exp. Med.*, 1956, **103**, 87.
4. Dubos, R. J., and Schaedler, R. W., Effects of cellular constituents of mycobac-

teria on the resistance of mice to heterologous infections, *J. Exp. Med.*, 1957, **106,** 703.

5. Dubos, R. J., and Schaedler, R. W., Effect of nutrition on the resistance of mice to endotoxin and on the bactericidal power of their tissues, *J. Exp. Med.*, 1959, **110,** 935.
6. Kelly, M. G., Smith, N. H., Wodinsky, I., and Rall, D. P., Strain differences in local hemorrhagic response (Shwartzman-like reaction) of mice to bacterial polysaccharides, *J. Exp. Med.*, 1957, **105,** 653.
7. Arndt, W. F., and Schneider, H. A., Two local hemorrhagic skin responses to bacterial endotoxin in an inbred mouse strain, *Proc. Soc. Exp. Biol. and Med.*, 1958, **99,** 127.
8. Arndt, W. F., and Schneider, H. A., The extension of the Shwartzman phenomenon to the mouse and some ecological determinants of the single-injection reaction, *J. Exp. Med.*. 1960, **112,** 167.
9. Fine, J., Ruttenburg, S., and Schweinburg, F. B., The role of the reticuloendothelial system in hemorrhagic shock, *J. Exp. Med.*, 1959, **110,** 547.
10. Berry, J., and Smythe, D. S., Effects of bacterial endotoxins on metabolism. III. Nitrogen excretion after ACTH as an assay by endotoxins, *J. Exp. Med.*, 1961, **113,** 83.
11. Suter, E., and While, R. G., The response of the reticuloendothelial system to the injection of the "purified wax" and the lipopolysaccharide of tubercle bacilli, *Am. Rev. Tuberc.*, 1954, **70,** 793.
12. Suter, E., Ullman, G. E., and Hoffman, R. G., Sensitivity of mice to endotoxin after vaccination with BCG (Bacillus Calmette-Guérin), *Proc. Soc. Exp. Biol. and Med.*, 1958, **99,** 167.
13. Halpern, B. N., Biozzi, G., Howard, J. G., Stiffel, C., and Mouton, D., Exaltation du pouvoir toxique d'*Eberthella typhosa* tuée chez la Souris inoculée avec le B.C.G. vivant. Relation entre cette augmentation de la susceptibilité et l'état, *Compt. Rend. Soc. Biol.*, 1958, **152,** 899.
14. Howard, J. G., Biozzi, G., Halpern, B. N., Stiffel, C., and Mouton, D., The effect of *Mycobacterium tuberculosis* (BCG) infection on the resistance of mice to bacterial endotoxin and *Salmonella enteritidis* infection, *Brit, J. Exp. Path.*, 1959, **40,** 281.
15. Rzucidlo, L., Lichtensztejn, A., and Kwiek, S., Influence of various extraction methods on the biological properties of *Mycobacterium tuberculosis*, *Bull. Acad. Polon. Sc.* 1957, **5,** 419.
16. Williams, C. A., Jr., and Dubos, R. J., Studies on fractions of methanol extracts of tubercle bacilli. I. Fractions which increase resistance to infection, *J. Exp. Med.*, 1959, **110,** 981.
17. Williams, C. A., Jr., Studies on fractions of methanol extracts of tubercle bacilli. II. Toxic and allergenic properties of fractions employed as antituberculous vaccine, *J. Exp. Med.*, 1960, **111,** 369.
18. Benacerraf, B., Thorbecke, G. J., and Jacoby, D., Effect of zymosan on endotoxin toxicity in mice, *Proc. Soc. Exp. Biol. and Med.*, 1959, **100,** 796.