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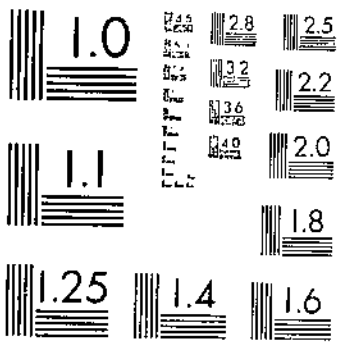

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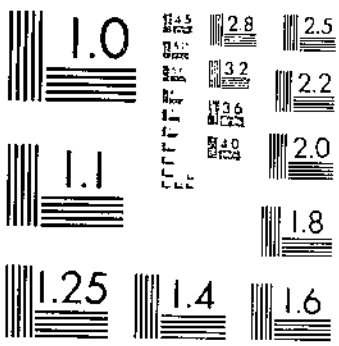

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EFFICACY OF ANTHRAX BIOLOGICS IN PRODUCING IMMUNITY IN PREVIOUSLY UNEXPOSED ANIMALS

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INTRODUCTION

Immunization against anthrax dates from Louis Pasteur's epoch-making demonstration in 1881 at Pouilly le Fort, France. The perfect results of that test are well known; all the vaccinated animals successfully withstood artificial exposure to anthrax, whereas all the controls died.

Undoubtedly, Pasteur's ability to protect animals against anthrax by vaccination was heralded at the time as a sure means of preventing that dreaded disease of livestock. The vaccines subsequently prepared by Pasteur did much to control the disease. Experience has shown, however, that the Pasteur vaccine had definite limitations. The product was subject to rapid deterioration, especially if kept under unfavorable conditions. Furthermore, a relatively long time, approximately 3 weeks, was required for the product to impart its full measure of protection. The double handling of the animals was also a disadvantage, especially in the treatment of range animals. Objections were raised likewise to a product composed of living anthrax organisms which, under certain conditions, might produce the disease in unusually susceptible animals and thereby actually spread the disease that was to be combated. Accordingly, numerous investigators undertook the task of developing anthrax biologics that would meet the above-mentioned objections, such researches being continued

[1] Acknowledgment with appreciation is made of the assistance rendered by Thomas Castor, inspector in charge of the Bureau's station at Philadelphia, Pa., in obtaining the uniform lots of test sheep used in the three experiments reported in the latter part of this bulletin. Acknowledgment is made also of the laboratory assistance given by C. N. Dale, W. T. Miller, and M. S. Shulman, of the Pathological Division.

up to the present time. As a result of these long-continued studies, a number of products have been developed for the control of anthrax.

For the immunization of animals against anthrax, the veterinarian has, therefore, a number of biologics at his command, namely anti-anthrax serum, antianthrax serum and anthrax-spore vacci used simultaneously, anthrax-spore vaccine (single injection), anthrax-spore vaccine (intradermic), anthrax-spore vaccines (2, 3, or 4 injection) anthrax-spore vaccine in saponin solution, anthrax aggressin, and two kinds of killed-organism anthrax bacterins, one being a whole-culture anthrax bacterin, and the other a washed-culture anthrax bacterin.

The intelligent use of these products depends on a knowledge of the efficacy of each under conditions prevailing in the field, which include the presence or absence of anthrax at the time, the previous existence of anthrax on the premises, the degree of danger of infection that is impending, the history and virulence of the outbreaks, and other pertinent matters. Taking these conditions into account one should select the biologic from the standpoints of safety, possibility of sensitization, rapidity of immunity production, and the degree and duration of the immunity produced.

OBJECT OF INVESTIGATION

Although it had been reported both experimentally and from the field that each of the products enumerated was capable of producing an immunity to anthrax, no comparative evaluations had been made, so far as the writers were aware, either experimentally or through carefully controlled field tests. Accordingly, to obtain information which would serve as a rational basis for the proper use of these products under various field conditions, the writers made comparative evaluations of six commercial anthrax biologics or combinations of them from the viewpoints of safety, possibility of sensitization, rapidity of immunity production, and the degree and duration of the immunity produced.

The phase of the project here reported on was conducted under conditions in which the test animals had had no previous exposure to or contact with anthrax infection. Work is in progress on comparative tests under experimental conditions wherein vaccination is performed on animals which have been previously exposed to the disease.

EARLY POTENCY TESTS WITH ANTHRAX BIOLOGICS

It appears appropriate to review some earlier potency tests of anthrax biologics that were conducted by the senior author at the Bureau's Experiment Station, Bethesda, Md. For the most part these tests, beginning in 1925, were made separately rather than on a comparative basis and in all instances were conducted for the sole purpose of determining whether the products possessed immunizing value.

RESULTS OF TESTS

Table 1 shows the results of a potency test of anthrax aggressin on cattle and horses. Tables 2, 3, 4, 5, and 6 show the results of potency tests of various biologics on sheep. Table 7 is a summary of the results of the earlier potency tests of various anthrax biologics.

TABLE 1.—*Results of potency tests of anthrax aggressin on cattle and horses*

[Exposure: Subcutaneous injection of 3 cc of a 24-hour bouillon culture of *Bacillus anthracis* no. 8052, Nov. 20, 1925]

Experimental animals	Animals in test	Vaccination			Time between vaccination and exposure	Animals surviving
		Date (1925)	Quantity of aggressin injected	Method of injection		
Cattle:	*Number*		*Cc*		*Days*	*Percent*
Principals	6	July 25	5	Subcutaneous	118	50
Controls	4					0
Horses:						
Principals	2	Aug. 8	5	Subcutaneous	104	50
Controls	2					0

TABLE 2.—*Results of potency tests of anthrax aggressin and anthrax-spore vaccine (2-injection) on sheep*

[Exposure: Subcutaneous injection of one-forty-thousandth part of one platinum loop of 24-hour agar culture growth of *Bacillus anthracis* Oct. 20, 1925]

Experimental sheep	Sheep in test	Vaccination				Time between vaccination and exposure	Sheep surviving
		Biologic used	Date (1925)	Quantity injected	Method of injection		
	Number			*Cc*		*Days*	*Percent*
Principals	6	Anthrax aggressin	Sept. 17	2	Subcutaneous	33	50
	6	do	do	5	do	33	33
		Anthrax-spore vaccine (2-injection):					
	3	Spore no. 1	do	1	do	23	100
		Spore no. 2	Sept. 27	1	do		
Controls	4						25

TABLE 3.—*Results of potency test of anthrax bacterin (whole culture) on sheep and final preliminary titrations of the exposure culture used*

[Exposure: Subcutaneous injection of 1 cc of a 24-hour bouillon culture of *Bacillus anthracis* no. 1, Feb. 17, 1932]

RESULTS OF POTENCY TEST

Experimental sheep	Sheep in test	Vaccination			Time between vaccination and exposure	Sheep surviving
		Date (1932)	Quantity of aggressin injected	Method of injection		
	Number		*Cc*		*Days*	*Percent*
Principals	10	Feb. 4	10	Subcutaneous	13	40
		Feb. 5			12	
Controls	5					20

RESULTS OF FINAL PRELIMINARY TITRATIONS OF THE EXPOSURE CULTURE

Sheep no.	Quantity injected	Date of injection (1932)	Result
	Cc		
880	1.0	Jan. 21	Died of anthrax Jan. 23.
881	1.0	do	Do.
882	1.0	do	Died of anthrax Jan. 25.
894	.8	Feb. 5	Remained normal.
890	.8	do	Died of anthrax Feb. 8.
898	.8	do	Remained normal.
884	1.0	Feb. 12	Died of anthrax Feb. 14.
886	1.0	do	Died of anthrax Feb. 15.
887	1.0	do	Do.

TABLE 4.—*Results of potency tests of 4 anthrax biologics on sheep, and final preliminary titration of the exposure culture used*

[Exposure: Subcutaneous injection of 3 cc of a 1:100 dilution of a 24-hour broth culture of *Bacillus anthracis* no. 02, June 6, 1932]

RESULTS OF POTENCY TEST

Experimental sheep	Sheep in test	Vaccination				Time between vaccination and exposure	Sheep surviving
		Biologic used	Date (1932)	Quantity injected	Method of injection		
	Number			*Cc*		*Days*	*Percent*
Principals	5	Anthrax aggressin	Apr. 30	3	Subcutaneous	37	60
	4	Anthrax bacterin (whole culture).	do	10	do	37	50
	2	Anthrax-spore vaccine (intradermic).	Apr. 13	.25	Intradermic	54	100
	3	Anthrax-spore vaccine (single injection).	do	1	Subcutaneous	54	100
Controls	7						57

RESULTS OF FINAL PRELIMINARY TITRATION OF THE EXPOSURE CULTURE

Sheep no.	Quantity injected	Date of injection (1932)	Result
	Cc		
965	3	May 31	Died of anthrax June 5.
965A	3	do	Died of anthrax June 3.

TABLE 5.—*Results of potency tests of 4 anthrax biologics on sheep, and final preliminary titration of the exposure culture used*

[Exposure: Subcutaneous injection of 1 cc of a 1:100 dilution of frozen anthrax culture 1884 M. lot 1, No. 12, 1932]

RESULTS OF POTENCY TEST

Experimental sheep	Sheep in test	Vaccination				Time between vaccination and exposure	Sheep surviving
		Biologic used	Date (1932)	Quantity injected	Method of injection		
	Number			*Cc*		*Days*	*Percent*
Principals	6	Anthrax bacterin (washed culture).	Oct. 13	2	Subcutaneous	30	83.3
	6	do	do	4	do	30	100.0
	6	Anthrax bacterin (whole culture).	do	5	do	30	33.3
	6	do	do	10	do	30	33.3
	5	Anthrax-spore vaccine (intradermic).	do	.25	Intradermic	30	100.0
	6	Anthrax-spore vaccine (single injection).	do	2	Subcutaneous	30	83.3
Controls	12						41.7

RESULTS OF FINAL PRELIMINARY TITRATION OF THE EXPOSURE CULTURE

Sheep no.	Quantity injected	Date of injection (1932)	Result
	Cc		
1022	1	Nov. 1	Died of anthrax Nov. 4.
1025	1	do	Remained normal.
1040	1	do	Died of anthrax Nov. 8.
1046	1	do	Died of anthrax Nov. 5.
1047	1	do	Died of anthrax Nov. 4.
1095	1	do	Remained normal.

TABLE 6.—*Results of potency tests of anthrax-spore vaccine in saponin solution on sheep, and results of final preliminary titration of exposure culture used*

[Exposure: Subcutaneous injection of 1 cc of a 1:10 dilution of a 24-hour broth culture of *Bacillus anthracis* no. 3733, May 4, 1933]

RESULTS OF POTENCY TEST

Experimental sheep	Sheep in test	Vaccination			Time between vaccination and exposure	Sheep surviving
		Date (1932)	Quantity injected	Method of injection		
	Number		*Cc*		*Days*	*Percent*
Principals	6	Mar. 23	0.25	Subcutaneous	43	100
Controls	6					50

RESULTS OF FINAL PRELIMINARY TITRATIONS OF THE EXPOSURE CULTURE

Sheep no.	Culture injected			Result
	Dilution	Quantity	Date (1933)	
		Cc		
1199	1:10	1	Apr. 28	Died of anthrax May 2.
1204	1:10	1	do	Died of anthrax May 1.
1197	1:25	1	do	Remained normal.
1192	1:25	1	do	Died of anthrax May 5.

TABLE 7.—*Summary of the results of early potency tests of various anthrax biologics (tables 1 to 6)*

Biologic	Tests conducted	Animals in tests		Animals surviving experimental exposure	
		Principals	Controls	Principals	Controls
	Number	*Number*	*Number*	*Percent*	*Percent*
Anthrax aggressin	3	25	17	48	29
Anthrax bacterin (washed culture)	1	12	12	92	42
Anthrax bacterin (whole culture)	3	26	24	38	42
Anthrax spore vaccine (intradermic)	2	7	10	100	47
Anthrax spore vaccine in saponin solution	1	6	6	100	50
Anthrax spore vaccine (single injection)	2	9	19	89	47
Anthrax spore vaccine (two injections)	1	3	4	100	25

DISCUSSION OF EARLY POTENCY TESTS

In the foregoing tests, anthrax bacterin (washed culture), anthrax-spore vaccine (intradermic), anthrax-spore vaccine in saponin solution, anthrax-spore vaccine (single injection), and anthrax-spore vaccine (two injections) produced well-marked immunity to anthrax.

The results of the tests made with anthrax aggressin showed that the product is capable of producing some degree of immunity to anthrax. In these tests, however, the immunity produced was not so strong as that conferred by the living anthrax-spore vaccines and the anthrax bacterin (washed culture).

Although anthrax bacterin (whole culture) increased resistance to anthrax in some of the tests, the immunity produced was less than that of the other anthrax biologics tested.

The underactivity of the exposure dose of anthrax culture used in the tests reported in table 4, in which 57 percent of the controls sur-

vived, was rather disappointing since the preliminary titrations indicated a higher degree of infectivity. This represents but one of a number of examples of the instability of the virulence of cultures of *Bacillus anthracis* as ordinarily prepared, which prompted the use in the subsequent immunity tests of a specially prepared culture of *B. anthracis* which had been found to be of stable virulence.

COMPARATIVE EXPERIMENTS WITH ANTHRAX BIOLOGICS

To obtain definitely comparable data on anthrax biologics, the writers conducted a series of experiments in 1933 and 1934 at the Bureau's Experiment Station, Bethesda, Md.

BIOLOGICS AND TEST ANIMALS USED

The biologics used were antianthrax serum, antianthrax serum and anthrax-spore vaccine used in combination, anthrax-spore vaccine (single injection), anthrax-spore vaccine (intradermic), anthrax-spore vaccine in saponin solution, and anthrax bacterin (washed culture). These were all of commercial manufacture and were found to be satisfactory to such laboratory tests as were applicable to each product. The size of dose recommended by the manufacturers for sheep, the test animals employed (fig. 1), was used in each instance. The limited space and the need for a considerable number of animals for each test made it necessary to limit the investigation to the number of products named.

The test animals were 2-year-old Merino wethers of uniform weight and in good condition (fig. 2). After being exposed to anthrax, the sheep were housed in a large, tightly screened, concrete barn adjacent to the incinerator (figs. 3 and 4).

PLAN OF WORK

To obtain the desired information, tests were projected to compare the immunities produced by the several anthrax biologics at intervals of 4, 14, 60, 120, and 180 days after vaccination. By reason of the large number of test animals involved, the work was divided into three experiments.

Because more time than had been anticipated was consumed in the preliminary titrations of the exposure culture of *Bacillus anthracis* to establish a satisfactory infective dose, the originally planned 60-day interval between vaccination and exposure had to be lengthened to 108 days and the 120-day interval to 155 days. Accordingly, the 180-day interval was lengthened to 300 days, and the need for replacement of some animals caused an extension to 360 days in some cases.

In the first of the three experiments the comparison of efficacy was made by exposing at one time, to the same previously determined infective dose of *B. anthracis* virus, one group of sheep that had been vaccinated with various anthrax biologics 4 days previously, a second group vaccinated 16 days previously, and a third group vaccinated 108 days previously.

In the second experiment a group of sheep that had been vaccinated for a period of 155 days was given an exposure to anthrax equal to that of the first experiment.

In the third experiment, in which the date of vaccination was February 21, 1933, a group of sheep that had been vaccinated for a

period of 300 days was given a similar exposure to anthrax. Within 60 days after vaccination, some of the sheep succumbed to intercurrent disease. These vacancies were filled with sheep vaccinated April 21,

FIGURE 1.—A group of the sheep used in the comparative immunity tests of anthrax biologics.

1933. Between April 21 and the date of exposure to anthrax, February 16, 1934, further losses were sustained from intercurrent disease but the animals that died were not replaced. Accordingly, each of the

FIGURE 2.—One of the sheep, a 2-year-old Merino wether, illustrating the type and condition of the test animals used.

vaccinated groups in experiment 3 contained fewer than the originally planned number of sheep, and some groups contained sheep that were vaccinated February 21 and April 21, 1933, representing intervals between vaccination and exposure of 300 and 360 days. Since both

of these periods materially exceed the duration of the usual anthrax period, from 6 to 9 months, there is no difference in the significance of the data for 300 days and for 360 days. The results are accordingly grouped.

PREPARATION OF ANTHRAX CULTURES USED FOR EXPOSURE

It has been the experience of the writers, as well as others, that success in conducting anthrax-immunity tests depends in a large measure on the stability of the exposure material that is to be used. It is a well-known fact that a culture of anthrax of a certain degree of infectivity cannot be depended on to retain that degree of infectivity

FIGURE 3.—Barn used to house test animals exposed to anthrax; incinerator on the left.

over any appreciable period by the means ordinarily used for maintaining cultures. Recently Reichel and Schneider [2] found that the infectivity of an anthrax culture could be maintained with little change by holding the culture in a frozen state from the time of its preparation until it was used. The exposure culture used in the first experiment and designated "Frozen anthrax culture 1864, M. lot 3" was prepared at the Mulford Biological Laboratories, Sharp & Dohme, Glenolden, Pa., and was made available for this investigation through the courtesy of John Reichel, the director. As the supply of this culture became exhausted, a new lot, designated "Frozen anthrax culture 1864, B. A. I. lot 1", was prepared in the laboratory of the Pathological Division, Bureau of Animal Industry, following the procedure described by Drs. Reichel and Schneider.[2] This lot was used in the second and third experiments and was prepared in the following manner.

Sheep no. 993 was inoculated March 22, 1933, with 1 cc of a one-fiftieth dilution of frozen anthrax culture 1864, M. lot 3, and died March 30, 1933. A culture, on plain meat-infusion agar, recovered

[2] REICHEL, J., and SCHNEIDER, J. E. ANTHRAX-PROTECTION TESTS. Jour. Amer. Vet. Med. Assoc. 82: 376-383. 1933.

from the blood of the ear, was transferred at 24-hour intervals on March 31 and April 1 and 2. On April 3, the entire 24-hour growth on a ⅝ by 5-inch meat-infusion agar slant was removed with a platinum loop and directly transferred to 3,000 cc of nutrient broth which had been previously sterilized in a narrow-mouthed 4,500-cc bottle. After 15 hours' incubation at 37.5° C., the bottle was promptly removed from the incubator and handled as follows:

(1) Seven hundred and fifty cc of previously filtered, sterile horse serum was added.

(2) The culture and serum were shaken vigorously after the mouth of the bottle had been closed with a sterile rubber stopper.

FIGURE 4.—Interior view of barn used for anthrax experiments; capacity 110 sheep.

(3) A previously sterilized bottling apparatus was fitted to the bottle, aseptic precautions being used.

(4) Immediately before commencing bottling operations the contents were shaken thoroughly, this step being repeated in the middle of the bottling procedure.

(5) Sixty cc of the serum-and-broth-culture mixture was placed in each of 30 bottles of 100-cc capacity, rubber-stopper caps being used.

(6) The bottles were placed in beakers and packed in a box which was put in the freezer at −15° C. When observed 4 hours later the product was not uniformly solidified, but after 20 hours the contents of all bottles were solidly frozen.

(7) On April 8, the frozen product in bottle no. 1 was thawed at room temperature, thoroughly shaken, diluted 50 times with saline, and cultured on the surface of meat-infusion agar in Petri dishes. The colony count indicated the presence of 180,000 organisms per cubic centimeter of undiluted material. No colonies of organisms other than anthrax were observed.

PRELIMINARY TESTS OF INFECTIVITY OF EXPOSURE CULTURES USED

Because of the importance of the degree of infectivity of the exposure culture for the comparative tests that were to be undertaken, a number of preliminary titrations were made of the two lots of frozen anthrax culture in order to establish an infective dose that could be depended on to cause death from anthrax in a considerable majority of unvaccinated animals taken from the same lots as those used in the tests proper. A total of 71 sheep were used in this preliminary work.

It was found that a dose of 1 cc of undiluted anthrax culture 1864, M. lot 3, administered subcutaneously, caused death regularly in from 67 to 100 percent of the sheep. These titrations extended over a period of several months, during which time no appreciable variation in the degree of infectivity of the culture was noted. A subcutaneous injection of 1 cc of this culture was believed, therefore, to constitute a suitable exposure to anthrax. The results obtained with frozen anthrax culture 1864, B. A. I. lot 1, prepared by the writers, were found to be in close conformity with those obtained with the frozen anthrax culture 1864, M. lot 3, prepared by Reichel and Schneider. A subcutaneous injection of 1 cc of the former culture was considered, therefore, to constitute an exposure to anthrax equal to that of the latter culture.

Inasmuch as all the animals in experiment 1, consisting of 96 vaccinated sheep and 12 controls, were to be exposed on the same date, it was realized that approximately 2 hours would be required to administer the exposure dose to this number of animals, which would mean that the time between thawing the exposure culture and the actual injection into the sheep would vary from a few minutes in the case of the first sheep exposed to approximately 2 hours in the case of the last. Accordingly, this time factor between thawing of the culture and administration was taken into consideration in the titration of frozen anthrax culture 1864, M. lot 3. The injections were made over a period of 2 hours, and the culture was kept in an ice-water bath in the meantime. The results of the titration indicated no difference in the infectivity of the culture when used immediately after thawing or 2 hours afterward. Table 8 shows the last preliminary titrations of the exposure cultures used in the three experiments.

In the titrations of the exposure culture used in experiment 1, two bottles, each containing 60 cc, were thawed at room temperature, thoroughly shaken, and the entire contents poured into a 500-cc sterile Erlenmeyer flask and thoroughly mixed. In the titrations of the exposure cultures used in experiments 2 and 3, the procedure was the same except that 4 bottles of the culture, instead of 2, were used in each titration. To make the dilution of 1:2½, 4 cc of the mixed culture was placed in a sterile wide-mouthed bottle and 6 cc of sterile saline solution added.

In connection with the third experiment, a preliminary titration was made February 5, 1934, of the frozen anthrax culture 1864, B. A. I. lot 1, by injecting subcutaneously into each of five sheep 1 cc of the undiluted culture. One of the five sheep died of anthrax on February 9. The remaining four became visibly sick but recovered. The underactivity of the exposure culture in this titration appeared to be due to the low temperature, —10° F., that prevailed at the time,

since in the final preliminary titration (table 8) when the temperature was 24°, all the sheep succumbed to anthrax from the same quantity of the same culture. Also in the preliminary titration of the same exposure culture made in July (table 8), 57 percent of the animals died.

TABLE 8.—*Results of final preliminary titrations for infectivity of frozen anthrax cultures used in experiments 1, 2, and 3*

[1-cc amounts injected subcutaneously]

EXPERIMENT 1.—FROZEN ANTHRAX CULTURE 1804, M. LOT 3, INJECTED MAY 29, 1933

Sheep no.	Dilution of virus	Time of injection	Results
1305	Undiluted	2:30 p. m.	Died of anthrax June 2.
1306	do	do	Remained normal.
1307	do	3:30 p. m.	Died of anthrax June 2.
1308	do	do	Remained normal.
1309	do	4:35 p. m.	Died of anthrax June 1.
1310	do	do	Died of anthrax June 5.
1301	1:2½	2:30 p. m.	Died of anthrax June 3.
1302	do	3:30 p. m.	Died of anthrax June 2.
1303	do	do	Do.
1304	do	4:35 p. m.	Remained normal.

EXPERIMENT 2.—FROZEN ANTHRAX CULTURE 1804, B. A. I. LOT 1, INJECTED JULY 14, 1933

1357	Undiluted		Died of anthrax July 18.
1382	do		Died of anthrax July 16.
1381	do		Remained normal.
1384	1:2½		Do.
1386	do		Do.
1383	do		Died of anthrax July 17.
1390	do		Died of anthrax July 18.

EXPERIMENT 3.—FROZEN ANTHRAX CULTURE 1804, B. A. I. LOT 1, INJECTED FEB. 12, 1934

1444	Undiluted		Died of anthrax Feb. 16.
1445	do		Died of anthrax Feb. 15.
1440	do		Died of anthrax Feb. 14.

TESTS OF EFFICACY OF BIOLOGICS

Table 9 presents the conditions and results of the tests involving the six anthrax biologics when the sheep were exposed to anthrax 4, 16, 108, 155, 300, and 360 days after vaccination. Observations were made for periods of 28, 31, and 30 days in experiments 1, 2, and 3, respectively. As in the preliminary titration, the time at which deaths occurred among the controls in the test proper gave no indication of any difference in the infectivity of the exposure culture so far as the time interval between thawing and inoculation was concerned.

TABLE 9.—*Results of comparative tests of 6 anthrax biologics on sheep*

EXPERIMENT 1.—EXPOSURE 4, 16, AND 108 DAYS AFTER VACCINATION; SUBCUTANEOUS INJECTION OF 1 CC OF FROZEN ANTHRAX CULTURE 1864, M. LOT 3, JUNE 9, 1933

Experimental sheep	Sheep in test	Vaccination				Time between vaccination and exposure	Sheep surviving
		Date (1933)	Biologic used	Quantity injected	Method of injection		
	Number			*Cc*		*Days*	*Percent*
Principals	6	June 6	Anthrax bacterin (washed culture).	4	Subcutaneous	4	33
	6	do	Anthrax-spore vaccine in saponin solution.	.25	do		50
	6	do	Anthrax-spore vaccine (intradermic).	.50	Intradermic		100
	6	do	Anthrax-spore vaccine (single injection).	2	Subcutaneous		67
	6	do	Antianthrax serum	20	do		100
	6	do	Antianthrax serum and Anthrax-spore vaccine	10 1	do		67
	6	May 24	Anthrax bacterin (washed culture).	4	do	16	100
	6	do	Anthrax-spore vaccine in saponin solution.	.25	do		100
	6	do	Anthrax-spore vaccine (intradermic).	.5	Intradermic		100
	6	do	Anthrax-spore vaccine (single injection).	2	Subcutaneous		67
	6	do	Antianthrax serum	20	do		50
	6	do	Antianthrax serum and Anthrax-spore vaccine	10 1	do		100
	6	Feb. 21	Anthrax bacterin (washed culture).	4	do	108	17
	6	do	Anthrax-spore vaccine (intradermic).	.5	Intradermic		100
	6	do	Anthrax-spore vaccine (single injection).	2	Subcutaneous		83
	6	do	Antianthrax serum and Anthrax-spore vaccine	10 1	do		83
Controls	12						25

EXPERIMENT 2.—EXPOSURE 155 DAYS AFTER VACCINATION; SUBCUTANEOUS INJECTION OF 1 CC OF FROZEN ANTHRAX CULTURE 1864, B. A. I. LOT 1, JULY 26, 1933

Principals	6	Feb. 21	Anthrax bacterin (washed culture).	4	Subcutaneous	155	50
	6	do	Anthrax-spore vaccine in saponin solution.	.25	do		67
	6	do	Anthrax-spore vaccine (intradermic).	.5	Intradermic		83
	6	do	Anthrax-spore vaccine (single injection).	2	Subcutaneous		67
	6	do	Antianthrax serum and Anthrax-spore vaccine	10 1	do		50
Controls	12						17

EXPERIMENT 3.—EXPOSURE 300 AND 360 DAYS AFTER VACCINATION; SUBCUTANEOUS INJECTION OF 1 CC OF FROZEN ANTHRAX CULTURE 1864, B. A. I. LOT 1, FEB. 16, 1934

Principals	3	Feb. 21	Anthrax bacterin (washed culture).	4	Subcutaneous	300 and 360	33
	3	do	Anthrax-spore vaccine in saponin solution.	.25	do		80
	2	Apr. 21		.25	do		
	3	Feb. 21	Anthrax-spore vaccine (intradermic).	.5	Intradermic		100
	2	Apr. 21		.5	do		
	3	Feb. 21	Anthrax-spore vaccine (single injection).	2	Subcutaneous		100
	2	Apr. 21		2	do		
	1	Feb. 21	Antianthrax serum and Anthrax-spore vaccine	10 1	do		40
	4	Apr. 21	Antianthrax serum and Anthrax-spore vaccine	10 1	do		
Controls	6						33

DISCUSSION OF COMPARATIVE TESTS

Anthrax organisms were recovered from the blood of each sheep that died with the exception of two, one of which was exposed 16 days and the other 108 days after vaccination with anthrax-spore vaccine (single injection). The failure to recover the anthrax organisms in these two instances was in the writers' opinion due to the culturing of an inadequate quantity of blood. Subsequent tests showed that a light swab of blood might fail to reveal the anthrax organism, whereas a heavy swab of the same blood gave positive results. For this reason the collection of blood from the ear, the method used in the case of the two sheep in question, was discontinued in favor of collection from the axillary space, where ample blood could be obtained. In no case were any ill effects observed from the injection of the biologics.

None of the anthrax biologics produced a sensitization to anthrax that was evident 4 days after vaccination. All the anthrax biologics produced an increased resistance to anthrax that was demonstrable 4 days after vaccination. A difference in the rapidity with which increased resistance to anthrax was established was noted in favor of the antianthrax serum and the anthrax-spore vaccine (intradermic).

All the anthrax biologics produced an increased resistance to anthrax demonstrable at 16 days after vaccination. The immunity conferred by antianthrax serum appeared to be on the wane at this time. The immunity conferred by the killed-culture anthrax products was equal, at 16 days after vaccination, to that produced by the living-spore vaccines.

The immunity conferred by the living-spore vaccines was well maintained 108 days after vaccination, especially that produced by anthrax-spore vaccine (intradermic).

The immunity conferred by the living-spore vaccines, especially anthrax-spore vaccine (intradermic), was well maintained at 155 days.

The immunity conferred by the living-spore vaccines was especially well maintained at 300 and 360 days in the instances of the anthrax-spore vaccines (single injection) and the anthrax-spore vaccine (intradermic), and was well maintained also in the instance of the anthrax-spore vaccine in saponin solution. No appreciable immunity, however, remained in the animals vaccinated with antianthrax serum and anthrax-spore vaccine used in combination.

The anthrax bacterin (washed culture) at 108 days failed to afford any protection, whereas at 155 days distinct protection was afforded. The writers have no explanation to offer for this unusual result. However, at 360 days protection could not be demonstrated with the number of sheep used, the lot being smaller than in the case of the other products in this same group.

SUMMARY AND CONCLUSIONS

The results of early experimental tests, beginning in 1925, with various anthrax biologics indicated that several produced well-marked immunities to anthrax. Cattle, horses, and sheep were used as test animals. The biologics which produced well-marked immunity were: Anthrax bacterin (washed culture), anthrax-spore vaccine (intradermic), anthrax-spore vaccine in saponin solution, anthrax-spore

vaccine (single injection), and anthrax-spore vaccine (double injection). Anthrax aggressin produced a lesser degree of immunity, and anthrax bacterin (whole culture) produced comparatively little.

To obtain specific information on a comparative basis, a series of experiments with six types of anthrax immunizing agents was conducted in 1933–34. The biologics were: Antianthrax serum, antianthrax serum and anthrax-spore vaccine in combination, anthrax-spore vaccine (single injection), anthrax-spore vaccine (intradermic), anthrax-spore vaccine in saponin solution, and anthrax bacterin (washed culture). These products were subjected to comparative tests to determine their relative safety, sensitizing effect, rapidity of immunity production, and the degree and duration of immunity which they produced under experimental conditions in which the test animals had had no previous contact with or exposure to anthrax infection.

This information was sought through a comparison of the immunities produced by these biologics at 4, 16, 108, 155, 300, and 360 days after vaccination. The test animals exposed 4, 16, and 108 days after vaccination were injected with the same anthrax culture at the same time. The animals exposed 155, 300, and 360 days after vaccination received an equal injection through the use of a culture prepared from the same culture of *Bacillus anthracis* and in the same manner as the exposure culture used in the first three groups. Preliminary titrations showed these exposure cultures to be equal in infectivity.

A total of 250 sheep were used in the three experiments, 71 of which were used in the titrations of the exposure cultures, 149 were vaccinated animals, and 30 were used as controls.

With antianthrax serum there were 100-percent survivals at 4 days and 50-percent survivals at 16 days after vaccination, as compared with 25-percent survivals in the control group.

With antianthrax serum and anthrax-spore vaccine in combination there were 67-percent survivals at 4 days, 100 percent at 16 days, and 83 percent at 108 days, as compared with 25-percent survivals in the control group. At 155 days there were 50-percent survivals, as compared with 17-percent survivals in the control group. At 300 and 360 days there were 40-percent survivals, as compared with 33-percent survivals in the control group.

With anthrax-spore vaccine (single injection) there were 67-percent survivals at 4 and 16 days and 83-percent survivals at 108 days, as compared with 25-percent survivals in the control group. At 155 days there were 67-percent survivals, as compared with 17-percent survivals in the control group, and at 300 and 360 days there were 100-percent survivals, as compared with 33-percent survivals in the control group.

With anthrax-spore vaccine (intradermic) there were 100-percent survivals at 4, 16, and 108 days, as compared with 25-percent survivals in the control group. At 155 days there were 83-percent survivals, as compared with 17-percent survivals in the control group. At 300 and 360 days there were 100-percent survivals, as compared with 33-percent survivals in the control group.

With anthrax-spore vaccine suspended in saponin solution there were 50-percent survivals at 4 days and 100-percent survivals at 16 days, as compared with 25-percent survivals in the control group. At 155 days there were 67-percent survivals, as compared with 17-

percent survivals in the control group, and at 300 and 360 days there were 80-percent survivals, as compared with 33-percent survivals in the control group.

With anthrax bacterin (washed culture) there were 33-percent survivals at 4 days, 100-percent at 16 days, and 17-percent survivals at 108 days, as compared with 25-percent survivals in the control group. At 155 days there were 50-percent survivals, as compared with 17-percent survivals in the control group, and at 300 and 360 days there were 33-percent survivals, as compared with 33-percent in the control group.

None of the biologics used for vaccinating produced any ill effects on the test animals. None of the biologics produced any sensitization to anthrax that was demonstrable in these tests.

The results obtained must be considered in the light that none of the test animals had had any previous contact with or exposure to anthrax infection whatsoever.

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