EXPERIMENTAL ANTHRAX IN THE RAT

II. The Relative Lack of Natural Resistance in Germ-Free (Lobund) Hosts

Martha J. Taylor, A.B.; James R. Rooney, D.V.M.,* and George P. Blundell, M.D.†

From the U.S. Army Chemical Corps Biological Laboratories, Fort Detrick, Frederick, Md.

The preceding report in this series[1] described an age-correlated increase in the natural resistance of conventionally reared albino rats to lethal infection with spores of *Bacillus anthracis*. The average rate at which a high degree of resistance developed was calculated for the Fischer 344 strain of rat, revealing that for every 1-day increase in injection age from the sixth through the 31st postnatal day, the spore dose corresponding to 50 per cent survival increased at a rate of 1.5-fold daily—from 10^4 to 10^9 spores. The LD_{100} for 1-day-old Fischer rats was less than 10^4 spores, while rats more than 5 weeks old at injection usually survived 10^9 spores.

The various factors affecting this level of resistance in the conventionally reared rat are not understood and are difficult to define. There is a need to analyze the morphologic and physiologic aspects of maturation of the host defense mechanisms and a variety of factors in the environment. The problems involved in designing adequately controlled experiments to investigate the development of nonspecific resistance to infection in laboratory animals are numerous. A controlled environment is one obvious essential. Experiments have now been performed with germ-free albino rats reared under conditions that eliminated exposure of the animals to micro-organisms present in the normal (conventional) rat colony maintained here. The results permit comparisons between germ-free and conventional rats, at age 5 weeks, with respect to (a) their level of resistance to lethal infection with *B. anthracis* spores, and (b) electrophoretically determined protein patterns in serums of noninfected animals. The level of resistance was investigated further by analyzing gross and microscopic features of the rats at death.

Presented in part before the 60th Annual Meeting of the Society of American Bacteriologists, May 4, 1960, Philadelphia.

Accepted for publication, December 30, 1960.

* Present address: Department of Animal Pathology, University of Kentucky, Lexington, Ky.

† Present address: Oscar B. Hunter Memorial Laboratory, 915 Nineteenth Street, N.W., Washington 6, D.C.

The results indicate that nonspecific factors in a conventional environment play a role in producing the naturally acquired resistance of the adult albino rat to lethal infection with *B. anthracis* spores.

Material and Methods

Breeding and Care of Rats

The germ-free rats, descendants of inbred Wistar stock, now bred at the Lobund Institute, were in the fourth to ninth generations of germ-free life, and conformed to Lobund standards for germ-free animals.[3] Five closely related litters were brought to and maintained at Fort Detrick in a Trexler flexible plastic isolator unit (Trexler,[3] Fig. 1), ventilated only with filtered air. Litter mates, and later the experimental groups of 3 to 7 rats, were housed in lucite cages equipped with the necessary bedding, lids, food, and bottles of water. They were fed Lobund diet L-356,[4] which is similar to the Purina Chow fed the Fischer rats with respect to analysis of protein, fat, and ash, but contains additional salts and minerals.

All equipment taken into the unit was autoclaved at 121° C. for at least 30 minutes, and then held for 30 minutes in an air lock attached to the unit and filled with peracetic acid vapor. The only cultural tests made at Fort Detrick were for bacteria that would grow aerobically on the surface of blood agar (made by adding 7 ml. of fresh, defibrinated, whole rabbit blood to each 100 ml. of Difco blood agar base). Tests for bacteria in the rats' environment were made by culture of swabs from cages, and of feces, on plates of blood agar. These samples were taken immediately prior to (a) sacrifice of the controls, (b) inoculation of the other animals with *B. anthracis* spores, and at intervals thereafter. The consistent failure to find colonies, other than those of *B. anthracis*, was considered adequate confirmation of the continuing, successful exclusion of air-borne bacterial contaminants.

Our colony of the genetically homogeneous Fischer 344 strain of rats has been described.[1]

Experimental Rats

In the first experiment, one litter of 7 Lobund rats was challenged with 10^7 spores. In the second experiment, 3 of 4 litters which came from the Lobund Institute 8 months later were challenged. These rats were identified with toe or ear markings on the day of arrival, and distributed to cages for 6 dose groups (Table II).

Control Rats

The first shipment of Lobund rats consisted of the litter of 7 mentioned above, and 1 extra male of unknown age. He was held in the unit, caged alone, as a noninoculated control. On the day the injected litter died of anthrax, this rat was killed. His spleen and feces were cultured on blood agar. As additional controls, a 38-day-old litter (2 males and 1 female) in the second shipment of Lobund rats was removed from the unit on the day after arrival. These rats equaled Fischer rats of the same age and sex in weight and sexual development. Heart's blood was drawn from each to provide serums for electrophoretic analysis and for control on tests for antibody. The rats were weighed, killed, and necropsied. Samples of liver and feces were cultured aerobically on blood agar. Tissues of these 4 Lobund rats were prepared for comparative histologic study with similar tissues of the experimental animals.

Serums from conventionally reared Fischer rats, some the same age as the Lobund rats, and some older (Table V), were obtained to run concurrently with the Lobund serums in the electrophoretic analyses of serum proteins.

Gross necropsy records and histologic sections of normal and *B. anthracis*-infected tissues of Fischer rats, of nearly the same age at sacrifice or death as the Lobund rats, were available from earlier experiments for comparative studies.

Pathogen, Dose and Injection Route

The spores constituting the inoculums for these experiments were from the same suspension of spores of the virulent 1B sib-progeny of the Vollum strain of *B. anthracis* previously described.[1] The number of viable spores was counted repeatedly during 18 months of use and consistently found to be between 7×10^9 and 9×10^9 per ml. The suspension was heat-shocked at 60° C. for 30 minutes in May, 1957, and again in October, 1958. Storage was always in the refrigerator. This stock suspension was diluted in distilled water to the concentration intended for each inoculum. The calculated concentration was confirmed by plate counts for viable spores made within 24 hours of use. In the first experiment each rat received a dose of 10^7 spores in a volume of 0.1 ml. In the second experiment, each member of the designated groups of rats received 10^1, 10^2, 10^3, 10^4, 10^5, or 10^6 spores. Each dose was in 0.1 ml. of water (Table II).

On the day of injection, fur was snipped from a 1.5 $cm.^2$ area on the left flank, where each rat was then dosed with 0.1 ml. of spore suspension intradermally as superficially as a 27-gauge hypodermic needle could be introduced without leakage of inoculum. This raised a clearly visible bleb. Identical injection techniques had been used to challenge conventional Fischer rats with 10^6 and 10^9 spores.[1] In those experiments the 10^7 dose was delivered subcutaneously over the abdomen.

Time Between Inoculation and Death

Surveys for dead rats were made several times a day during the first 96 hours after injection and daily thereafter. Time to death was estimated to the nearest 6-hour interval.

Diagnostic Procedures

Dead animals were first weighed and then opened and necropsied aseptically. Tissue was taken for culture, impression smears were made, and the condition of inoculation site, lymph nodes, and viscera noted. The viscera were stripped from the carcass, the brain case opened, and everything placed in buffered 10 per cent formalin for fixation. Selected tissues from each animal were prepared for histologic study.

For culture, the cut surface of the tissue to be studied, or a fecal pellet was touched several times to the surface of blood agar plates. Part of the touched surface was loop-streaked to insure isolated colonies for observation of structure. Plates were read at 24 and 48 hours. The typical Vollum colony (on this agar, at 24 hours: 2 to 4 mm., abruptly raised, finely granular surface, with comma- or tail-like peripheral processes; at 48 hours: much larger, thick and cream colored), with no contaminants present, was accepted as evidence that a pure culture of *B. anthracis* had been recovered.

Impression smears were made from all livers, and from certain other organs in 1 case. Smears were air-dried, fixed in buffered formalin for 30 minutes to overnight, washed in running water, drained dry in the 37° C. incubator, and stained with Jenner-Giemsa.

For histologic study, all tissue sections were stained by a modified Lillie-Giemsa method. Comparisons were made as follows: (1) Unchallenged *versus* fatally infected Lobund rats 33 to 51 days old. (2) Unchallenged *versus* fatally infected Fischer rats 28 to 38 days old. (3) Lobund rats, fatally infected at age 33 to 37 days with 10^7 spores *versus* Fischer rats, fatally infected at age 27 to 32 days with 10^7, 10^8, or 10^9 spores (Table I). (4) Among groups of Lobund rats, fatally infected at age 33 to 37 days, with spore doses of 10^1 through 10^7 separated by 1-log intervals.

Analysis of Serums

Paper electrophoretic determinations were made on 0.01 ml. of serum in a hanging strip cell in 0.075Γ/2 veronal at pH 8.6. Eight strips of Whatman 3 MM paper were

run simultaneously in 1 cell for 16 hours at room temperature under constant current conditions of 8 ma. per cell. The strips were stained in bromphenol blue and evaluated by transmission densitometry with the Analytrol under the conditions recommended by the manufacturer.

TABLE I

CONVENTIONAL FISCHER 344 RATS DEAD WITH ANTHRAX * THAT WERE SELECTED FOR HISTOLOGIC COMPARISON WITH ANTHRAX IN THE GERM-FREE LOBUND RATS

Log of spore dose	Age at injection (days)	Sex	Weight at injection (gm.)	Time to death (hr.)	Edema at injection site
7†	28	F	52	27	±
7†	29	M	72	20	2+
7†	30	F	60	60	1+
8‡	32	M	85	32	1+
9‡	27	M	55	44	1+
9‡	27	M	51	37	2+

*Diagnosis confirmed by culture and Jenner-Giemsa-stained impression smears, both of liver. Individuals were selected on a basis of injection age and dose of spores most nearly comparable with the germ-free experiment.

† Injection was subcutaneous.

‡ Injection was intradermal.

RESULTS

Successful Control of Bacterial Environment

Pre-inoculation cultures from cages and feces, and cultures at necropsy of the 4 uninoculated Lobund rats' feces and tissues were entirely negative. Periodic postinoculation cultures from cages, feces, and various inside surfaces of the plastic unit always revealed the presence of *B. anthracis* and no other bacteria. The unit was still in this state of monocontamination when the 1 surviving rat was sacrificed 3 months after challenge.

Death and Survival

The hours between inoculation and death of each rat are listed in Table II. The smaller doses tended to prolong the time to death, although the animal that died a week later than any others received 10^6 spores. The 1 survivor received 10 spores. These 2 animals were litter mates.

Gross Necropsy and Diagnosis

Certain necropsy findings for the infected Lobund rats are included in Table II. The following statements are based upon a review of the full necropsy records for these rats, and for the many Fischer rats of comparable age dead of anthrax in previous experiments. Instead of the localized, necrotic, slightly edematous and hyperemic injection-site reaction observed in the conventional Fischer rats, the majority of the

Lobund site lesions were characterized by a thickened, dull-red area 1 to 3 cm. in diameter immediately under the point of needle entry. The lesion was surrounded by an extensive zone of gelatinous edema and hyperemia. This closely resembled the site lesions customarily seen in fatally infected mice, guinea pigs, and other species for which the LD_{50} of *B. anthracis* is approximately 10 spores injected intradermally or

TABLE II

SUMMARY DATA FOR GERM-FREE LOBUND RATS TESTED INTRADERMALLY WITH SPORES OF *B. anthracis*

Log of spore dose	Age at injection (days)*	Sex	Weight at death (gm.)	Time to death (hr.)	Edema at site of injection	Fluid in chest
1	34	M	Survived †			
	36	M	121	132	0	0
	36	F	96	54	0	0
	37	F	109	138	0 ‡	0
2	34	M	101	72	0 §	Present
	36	M	80	48	2+	0
	36	F	84	48	+	0
	37	F	89	66	3+	0
3	34	F	96	54	3+	0
	36	M	110	144	4+	0
	37	M	99	48	4+	0
	37	F	99	54	2+	0
4	34	M	113	54	2+	0
	36	F	100	30	±	0
	37	M	118	60	4+	0
5	34	M	98	30	3+	0
	36	F	85	42	4+	0
	37	M	106	54	4+	0
6	34	F	129	390	0	0
	36	M	89	30	4+	0
	37	M	98	30	4+	0
7	33	F	69	36	4+	0
	33	F	72	30	4+	0
	33	F	74	30	4+	0
	33	F	72	24	4+	0
	33	F	75	24	4+	0
	33	F	74	30	4+	0
	33	F	71	30	4+	0

* Rats the same age are litter mates. The first experiment (33-day-old litter) was inoculated in February, 1958. The second experiment (other 3 litters) was inoculated in October, 1958.

† Killed for special study 3 months after challenge.

‡ None at injection site, but considerable amount centered around the left eye. See text.

§ Small necrotic lesion in subcutaneous tissue; this resembled the conventional Fischer rat reaction.

subcutaneously. The site lesion was notably smaller in the Lobund rats dosed with 100 spores than with larger numbers of spores, and absent from those rats given 10 spores and from the one that died on the 17th post challenge day. One of the rats with no injection-site lesion had subcutaneous edema around the left eye. All conventional rats that died consistently exhibited copious, clear pleural fluid (hydrothorax); only 1 Lobund rat (a litter mate of the survivor) had pleural fluid. The Lobund rat spleens were generally dark red and plump—not notably different from those of conventional rats and other rodents with anthrax. The lymph node (left axillary) receiving drainage from the injection site was usually enlarged; all others appeared relatively nonreactive.

Jenner-Giemsa-stained impression smears of liver from each rat, and of several additional tissues from the rat dead at 17 days, revealed the omnipresence of characteristic bacilli.

Of the 59 cultures (27 liver, 21 fecal, 3 lung, 3 spleen, 2 lymph node, 3 tail-blood) of necropsy material from 27 animals, 58 gave pure *B. anthracis*. One (of an extremely autolyzed liver) was negative, but stained smears confirmed the presence of morphologically characteristic bacilli, and other tissues cultured from this rat gave *B. anthracis* colonies. No other bacterial species was recovered.

Histologic Features

The typical histologic findings in tissues of 6 Fischer (Table I) and 22 Lobund rats* dead following injection with *B. anthracis* spores are listed in Table III, and illustrated in Figures 1 to 5. The table shows that the Fischer rats reacted with the development of a localized skin lesion that included necrosis of the epidermis. Focal lesions also occurred in the regional lymph node, liver, and spleen. The Lobund rats reacted with (a) a larger, more diffuse skin lesion without necrosis, (b) diffuse necrosis of the regional lymph node and other lymph nodes, and (c) massive diffuse necrosis of the spleen. Reactions were also present in the adrenal and thymus of the Lobund rats that were not noted in the Fischer rats. The degree of "sequestration" of mononuclear cells and bacilli within the pulmonary vessels of the Fischer rats was greater than in the Lobund rats. The intra-alveolar edema and fluid within the pleural cavity were features of the Fischer rats only. The bacilli were seen in greater numbers in the blood vessels and diseased organs of the Lobund than in the Fischer rats. The renal lesions were similar in both rat types.

The 34-day-old litter of 6 Lobund rats, with 1 representative in each of the second experiment's dose groups, was atypical in its resistance

* The 34-day-old litter of 6 was atypical in certain respects, and is discussed separately below.

level and in its histologic pattern. The rat given 10 spores survived, although there was serologic and histologic evidence of infection and recovery. The lesions in the 4 rats receiving 10^5 through 10^2 spores showed a consistently definite trend toward the reaction pattern observed in conventionally reared Fischer rats—intra-alveolar edema and "sequestration" were increased in severity with decreasing spore dose, as was epidermal necrosis at the inoculation site. The adrenal hemor-

TABLE III

HISTOLOGIC FINDINGS IN TISSUES OF CONVENTIONAL FISCHER 344 AND GERM-FREE LOBUND RATS OF COMPARABLE AGES, DEAD FOLLOWING INJECTION OF *B. anthracis* SPORES

Tissues	Fischer *	Lobund †
Site of injection	Acute cellulitis Epidermal necrosis Few bacilli	Acute cellulitis No epidermal necrosis Masses of bacilli
Regional lymph node	Focal, subcapsular necrosis Mononuclear cell accumulation	Liquefaction necrosis Hemorrhage Many bacilli
Other lymph nodes	Normal	Variably affected by lymphoid necrosis, hemorrhage, and bacilli
Liver	Kupffer cell hypertrophy Phagocytosis of bacilli Intralobular mononuclear cell foci, frequently undergoing necrosis	Little or no Kupffer cell response Masses of bacilli pack the sinusoids
Spleen	Demarcated mononuclear cell foci, with necrosis Moderate hyperemia Few bacilli	Massive, diffuse necrosis Severe hyperemia Many bacilli
Kidney	Same picture in both sets of rats: Acute glomerulitis—necrosis, thrombosis, bacilli Acute segmental necrosis of the cortical convoluted tubules	
Adrenal	Normal	Marked cortical hemorrhages
Thymus	Normal	Hyperemia Necrosis of thymocytes
Lung	Striking "sequestration" of mononuclear cells and of bacilli in the pulmonary arteries Hyperemia Intra-alveolar edema	Minimal "sequestration" Marked hyperemia No alveolar edema Large numbers of bacilli in blood vessels

* Three rats, 28, 29, 30 days old, dosed subcutaneously with 10^7 spores. Three rats, 27, 27, 32 days old, dosed intradermally with 10^6, 10^5 and 10^5 spores respectively; 4 experiments represented.

† Twenty-two rats, 33, 36, 37 days old, dosed intradermally with 10^1 through 10^7 spores at 1-log intervals. See text for discussion of 1 atypical 34-day-old litter.

rhages and thymic involution, however, which had not been observed in the Fischer rats but which were typical of the Lobund rats, were observed even at the 10^2 spore dose level. The rat given 10^6 spores died on the 17th postinjection day. The cellular response in this rat more closely resembled that of the conventional Fischer than of the Lobund rats, the long course of the disease having allowed a definite chronic

TABLE IV

EVIDENCE FOR THE PRODUCTION OF ANTIBODY * TO THE PROTECTIVE ANTIGEN OF *Bacillus anthracis* BY GERM-FREE LOBUND RATS FOLLOWING INJECTION OF SPORES

Rat				
Strain	Treatment	Age (days)	Number of days after injection	Antibody titer †
Fischer	None	38		Nil
Lobund	None	38		Nil
Fischer	None	141		Nil
Lobund	10^6 spores	50	16	1/8
Lobund	10^1 spores	50	16	1/4
Lobund	10^1 spores	127	93	1/16

* Serums were tested by the agar-diffusion method of Thorne and Belton.[5]

† Highest dilution of serum neutralizing the reaction of standard amounts of protective antigen with standard antiserum.

reaction to develop. Again, though, the necrosis of cells in the thymus was like that of the other Lobund rats.

Antibody Production

Neutralizing antibody to the *B. anthracis* protective antigen was demonstrated in the serums of 2 Lobund rats that lived to supply blood samples on the 16th postinjection day, and in the serum of the one of these that was tested again 2½ months later (Table IV). This finding is reported as evidence that at age 34 to 50 days rats reared germ-free responded effectively (produced antibody promptly) to antigenic stimulation.

Gamma Globulin

The electrophoretic patterns obtained for serum proteins of the 38-day-old noninoculated germ-free Lobund and the 36 and 38-day-old noninoculated conventional Fischer rats were indistinguishable from each other and corresponded to those figured by Gustafsson and Laurell [6] for 50- to 150-day-old germ-free rats, notably regarding the faintness of the gamma globulin bands. This band was more intense in the serums of our 128- and 333-day-old Fischer rats, appearing about as it does in

serums of Gustafsson and Laurell's 50- to 150-day-old conventional rats. The values obtained for gamma globulin relative to total serum protein are shown in Table V. Other serum protein fractions in our samples of Lobund and young Fischer rats exhibited no obvious deviations from those present in the older Fischer rats. Serums from additional animals would have to be analyzed to determine whether smaller, but possibly significant, differences exist.

Discussion

The small numbers of germ-free rats tested do not permit calculation of a spore LD_{50}, but the results place the LD_{100} at less than 100 spores. This is 1/10,000,000th the LD_{50} for a 31-day-old and 1/100th the LD_{50}

Table V

GAMMA GLOBULIN IN SERUM PROTEIN OF NONINOCULATED GERM-FREE LOBUND AND CONVENTIONAL FISCHER 344 RATS

Rats	Age (days)	Sex	Weight (gm.)	Gamma globulin (%)* Individual	Gamma globulin (%)* Average	Measure of resistance to *B. anthracis*
Germ-free	38	M	107	5.4	4.8	LD_{100}† = less than 100 spores
	38	M	98	3.8		
	38	F‡	91	5.1		
Conventional	38	F‡	87	4.7	5.6	98 to 100% survive 10^9 spores
	38	F‡	98	6.0		
	38	F§	83	5.3		
	36	F‡	85	5.1		
	36	F‡	86	6.8		
Conventional	128	F	194	9.7	8.9	Not tested
	128	F	181	9.0		
	128	F	173	8.1		
Conventional	333	M	415	10.8	10.0	Not tested
	333	F	262	9.4		
	333	F	297	9.8		

* Values obtained by transmission densitometry for gamma globulin divided by the value for total serum protein, times 100.

† Dose per rat required to kill 100 per cent of the animals tested, in this case by the intradermal route.

‡ Vagina open.

§ Vagina closed.

for 6-day-old conventional Fischer rats. Unpublished results from a limited number of experiments indicate the LD_{100} for 2- to 5-day-old Fischer rats to be betwen 10^2 and 10^3 spores. Thus, the 33- to 37-day-old Lobund rats resist fewer spores than 2- to 5-day-old conventional rats. The 35- to 40-day-old Fischer rats, which were entirely comparable in weight and sexual development with the Lobund rats, usually survived

10^9 spores. Although genetic differences must exist between the Lobund and Fischer rat, there is no reason to suppose that they are responsible for the tremendous difference observed in resistance levels. The bacteria in the conventional environment are likely candidates for a causal role in the production of this naturally acquired resistance of the adult white rat to infection with *B. anthracis* spores. Since adults of several other rodent species do not develop such resistance, in spite of exposure to similar environmental factors, more attention must be directed toward learning how this is accomplished by the rat.

The mechanisms by which gamma globulins promote resistance remain unknown. Gustafsson and Laurell provided evidence that strongly suggested that "the normal flora of microorganisms is an important stimulant for the gamma globulin-producing cells."[6] If their observed values for gamma globulin, which were reported as gm. per hundred ml. of serum, are converted to per cent of total serum protein, the mean value for this component in germ-free serums is 3.8 and in the conventional serums 8 per cent. Wostmann and Gordon's data,[2] similarly recalculated, show 3 and 8 per cent gamma globulin in germ-free and conventional Lobund rats. These values serve to substantiate ours, which were obtained from small numbers of individuals. Our results suggest that there is no correlation between electrophoretically measured serum gamma globulin levels and the degree of resistance in the rat to the spores of *B. anthracis*—a high level of resistance to anthrax exists in the Fischer rat at an age when its serum gamma globulin is still as low, within the limits of the reliability of the method, as that of the highly susceptible Lobund rats. If environmental bacteria enhance natural resistance to *B. anthracis* through stimulating production of nonspecific antibody gamma globulin, the product cannot be detected by paper electrophoresis.

Histologic examination revealed that anthrax bacilli produced focal lesions in specific tissues in the Fischer rats. Corresponding tissues in the Lobund rats showed diffuse involvement, and additional organs evidenced a reaction. It thus appears that the conventional Fischer rat possesses greater ability to localize and contain the destructive action of the bacilli.

The "sequestration" of mononuclear cells and bacilli within many of the pulmonary blood vessels was greater in the Fischer than in the Lobund rats. This condition takes on added significance as a major contributing cause of death when one considers the gross finding of excess fluid in the pleural cavity and the microscopic feature of intra-alveolar edema in the Fischer rat. Although "sequestration" was occasionally observed in Lobund rats, hydrothorax and intra-alveolar edema were

absent. The more severe cutaneous edema in the Lobund rat may have prevented the occurrence of the intra-alveolar edema and pleural effusion found in the Fischer rats. It has been suggested that death of guinea pigs with anthrax may be due to secondary shock.[7] This is acceptable for the Lobund rat. The more resistant Fischer rat dies with pulmonary edema associated with pulmonary vascular sequestration and shows no evidence of shock.

Summary

Albino rats, reared germ-free at the Lobund Institute, were transported in an isolator unit to our laboratory and tested at the age of 5 weeks for their susceptibility to intradermally injected spores of *Bacillus anthracis*. The LD_{100} for these rats was found to be less than 100 spores, which is 1/10,000,000th the LD_{50} for 31-day-old and 1/100th the LD_{50} for 6-day-old conventionally reared Fischer rats. This places the level of the 5-week-old germ-free rat's resistance at least as low as that of conventional rats less than 1 week old, and suggests that the rat maturing in a conventional environment may improve its resistance to *B. anthracis* spores through contact with this environment.

The presence of antibody to the *B. anthracis* protective antigen in serums of 2 rats alive on the 16th day after challenge demonstrated that these rats were capable of producing antibody in response to antigenic stimulation.

Gamma globulin levels, determined electrophoretically, were equally low in serums of 5-week-old germ-free and conventional rats compared with the higher level found in 4- and 11-month-old conventional rats. This means that the development of high resistance to *B. anthracis* in the conventional rat precedes the detected rise in serum gamma globulins.

As a result of histologic studies, it is suggested that death may result from different causes in the germ-free and in the conventional rat.

References

1. Taylor, M. J.; Kennedy, G. H., and Blundell, G. P. Experimental anthrax in the rat. I. The rapid increase of natural resistance observed in young hosts. *Am. J. Path.*, 1961, **38**, 469–480.
2. Wostmann, B. S., and Gordon, H. A. Electrophoretic studies on the serum protein pattern of the germfree rat and its changes upon exposure to a conventional bacterial flora. *J. Immunol.*, 1960, **84**, 27–31.
3. Trexler, P. C. The use of plastics in the design of isolator systems. *Ann. New York Acad. Sc.*, 1959, **78**, 29–36.
4. Larner, J., and Gillespie, R. E. Gastrointestinal digestion of starch. III. Intestinal carbohydrase activities in germ-free and non-germ-free animals. *J. Biol. Chem.*, 1957, **225**, 279–285.

5. THORNE, C. B., and BELTON, F. C. An agar-diffusion method for titrating *Bacillus anthracis* immunizing antigen and its application to a study of antigen production. *J. Gen. Microbiol.*, 1957, 17, 505–516.

6. GUSTAFSSON, B. E., and LAURELL, C.-B. Gamma globulins in germ-free rats. *J. Exper. Med.*, 1958, **108**, 251–258.

7. SMITH, H.; KEPPIE, J.; STANLEY, J. L., and HARRIS-SMITH, P. W. The chemical basis of the virulence of *Bacillus anthracis*. IV. Secondary shock as the major factor in death of guinea-pigs from anthrax. *Brit. J. Exper. Path.*, 1955, **36**, 323–335.

We are indebted to Professor P. C. Trexler for the Lobund rats; to Mr. Elliott Purlson whose active cooperation made it possible for us to control their bacterial environment after arrival; to Dr. Leonard Spero who made and interpreted the electrophoretic analyses; to Dr. Martha K. Ward who made the agar diffusion tests for antibody; and to the Armed Forces Institute of Pathology, Washington, D.C., for making the photomicrographs of our sectioned tissues.

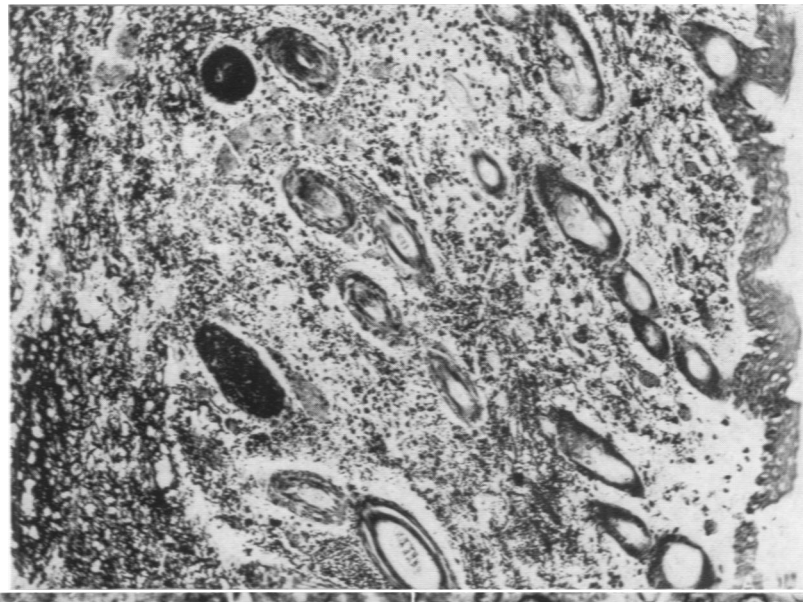
LEGENDS FOR FIGURES

The interval between inoculation with *B. anthracis* spores and death is enclosed in parentheses. All sections were stained by a modified Lillie-Giemsa method.

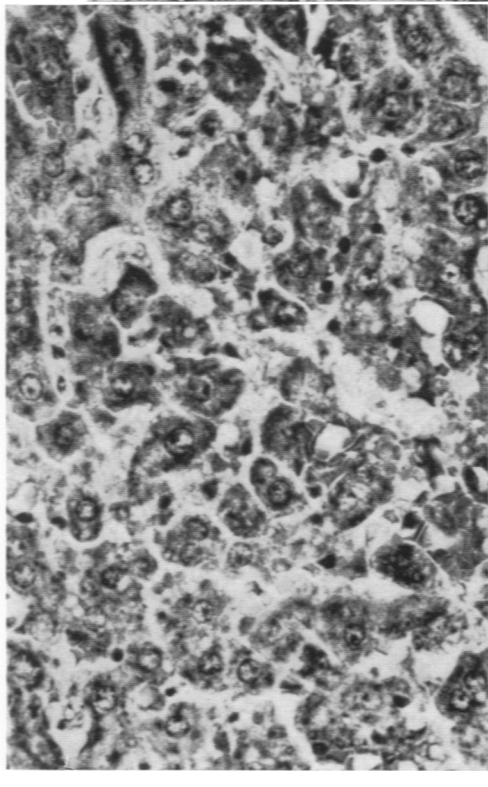
FIG. 1. AFIP 18–1R. Skin of a Lobund rat at the injection site. Large numbers of bacilli and moderate cellulitis are evident. (25 hr.) × 115.

FIG. 2. AFIP 18–3R. Liver of a Fischer rat with marked Kupffer cell hyperplasia and few bacilli. (27 hr.) × 500.

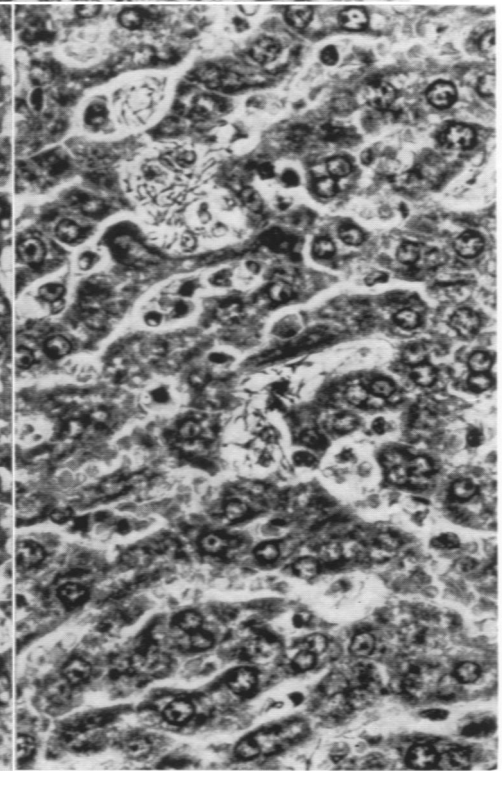
FIG. 3. AFIP 18–2R. Liver of a Lobund rat in which the sinusoids are filled with bacilli and the Kupffer cell reaction is slight. (25 hr.) × 500.



1

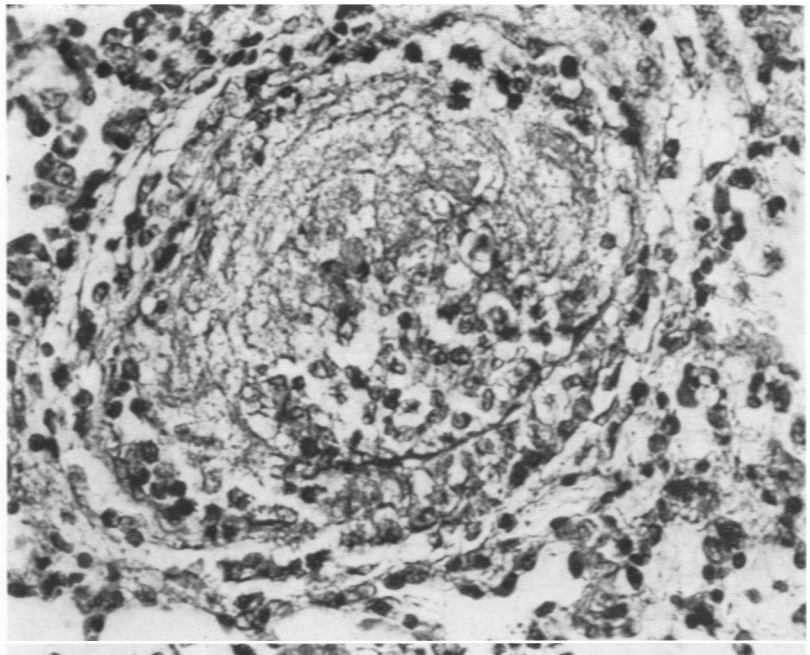


2



3

4



5

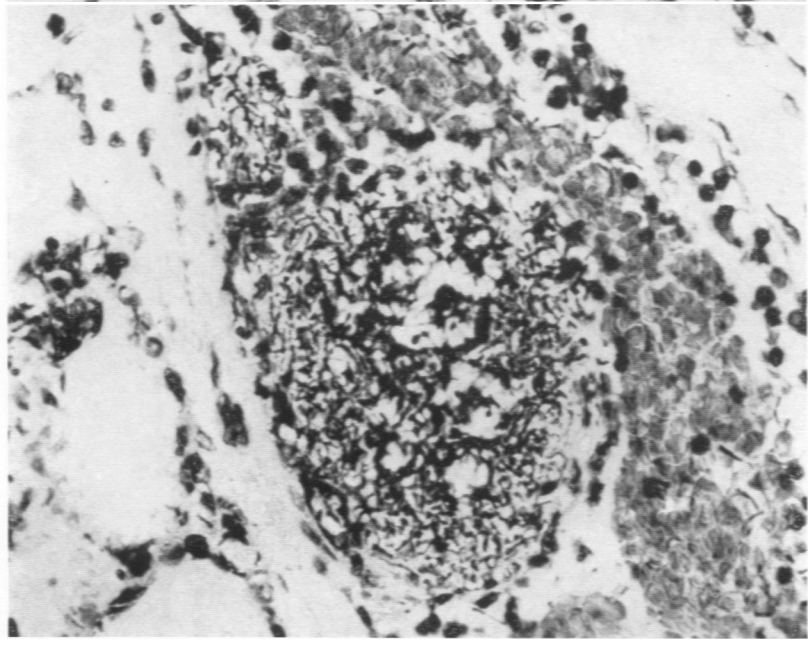


FIG. 4. AFIP 18–4R. Lung of a Fischer rat in which a small pulmonary artery is partially occluded by a thrombus and an aggregate of mononuclear cells. Bacilli are scarce. (20 hr.) × 500.

FIG. 5. AFIP 18–5R. Lung of a Lobund rat in which a small pulmonary artery is filled with bacilli. Note the absence of the mononuclear reaction and thrombus formation seen in the Fischer rat (Fig. 4). (25 hr.) × 500.