A bacteriological study of methods for the disinfection of hides infected with anthrax spores / by F.W. Tilley.

REPRINT FROM

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. IV WASHINGTON, D. C., APRIL 15, 1915 NO. 1

A BACTERIOLOGICAL STUDY OF METHODS FOR THE DISINFECTION OF HIDES INFECTED WITH ANTHRAX SPORES

By F. W. TILLEY

A BACTERIOLOGICAL STUDY OF METHODS FOR THE DISINFECTION OF HIDES INFECTED WITH ANTHRAX SPORES

By F. W. Tilley,

Senior Bacteriologist, Biochemic Division,
Bureau of Animal Industry[1]

INTRODUCTION

The number of hides and skins imported into this country each year amounts to many millions. Since these come to us from all quarters of the globe, it is evident that there is danger that they will bring with them infectious material which may cause disease among animals and human beings.

On account of the great resisting power of the anthrax spore, hides and skins imported from countries where anthrax is prevalent are regarded as especially dangerous; and inasmuch as methods of disinfection which will destroy the anthrax spore may be expected to kill other organisms with ease, considerable attention has been devoted to the problem of securing a disinfectant that will destroy the anthrax spores without damaging the hides and skins. Among the numerous processes which have been suggested, that devised in 1910 by Seymour-Jones (16)[2] has attracted much attention, while more recently the Schattenfroh (12) method has been declared by various investigators to be equally efficient and by some even more so.

As Eurich (1, 2), Ponder (9, 10), Seymour-Jones (16), and others have pointed out, the spores of anthrax are found chiefly in connection with blood stains, and as these, together with other material with which the spores are likely to be associated, are colloidal in nature, the problem, as Seymour-Jones expresses it, is to get at the anthrax spore "when imbedded in a gelatinous, albuminous, or other colloidal body without injury to the material or fabric to be disinfected."

OUTLINE OF SEYMOUR-JONES AND SCHATTENFROH METHODS OF DISINFECTION

Seymour-Jones (16) proposes to attain the desired result by the use of mercuric chlorid and formic acid. He holds that the acid causes the hide and the various associated colloidal substances to swell, absorb water, and become soft and tender, thus furnishing favorable conditions for the action of the mercuric chlorid. Under these conditions he con-

[1] The writer desires to express his obligations to Mr. F. P. Veitch, Chemist in Charge, Leather and Paper Laboratory, Bureau of Chemistry, for the work done under his direction in tanning pieces of disinfected hide, and to Dr. E. C. Schroeder, Superintendent, Bureau of Animal Industry Experiment Station, for facilities afforded in carrying out the experimental work upon animals.

[2] Reference is made by number to "Literature cited," p. 91–92.

siders a dilute solution of mercuric chlorid sufficient for disinfection. After disinfection hides are transferred to a saturated solution of common salt, whereby, it is claimed, they will be shrunk and brought to the "wet salted" state. The dilutions recommended are mercuric chlorid, 1 part in 5,000, with 1 per cent of formic acid; and the time of exposure to the disinfectant, 24 hours.

One of the first workers to investigate the Seymour-Jones process was C. W. Ponder (9, 10). He found that artificially infected pieces of hide were not disinfected in 24 hours by a solution of mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, in 4 cases out of 10 and concluded that the effective dilution of mercuric chlorid lay between 1 to 1,000 and 1 to 5,000. In spite of these results he recommends the service use of mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, on the ground that his tests were made more rigorous than was necessary to meet the conditions obtaining in actual routine disinfection. It is worthy of note that he made no attempt to neutralize the disinfectant before testing the results by cultures and by inoculation of animals. Moegle (7) and Schnürer (13) have also reported favorable results with the Seymour-Jones method.

The investigations of Ševčík (14) controvert all these favorable results. By neutralizing the disinfectant with sodium sulphid he was able to obtain living and virulent anthrax bacilli from spores treated with very strong dilutions of mercuric chlorid and formic acid, even when the time of exposure was extended to a number of days. Judging from his published results, it would require a dilution of mercuric chlorid, 1 to 500, plus 1 per cent of formic acid, to destroy anthrax spores in 24 hours. The use of sodium sulphid in this manner does not seem unreasonable, since, as a matter of fact, many tanners use this substance for dehairing hides. Hilgermann and Marmann (4) have obtained similar results with the Seymour-Jones method, using ammonium sulphid as a neutralizing agent.

Another method for the disinfection of hides which has recently come into prominence is the method of Prof. Schattenfroh (12), which depends upon the use of hydrochloric acid and sodium chlorid. The amounts recommended for use at room temperature are 2 per cent of the acid and 10 per cent of the salt, with a 48-hour exposure. At higher temperatures weaker dilutions may be employed.

Gegenbauer and Reichel (3) have carried on an extensive research with this method and report entirely favorable results. They state that they consider the Seymour-Jones method inefficient on account of the low concentration of mercuric chlorid and also object to its use because of the discoloration by mercuric sulphid when sodium sulphid is used for dehairing. Their statements regarding the Seymour-Jones method appear to be based upon experimental work not yet published. The favorable results obtained by Gegenbauer and Reichel with the Schattenfroh method are confirmed by the favorable results obtained by Hilger-

mann and Marmann (4) as the result of comparative experiments with the Seymour-Jones and Schattenfroh methods.

Ševčík's comparison (14) of the two methods is interesting, but is not fair to the Schattenfroh method, as he admits, because of the use of solutions based on the percentage of "hydrochloric acid" rather than on the percentage of hydrochloric-acid gas.

EXPERIMENTAL WORK ON GERMICIDAL EFFICIENCY OF DISINFECTANTS

The experimental work was undertaken primarily with a view to determining the value of the Seymour-Jones method, and for that reason this paper deals largely with work done with that method, although some attention was paid to others, especially the Schattenfroh method.

In the absence of a supply of naturally infected hides it was necessary to make the experiments upon pure cultures and artificially infected pieces of hide. Although Ševčík (14) states that naturally infected hides are better for test preparations than those artificially infected, it does not seem that the difference is as great as he claims. Certainly his results with naturally infected hides, where the disinfectant was not neutralized, correspond very closely to results obtained by Ponder (9, 10) with artificially infected hides.

EXPERIMENTAL PROCEDURE

For preliminary work the Hill "rod" method (5) seemed best adapted; so this was used, with some modifications. The method as modified is as follows: Glass rods three-sixteenths of an inch in diameter and 8 inches long are etched at one end, the etched portion being about 1 inch long. Cotton is wrapped about the rods near the end not etched and the rods thrust into test tubes so as to engage the cotton in the mouth of the tube. The tubes containing the rods are sterilized by dry heat (150° C.) for one hour or more. In making tests the rods are removed and the etched portion dipped into a suspension made from a culture of the organism employed and this allowed to dry on the rod.

Rods so infected are transferred to test tubes containing the disinfectant to be tested and there exposed to its action for varying lengths of time. After exposure the rods are washed with sterile water in order to remove traces of the disinfectant and are then transferred to tubes containing bouillon or agar, which are incubated for at least 48 hours at 37.5° C. The suspension used in infecting the rods is made from the surface growth on an agar tube by rubbing up in several cubic centimeters of sterile water enough of the growth to give a suspension of approximately the same density as a 24-hour bouillon culture of *Bacillus typhosus*. For a non-spore-bearing organism the culture should be 24 hours old, while for spore-bearing organisms cultures 1 to 2 weeks old are usually the most suitable.

In making tests with disinfectants containing mercury it is advisable to dip the rods into a saturated solution of hydrogen sulphid or an aqueous solution of some sulphid before placing them in subculture tubes. In this connection it should be mentioned that media of acid reaction have been found to exert an inhibitory action upon the growth of *Bacillus anthracis* after exposure to disinfectants. For that reason the media employed in these experiments have been neutral or slightly alkaline.

A considerable number of tests by the rod method were made with organic matter added to the disinfectant. This was done by removing a certain portion of the total volume of disinfectant and substituting a like amount of defibrinated blood.

Inasmuch as the use of a solution of sodium chlorid did not seem essential in experiments upon "naked" anthrax spores, since this salt is said by Seymour-Jones to be used in his method to reduce the swelling of the hides caused by formic acid, a common salt solution was not used in the "rod" method experiments.

MERCURIC CHLORID AND FORMIC ACID

I. EXPERIMENTS BY ROD METHOD, USING BUREAU OF ANIMAL INDUSTRY STRAIN OF BACILLUS ANTHRACIS

These experiments were designed to show the germicidal efficiency of mercuric chlorid ($HgCl_2$) with and without formic acid (CH_2O_2) and with and without the addition of defibrinated blood.

In experiment 1 (Table I) the rods were infected by using an agar culture 2 weeks old for making the spore suspension. Microscopical examination of the suspension showed that plenty of spores were present. Each rod was exposed to 5 c. c. of disinfectant for 24 hours and was then washed in 20 c. c. of hydrogen-sulphid solution or sterile distilled water.

The rods were then transferred to subculture tubes of exactly neutral broth and incubated at 37.5° C. for three days.

TABLE I.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, and of phenol by the rod method, without addition of organic matter* [a]

EXPERIMENT 1

Rod No.	Disinfectant (5 c. c.) and dilution.	Time of exposure.	Results after incubation for—		
			18 hours.	1 day.	3 days.
		Hours.			
1	Mercuric chlorid (1:5,000)	24	No growth	Growth	Strong growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	do	No growth	No growth.
3	Control rod	(b)	Strong growth	Strong growth	Strong growth.
4	Mercuric chlorid (1:5,000)	24	No growth	No growth	No growth.
5	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	do	do	Do.
6	Control rod	(b)	Strong growth	Strong growth	Strong growth.
7	Phenol (5 per cent)	24	No growth	Growth	Do.
8	Do	48	do	do	Do.

[a] Rods 1, 2, and 3 washed with hydrogen-sulphid solution, and Nos. 4, 5, 6, 7, and 8 with sterile water.
[b] Not exposed.

The results of the above experiment indicate that mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, is efficient where mercuric chlorid alone is not and that the hydrogen-sulphid solution should be used to neutralize the disinfectant before putting the rods into subculture tubes. The result after 48 hours' exposure to 5 per cent of phenol indicates the resisting power of the anthrax spores. The next experiment consisted of short exposures with the addition of defibrinated blood. This experiment was intended to test the efficiency of the method of disinfecting hides prescribed in Circular No. 23 of the Treasury Department, which consisted in immersion of hides for half an hour in a solution of mercuric chlorid, 1 to 1,000.

The technique was similar to that described for experiment 1, except that all rods were washed with hydrogen-sulphid solution and defibrinated blood was added so as to make up 10 per cent of the volume of the disinfectant in each tube. The results are given in Table II, experiment 2.

Experiment 2 indicates that anthrax spores are not destroyed in the presence of defibrinated blood by mercuric chlorid, 1 to 1,000, without formic acid, or by mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, even with an exposure of two hours.

An experiment with stronger dilutions of mercuric chlorid plus formic acid was now tried. Defibrinated blood was added in the proportion of 10 per cent of the total volume. (See Table II, experiment 3.)

TABLE II.—*Germicidal efficiency of mercuric chlorid, with or without formic acid, by the rod method, with the addition of defibrinated blood* [a]

EXPERIMENT 2

Rod No.	Disinfectant (5 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Mercuric chlorid (1:1,000)	¼	Growth.
2	Do	½	Do.
3	Do	1	Do.
4	Do	2	Do.
5	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	¼	Do.
6	Do	½	Do.
7	Do	1	Do.
8	Do	2	Do.
9	Control rod	(b)	Do.

EXPERIMENT 3

1	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	¼	No growth.
2	Do	½	Do.
3	Do	1	Do.
4	Do	2	Do.
5	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	¼	Do.
6	Do	½	Do.
7	Do	1	Do.
8	Do	2	Do.
9	Control rod	(b)	Growth.

EXPERIMENT 4

1	Mercuric chlorid (1:1,000)	24	No growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	24	Do.
3	Control rod	(b)	Growth.

[a] Subculture tubes incubated one week. Rods washed with hydrogen-sulphid solution.
[b] Not exposed.

Returning to conditions more closely resembling the Seymour-Jones method, experiment 4 was carried out with a 24-hour exposure to the disinfectant plus 10 per cent of defibrinated blood. The results are given in Table II, experiment 4.

The results of the preceding experiments indicated that in the presence of 10 per cent of defibrinated blood anthrax spores are not destroyed in 2 hours by mercuric chlorid, 1 to 1,000, without formic acid, nor by mercuric chlorid, 1 to 5,000, with 1 per cent of formic acid, but that they are destroyed by mercuric chlorid, 1 to 2,000, with 1 per cent of formic acid, under the same conditions. On the other hand, anthrax spores are destroyed by mercuric chlorid, 1 to 1,000, without formic acid, and by mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, even in the presence of defibrinated blood, when the time of exposure is 24 hours.

On account of the greatly increased germicidal power of mercuric chlorid in the presence of formic acid observed in the foregoing preliminary experiments, it was deemed advisable to test the germicidal power of mercuric chlorid and formic acid against anthrax spores dried upon pieces of hide. The Bureau of Animal Industry (B. A. I.) strain of *Bacillus anthracis*, which was employed in the previously described "rod" method experiments, was used in infecting the pieces of hide.

The results of these experiments, both by cultural methods and by inoculation of animals, were entirely unsatisfactory, the reason for this being apparently that the B. A. I. strain of *Bacillus anthracis* produced spores of comparatively low virulence and low vitality.

For this reason a culture of an entirely different strain of *Bacillus anthracis* was obtained from the Army Medical School (A. M. S.) through the courtesy of Capt. Craig, and spores of this strain were used in all further experiments. Experiments were made with "naked" spores by the "rod" method and with spores dried upon pieces of hide. As the subsequent records of these experiments will show, the spores of the A. M. S. strain were found to be very much more virulent and resistant to the action of disinfectants, drying, etc., than those of the B. A. I. strain.

II. EXPERIMENTS BY ROD METHOD, USING ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS

The technique of these experiments was exactly the same as for those with the B. A. I. strain, except that the quantity of disinfectant per rod was made 10 c. c. instead of 5 c. c.

Experiment 5 (Table III) indicates that mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, is able to destroy anthrax spores of the A. M. S. strain in 24 hours when no organic matter is added.

TABLE III.—*Germicidal efficiency of mercuric chlorid and formic acid by the rod method, without addition of organic matter*

EXPERIMENT 5[a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	No growth.
2	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	24	Do.
3	Control rod	(b)	Growth.

[a] Incubated 5 days. Hydrogen-sulphid solution used for neutralization of mercury.
[b] Not exposed.

Experiment 6, on the other hand, indicates that with an addition of defibrinated blood mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, is not able to kill spores of the A. M. S. strain in 24 hours (Table IV).

TABLE IV.—*Germicidal efficiency of mercuric chlorid, with or without formic acid, by the rod method, with and without addition of organic matter*

EXPERIMENT 6[a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Quantity of blood added.	Time of exposure.	Result.
		C. c.	*Hours.*	
1	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	None.	24	No growth.
2	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	1	24	Growth.
3	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	None.	24	No growth.
4	Mercuric chlorid (1:5,000)+formic acid (1 per cent)	1	24	Growth.
5	Mercuric chlorid (1:1,000)	None.	24	No growth.
6	Mercuric chlorid (1:1,000)	1	24	Growth.
7	Control rod	.....	(b)	Do.

[a] Incubated 3 days. Saturated aqueous solution of hydrogen sulphid used for neutralization of disinfectant.
[b] Not exposed.

In another experiment with various dilutions (Table V, experiment 7) there were 9 c. c. of disinfectant plus 1 c. c. of defibrinated blood in each tube.

Experiment 7 was repeated with the result given in Table V, experiment 8.

In experiment 9 the technique was the same as for experiment 8, the age of the culture being approximately the same (Table V, experiment 9).

TABLE V.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, by the rod method,[a] with addition of defibrinated blood*

EXPERIMENT 7[b]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	No growth.
2	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Growth.
3	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Do.
4	Mercuric chlorid (1:1,000)	24	Do.
5	Control rod	(c)	Do.

[a] Hydrogen-sulphid solution used to neutralize disinfectant.
[b] The quantity of disinfectant used in experiments 7, 8, and 9 included 1 c. c. of defibrinated blood.
[c] Not exposed.

TABLE V.—*Germicidal efficiency of mercuric chlorid, with and without formic acid, by the rod method, with addition of defibrinated blood*—Continued.

EXPERIMENT 8[a]

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
1	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Growth.
2	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
3	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Do.
4	Mercuric chlorid (1:1,000)	24	Do.
5	Control rod	(b)	Do.

EXPERIMENT 9[a]

1	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	No growth.
2	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Growth.
3	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
4	Mercuric chlorid (1:1,000)	24	Do.
5	Control rod	(b)	Do.

[a] The quantity of disinfectant used in experiments 7, 8, and 9 included 1 c. c. of defibrinated blood.
[b] Not exposed.

The discrepancy between the results of experiment 7 and those of experiments 8 and 9 appeared to be due to the use of a culture only 7 days old for making the spore suspension used in experiment 7, while the cultures used in experiments 8 and 9 were 17 and 22 days old, respectively.

In all these experiments spore suspensions were examined microscopically to make certain that plenty of spores were present, and it was noted that where cultures were less than 10 days old the suspensions generally contained a greater number of bacilli in relation to the spores than suspensions made from cultures 2 to 3 weeks old. The older cultures were therefore better adapted for this work.

The results of these experiments and a number of other similar experiments indicated that the A. M. S. strain of *Bacillus anthracis* was much more vigorous than the B. A. I. strain, which was used in experiments 1 to 4, and consequently was better suited for the purpose of this work. The following experiments, in which pieces of infected hide were employed, were therefore carried on with spores of the A. M. S. strain.

III. EXPERIMENTS UPON PIECES OF HIDE INFECTED WITH SPORES OF THE ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS WITHOUT NEUTRALIZATION OF DISINFECTANT

Some of the pieces of hide were prepared by a method essentially the same as that described by C. W. Ponder (9, 10), the details being as follows: The test preparations were made by cutting out pieces of hide so that each piece weighed about 2½ gm. Blood was drawn from the ear of a rabbit and a good-sized drop allowed to fall on the center of the hair side of each piece. Before clotting occurred a loopful of a suspension of anthrax spores was mixed thoroughly into the drop of blood.

The loop used was 3 cm. in diameter of 23-gauge platinum wire. The preparations so made were dried in the incubator 23 hours and then kept at room temperature until used.

In view of statements made by Otsuki (8) that spores of anthrax are injured by drying at 37.5° C., and that the best method of preparation is by drying them at 10° C., another lot of test preparations of hide was made as follows: Pieces of hide were cut to weigh about 2½ gm. On each piece a good-sized drop of blood from a rabbit's ear was allowed to fall and into this was mixed a loopful of a suspension of anthrax spores. This suspension was prepared by rubbing up in sterile water enough of the surface growth from a 15-day agar culture to give a suspension approximately equal in density to a 24-hour bouillon culture of *Bacillus typhosus*. These pieces of hide were placed in Petri dishes with raised covers and were dried for three days in a desiccator over sulphuric acid at a temperature of 10° C. and in a vacuum equal to about 6 cm. of mercury.

Guinea pigs were inoculated with clots from pieces of hide dried by each method. In neither case were the spores found to possess sufficient vitality to infect the animals, and it seemed evident that the methods of preparation had in some way attenuated the virulence of the spores. In view of the statement made by Roos (11) that rabbit blood is bactericidal for anthrax bacilli, while guinea pig blood is not, it seemed that the lack of virulence might be due to the use of rabbit blood. Therefore new pieces of hide were prepared, using blood from a guinea pig instead of rabbit blood as before. The pieces of hide were dried for 24 hours at 37.5° C. and then kept several days at room temperature in a dark closet. The lower drying temperature was used in later experiments. The spores in these test preparations were found to be virulent for guinea pigs, although less virulent than the original A. M. S. culture when tested shortly after it was received.

The virulence of the cultures was therefore raised by successive inoculations until a culture was obtained which killed a guinea pig in about 36 hours after subcutaneous inoculation. This culture was then employed in preparing test pieces of hide by the method above described, guinea-pig blood being used and the pieces being dried at 37.5° C. The pieces of hide so prepared were subjected to the following tests:

Each piece of hide was exposed to 25 c. c. of disinfectant for 24 hours and then soaked in 25 c. c. of saturated salt solution for 24 hours. At the end of that time the clots were scraped off and inoculated into guinea pigs. The results are given in Table VI.

TABLE VI.—*Inoculation of guinea pigs with clots from pieces of hide*

EXPERIMENT 10

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots inoculated.	Result of inoculation.
		Hours.		
23237	Mercuric chlorid (1:2,000)+formic acid (1 per cent).	24	1	Lived.
23238	Do.	24	1	Do.
23239	Mercuric chlorid (1:3,000)+formic acid (1 per cent).	24	1	Do.
23240	Do.	24	1	Do.
23241	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Do.
23242	Do.	24	1	Do.
23243	Mercuric chlorid (1:5,000)+formic acid (1 per cent).	24	1	Died in 5 days. Anthrax.
23244	Do.	24	1	Do.
23245	No disinfectant.	(a)	1	Died in less than 48 hours. Anthrax.
23246	Do.	(a)	1	Do.

EXPERIMENT 11

23251	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
23252	Do.	24	1	Do.
23277	Do.	24	1	Do.
23278	Do.	24	1	Do.
23279	Do.	24	1	Do.
23280	Do.	24	1	Do.
23281	Do.	24	1	Do.
23282	Do.	24	1	Do.
23250	Do.	24	2	Died in 5 days. Anthrax.
23248	No disinfectant.	(a)	1	Died in 48 hours. Anthrax.
23249	Do.	(a)	1	Do.

EXPERIMENT 12

24354	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
24355	Do.	24	1	Do.
23456	Do.	24	1	Do.
23457	Do.	24	1	Do.
23458	Do.	24	1	Do.
23459	Do.	24	1	Do.
23452	Do.	24	2	Do.
23453	Do.	24	2	Do.
23451	Mercuric chlorid (1:2,500) +formic acid (1 per cent).	24	5	Do.
23449	No disinfectant.	(a)	1	Died in 48 hours. Anthrax.
23450	Do.	(a)	1	Do.

EXPERIMENT 13

24999	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
25300	Do.	24	1	Do.
25301	Do.	24	2	Do.
25302	Do.	24	2	Do.
25303	Do.	24	4	Died in 3 days. Not anthrax.
25304	Do.	24	4	Lived.
25315	Sodium chlorid, but no disinfectant.	(a)	1	Died in 4 days. Anthrax.
25316	Do.	(a)	1	Died in 5 days. Anthrax.

[a] Not exposed.

Since in experiment 10 mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, was shown to be efficient, while mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid, was not, further tests were made with the lower dilution.

Ten pieces of hide were exposed for 24 hours to 25 c. c. (for each piece) of mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, and then soaked 24 hours in a saturated common-salt solution. The clots were then scraped off and inoculated into guinea pigs. In one instance two clots were inoculated into one animal; in all other cases only one clot was used (Table VI, experiment 11).

Another experiment (Table VI, experiment 12) was made in which six guinea pigs were inoculated with one clot each from pieces of hide disinfected with mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid; two guinea pigs were inoculated with two clots each from pieces similarly disinfected; and one guinea pig was inoculated with five clots from pieces of hide disinfected with mercuric chlorid, 1 to 2,500, plus 1 per cent of formic acid. As in the preceding experiments, each piece of hide was exposed for 24 hours to 25 c. c. of disinfectant and soaked in 25 c. c. of saturated common-salt solution for 24 hours, after which the clots were scraped off and inoculated under the skin of the guinea pigs.

The apparent discrepancy between experiments 11 and 12 in connection with results obtained by inoculation into guinea pigs of clots from two pieces of hide disinfected with mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, may be explained on the ground that the pieces used in the second experiment had been kept longer than those used in the first and had consequently lost virulence by continued drying. Even in experiment 11 it will be seen that the disinfectant exercised a marked influence on the virulence of the spores, since the guinea pig remained alive until five days after inoculation.

The results of these experiments are confirmed by the results of a further experiment (Table VI, experiment 13) performed later with test preparations of a different lot. This later lot was prepared in exactly the same way as the earlier ones; but the culture used for infecting the pieces of hide was obtained from a guinea pig dying a little more than 48 hours after inoculation, while the culture used for the pieces first prepared was obtained from a guinea pig dying within 36 hours after inoculation. The difference in the vitality of the spores is clearly seen in the length of time necessary to kill the guinea pigs inoculated from the check pieces. As will be seen by reference to Table VI, this time was about 48 hours for the first lot, while for the second it was from 4 to 5 days.

In order to ascertain the effect of mercuric chlorid and formic acid upon hides from the standpoint of the tanner, pieces of hide about 4 by 5 inches in size and weighing about 50 gm. each were disinfected by the Seymour-Jones method, using mercuric-chlorid dilutions of 1 to 4,000 and 1 to 2,500 plus 1 per cent of formic acid. The proportion of disinfectant used was 10 times the weight of the hide. These were examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry. Immediately after dehairing, these pieces of hide were observed to be very much blackened, but after the full process of tanning

this was not evident, so it appeared that the coloring matter of the tanning liquid had covered up this discoloration.

Judged solely by the results of the various experiments previously described, it might seem that the Seymour-Jones method could be accepted as suitable for the disinfection of hides, provided that mercuric chlorid in a strength of 1 to 2,500 was substituted for the recommended dilution of 1 to 5,000. However, at this stage the writer's attention was called to the work of Ševčík (14), which appeared to controvert the favorable results obtained by various workers as well as his own previous results. Ševčík concluded that it is necessary to carefully neutralize the disinfectant before attempting, either by cultural methods or animal inoculation, to ascertain whether anthrax spores have been destroyed, and that the hydrogen-sulphid solution used for a short time is not sufficient to neutralize mercuric chlorid plus formic acid. The neutralizing agent which he recommended was sodium sulphid, which neutralizes both the mercury and the acid. The time which he allowed for the neutralizing process was two hours.

Ševčík's contention that the mercuric chlorid and formic acid used in the Seymour-Jones method should be neutralized by sodium sulphid in order to determine whether disinfection has been complete seemed reasonable in view of the fact that many tanners use sodium sulphid for dehairing hides; therefore, in order to verify his conclusions, the following experiments were undertaken.

IV. EXPERIMENTS UPON PIECES OF HIDE INFECTED BY SPORES OF ARMY MEDICAL SCHOOL STRAIN OF BACILLUS ANTHRACIS WITH SODIUM SULPHID AS A NEUTRALIZING AGENT

Pieces of hide were exposed to 25 c. c. of disinfectant for 24 hours, treated with 25 c. c. of saturated solution of sodium chlorid for one hour and with 25 c. c. of a 1 per cent sodium-sulphid solution for two hours. They were then washed with sterile water.

In experiment 14 (Table VII) the clots were scraped off and inoculated into guinea pigs.

TABLE VII.—*Inoculation of guinea pigs with clots from infected pieces of hide*

EXPERIMENT 14

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
25522	Mercuric chlorid (1:1,000)+formic acid (1 per cent).	24	Died in 3½ days. Anthrax.
25523	Do	24	Lived.
25524	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	24	Do.
25525	Do	24	Died in 3½ days. Anthrax.
25526	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	Died. Mixed infection.
25527	Do	24	Died in 4 days. Anthrax.
25528	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died in 3 days. Anthrax.
25529	Do	(a)	Died. Mixed infection.

[a] Not exposed.

The test preparations used in experiment 14 were made as follows: Pieces of hide were cut so as to weigh about 2½ gm. A good-sized drop of guinea-pig blood was allowed to fall upon the center of each piece, and, before this clotted, a loopful of a suspension of anthrax spores was thoroughly mixed in. The suspension of spores was obtained by rubbing up in sterile water enough of the surface growth of an agar culture of *Bacillus anthracis* obtained directly from the spleen of a guinea pig (No. 25386) to give a suspension rather more dense than a 24-hour bouillon culture of *B. typhosus*. The loop employed was of No. 23 gauge platinum wire 3 mm. in diameter. The pieces of hide thus infected were dried in an electric oven at a temperature of about 45° C., in order to prevent the spores from developing into vegetative forms, which would be destroyed by the drying.

In experiment 15 (Table VIII) the test pieces of hide were prepared as follows: Pieces cut to weigh 2½ gm. were placed in a rather dense suspension of anthrax spores with hair side down. After soaking in this solution for 10 minutes they were placed in Petri dishes hair side up and allowed to dry a few minutes. Then 0.1 c. c. of the spore suspension was dropped on each piece and they were allowed to stand at room temperature for one hour. The pieces of hide were then dried in an electric oven at 43° C. for two days, the covers of the Petri dishes being tilted to one side. They were then kept at room temperature until used. After exposure to the disinfectant a considerable part of the hair with some of the underlying hide was scraped off and inoculated subcutaneously into guinea pigs, instead of inoculating blood clots as before. In other respects the technique was the same as for experiment 14.

TABLE VIII.—*Inoculation of guinea pigs with portions of hide*

EXPERIMENT 15

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
25566	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Died in 3½ days. Anthrax.
25567	Do.....	24	Lived.
25568	Mercuric chlorid (1:2,500)+formic acid (1 per cent)	24	Died in 5 days. Anthrax.
25569	Do.....	24	Do.
25570	Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Died in 4 days. Anthrax.
25571	Do.....	24	Died in 3½ days. Anthrax.
25572	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Do.
25573	Do.....	(a)	Died in 2 days. Mixed infection.

EXPERIMENT 16

25717	Mercuric chlorid (1:500)+formic acid (1 per cent).....		Lived.
25718	Do.....	24	Do.
25719	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Do.
25720	Do.....	24	Do.
25721	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Do.
25722	Do.....	24	Do.
25723	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 4 days. Anthrax.
25724	Do.....	(a)	Do.

a Not exposed.

TABLE VIII.—*Inoculation of guinea pigs with portions of hide*—Continued

EXPERIMENT 17

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
		Hours.	
24598	Mercuric chlorid (1:500)+formic acid (1 per cent).....	24	Lived.
24599	Do.....	24	Died after 7 days. Anthrax.
25725	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Lived.
25726	Do.....	24	Do.
25727	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Died after 6 days. Anthrax.
25728	Do.....	24	Lived.
25729	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 3 days. Mixed infection.
25730	Do.....	(a)	Died after 5 days. Anthrax.

EXPERIMENT 18

27221	Mercuric chlorid (1:250)+formic acid (1 per cent).....	24	Died after 4 days. Anthrax.
27222	Do.....	24	Died after 6 days. Not anthrax.
27223	Mercuric chlorid (1:500)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27224	Do.....	24	Died after 6 days. Anthrax.
27225	Mercuric chlorid (1:1,000)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27226	Do.....	24	Lived.
27227	Mercuric chlorid (1:2,000)+formic acid (1 per cent).....	24	Died after 5 days. Anthrax.
27228	Do.....	24	Died after 6 days. Anthrax.
27231	Sodium chlorid followed by sodium sulphid. No disinfectant.	(a)	Died after 5 days. Anthrax.
27232	Do.....	(a)	Died. Mixed infection.

[a] Not exposed.

Experiment 16 (Table VIII) was similar to the preceding, except that the pieces of hide used were dried for three instead of two days. A culture from the heart blood of guinea pig 25515 was used in making the spore suspension.

Apparently the added duration of drying had an injurious action upon the spores. It should be noted, however, that cultures from one guinea pig (No. 25386) were used in preparing material in experiments 14 and 15, while the test preparations used in experiment 16 were infected by a culture derived from a different animal.

The available cultures from the same source as those used in preparing material for experiments 14 and 15 were now 1 month old. In experiment 17 (Table VIII) one of these was used in infecting pieces of hide in the following way: Pieces of hide of 2½ gm. weight were soaked in a suspension of anthrax spores for 10 minutes; then one-tenth c. c. of suspension was dropped on each, and the pieces of hide were dried in an electric oven at 43° C. for 24 hours and then kept at room temperature for 24 hours before use. The covers of the Petri dishes containing the pieces of hide were kept raised during all of this time.

The results of this experiment seem to indicate that cultures derived from one animal (guinea pig 25386) yielded spores of very great resisting power as compared with cultures from another animal (guinea pig 25515). The irregularities which will be noted in experiment 17 are probably due to the age of the culture used.

A further series of experiments having given unsatisfactory results, it was deemed advisable to undertake comparative tests of infected pieces of hide prepared by several different methods.

Further experiments were thereupon made to compare the infectivity of pieces of hide dried (1) in an electric oven at 44° C. for 40 hours; (2) in an incubator at 37° C. for 24 hours (spores in blood clots); and (3) in a desiccator over sulphuric acid at a temperature of about 10° C., the desiccator being exhausted of air down to a pressure of about 6 cm. of mercury, time of drying, 48 hours.

Of the above only those pieces dried at a low temperature proved infectious, the guinea pig inoculated dying after one week. As a guinea pig inoculated by pure culture also remained alive for a week, it seemed that the process of drying at 10° C. in a vacuum over sulphuric acid had not appreciably diminished the virulence of the spores. This process was therefore used in the preparation of all further test pieces of hide.

Previous experiments had shown a difference between the two strains of guinea pigs which had been used in these experiments, one strain being much more susceptible to infection by anthrax than the other. The comparatively low virulence of the pure culture mentioned above seemed to be due to passage through the less resistant strain of guinea pigs. Beginning, therefore, with a culture which had not been so treated, successive inoculations were made with the more resistant strain of guinea pigs until cultures of satisfactory virulence and vitality were obtained.

V. EXPERIMENTS UPON INFECTED PIECES OF HIDE DRIED AT 10° C.

A lot of pieces of hide were prepared as follows: Pieces of 2½ gm. in weight were washed and dried. These were infected by a suspension made from a 7-day agar culture, in the following manner: Pieces were placed in the suspension, hair side down, and allowed to soak for 10 minutes, and then 0.2 c. c. of the suspension was dropped on each. These pieces were left in Petri dishes in the ice box for half an hour with covers of dishes on. At the end of that time the dishes were placed in a desiccator over sulphuric acid and the covers raised. The desiccator was then exhausted of air and put into the ice box, where it remained 48 hours at a temperature of 10° C. The pieces of hide were then removed and kept at room temperature until used. A guinea pig inoculated with the pure culture used for infecting these pieces of hide died in four days. Using the pieces of hide prepared as described, the following experiments were performed:

In experiment 18, pieces of hide were exposed to the disinfectant for 24 hours, followed by a saturated salt solution for 1 hour. They were then treated with a 1 per cent sodium-sulphid solution for 2 hours and washed with sterile water. Material was then scraped from the surface of each and inoculated into a guinea pig. The results are given in Table VIII, experiment 18.

In this experiment, as in those of similar character preceding it, neutralization of the disinfectant by sodium sulphid was done within a comparatively few hours after the process of disinfection was complete. In view of the strong dilution (1 to 250) found to be inefficient under these circumstances, no further attempt was made to find a dilution strong enough to disinfect, with neutralization afterward. Instead of this, an attempt was now made to determine how long spores remained viable after treatment of the pieces of hide by much weaker dilutions of mercuric chlorid plus formic acid. This seemed worth while because the Seymour-Jones method was originally proposed to be employed at foreign ports, and in a voyage of ordinary length a considerable time would thus elapse between the time of disinfection and time of arrival at destination.

In experiment 19 (Table IX) a number of pieces of hide were exposed for 24 hours to mercuric chlorid, 1 to 4,000, plus 1 per cent of formic acid, treated with saturated common salt for 1 hour, and then laid aside and at intervals treated with sodium sulphid and inoculated into guinea pigs. In each case they were treated with 1 per cent of sodium sulphid for 2 hours and washed with sterile distilled water. Material was then scraped from each piece and inoculated subcutaneously into a guinea pig.

TABLE IX.—*Inoculation of guinea pigs with infected portions of hide*

EXPERIMENT 19 [a]

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Time before treatment with sodium sulphid.	Result of inoculation.
		Hours.	*Days.*	
27229	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
27229	Do	24	1	Do.
27257	Do	24	2	Died after 6 days. Anthrax.
27258	Do	24	2	Died. Pneumonia.
27259	Do	24	3	Died after 6½ days. Anthrax.
27260	Do	24	3	Lived.
27261	Do	24	4	Died after 10 days. Anthrax.
27262	Do	24	4	Died. Pneumonia.

EXPERIMENT 20 [b]

		Hours.	*Days.*	
27521	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
27522	Do	24	1	Do.
27525	Do	24	3	Died after 6 days. Anthrax.
27526	Do	24	3	Lived.
27531	Do	24	6	Died after 4 days. Anthrax.
27532	Do	24	6	Died after 5 days. Anthrax.
			Weeks.	
28004	Do	24	2	Lived.
28005	Do	24	2	Do.

[a] Control guinea pig died of anthrax in 5 days.
[b] Control guinea pig died of anthrax in 7 days.

TABLE IX.—*Inoculation of guinea pigs with infected portions of hide*—Continued

EXPERIMENT 21 [a]

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Time before treatment with sodium sulphid.	Result of inoculation.
		Hours.	*Days.*	
28006	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Lived.
28007	Do	24	1	Do.
28010	Do	24	4	Died after 9 days. Anthrax.
28011	Do	24	4	Died after 3 days. Anthrax.
28056	Do	24	9	Died after 7 days. Anthrax.
28057	Do	24	9	Died after 6 days. Anthrax.
			Weeks.	
28072	Do	24	2	Do.
28073	Do	24	2	Died after 8 days. Anthrax.

EXPERIMENT 22 [b]

		Hours.	*Days.*	
28012	Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	1	Died after 9 days. Anthrax.
28013	Do	24	1	Lived.
28008	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	24	1	Died after 13 days. Anthrax.
28009	Do	24	1	Lived.
28052	Do	24	2	Do.
28053	Do	24	2	Do.
28054	Do	24	4	Died after 6 days. Anthrax.
28055	Do	24	4	Lived.
28058	Do	24	6	Do.
28059	Do	24	6	Do.

[a] Control guinea pig died of anthrax in 4 days.
[b] Control guinea pig died of mixed infection.

The irregular results noted above might be due to variation in the extent of infection of the various pieces of hide.

In another experiment with similar technique, except that the pieces of hide were infected at a different time and by a different culture, the results were as given in Table IX, experiment 20.

In experiment 21 (Table IX), also, the procedure was the same as in experiment 19, except that the test pieces of hide were infected by a different culture.

Experiment 22 (Table IX) was similar to the preceding experiments, except in the use of a stronger dilution of the disinfectant.

In connection with experiments 20, 21, and 22 part of the material scraped from the pieces of hide was plated out to determine whether sterilization had been accomplished. Growth of some kind was obtained in every instance, although *Bacillus anthracis* was isolated in only about one-third of the cases. In one instance *B. anthracis* was recovered from material which failed to cause anthrax when inoculated into guinea pigs, but on the other hand, one guinea pig died from anthrax after inoculation with material which failed to yield *B. anthracis* by the plate method.

HYDROCHLORIC ACID AND SODIUM CHLORID

In view of the apparent inefficiency of the Seymour-Jones method and the favorable results reported by various workers using the Schattenfroh method, experiments were now undertaken to determine the germicidal power of hydrochloric acid and sodium chlorid against anthrax spores, both as "naked" spores and as contained on and in infected pieces of hide. The Schattenfroh method (12) as described by Prof. Schattenfroh consists of immersion of hides in solutions of hydrochloric acid and common salt, the proportions recommended varying according to temperature. The proportions recommended for use at room temperature are 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid, with the time of exposure 48 hours. At higher temperatures less of the acid is needed and the time of exposure is shortened, but inasmuch as special apparatus would be needed to maintain these higher temperatures it seemed that disinfection at these higher temperatures could be disregarded as being of little practical significance.

The experiments here described were therefore carried on at room temperature. In all cases dilutions were calculated upon the percentage of absolute hydrochloric acid, not upon the percentage of "concentrated hydrochloric acid." In accordance with Schattenfroh's recommendations, a sodium-carbonate solution was used after exposure to the disinfectant, in order to neutralize the hydrochloric acid.

1. EXPERIMENTS BY THE ROD METHOD, USING SPORES OF BACILLUS ANTHRACIS

A series of experiments was first made by the rod method, using various proportions of hydrochloric acid and sodium chlorid. The time of exposure in each case was 24 hours, and rods were washed with a 2 per cent solution of sodium carbonate to neutralize the hydrochloric acid. Experiment 23 (Table X) was made without the addition of organic matter; experiment 24 (Table X) was made with the addition of 1 c. c. of defibrinated blood to 9 c. c. of disinfectant in each tube. The results are given in Table X, together with the results of an experiment upon mercuric chlorid, alone and with acetic acid and formic acid, which was made at the same time, and with rods infected by the same spore suspension. This suspension was rather heavier than usual. In experiment 24 the hydrochloric-acid rods were washed in a 20 c. c. sodium-carbonate solution for one minute, and the mercuric chlorid rods in a 20 c. c. saturated hydrogen sulphid for one minute.

TABLE X.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid and mercuric chlorid, with and without formic acid, and with acetic acid, by the rod method, without addition of organic matter*[a]

EXPERIMENT 23

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
1	Hydrochloric acid (1 per cent)+sodium chlorid (5 per cent)	24	No growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (5 per cent)	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (5 per cent)	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (5 per cent)	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (5 per cent)	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent)	24	Do.
9	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent)	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent)	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent)	24	Do.
12	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent)	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent)	24	Do.
14	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent)	24	Do.
15	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent)	24	Do.
16	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent)	24	Do.
17	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent)	24	Do.
18	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent)	24	Do.
19	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent)	24	Do.
20	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent)	24	No.
21	Control rod	(b)	Growth.

EXPERIMENT 24[c]

1	Hydrochloric acid (1 per cent)+sodium chlorid (5 per cent)	24	Growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (5 per cent)	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (5 per cent)	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (5 per cent)	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (5 per cent)	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent)	24	No growth.
9	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent)	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent)	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent)	24	Growth.
12	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent)	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent)	24	No growth.
14	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent)	24	Do.
15	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent)	24	Do.
16	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent)	24	Do.
17	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent)	24	Growth.
18	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent)	24	No growth.
19	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent)	24	Growth.
20	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent)	24	No growth.
21	Mercuric chlorid (1:500) alone	24	Growth.
22	Mercuric chlorid (1:1,000)+acetic acid (1 per cent)	24	Do.
23	Mercuric chlorid (1:2,000)+acetic acid (1 per cent)	24	Do.
24	Mercuric chlorid (1:3,000)+acetic acid (1 per cent)	24	Do.
25	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	Do.
26	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Do.
27	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Do.
28	Control rod	(b)	Do.

EXPERIMENT 25 [c]

1	Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	No growth.
2	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	Do.
3	Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent)	24	Do.
4	Hydrochloric acid (4 per cent)+sodium chlorid (10 per cent)	24	Do.
5	Hydrochloric acid (5 per cent)+sodium chlorid (10 per cent)	24	Do.
6	Hydrochloric acid (1 per cent)+sodium chlorid (15 per cent)	24	Do.
7	Hydrochloric acid (2 per cent)+sodium chlorid (15 per cent)	24	Do.
8	Hydrochloric acid (3 per cent)+sodium chlorid (15 per cent)	24	Do.
9	Hydrochloric acid (4 per cent)+sodium chlorid (15 per cent)	24	Do.
10	Hydrochloric acid (5 per cent)+sodium chlorid (15 per cent)	24	Do.
11	Hydrochloric acid (1 per cent)+sodium chlorid (20 per cent)	24	Do.
12	Hydrochloric acid (2 per cent)+sodium chlorid (20 per cent)	24	Do.
13	Hydrochloric acid (3 per cent)+sodium chlorid (20 per cent)	24	Do.
14	Hydrochloric acid (4 per cent)+sodium chlorid (20 per cent)	24	Do.

[a] Percentage of hydrochloric acid means percentage of absolute hydrochloric acid.
[b] Not exposed.
[c] The quantity of disinfectant used (10 c. c.) included 1 c. c. of defibrinated blood.

TABLE X.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid and mercuric chlorid, with and without formic acid, and with acetic acid, by the rod method, without addition of organic matter*—Continued

EXPERIMENT 25—continued

Rod No.	Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.
		Hours.	
15	Hydrochloric acid (5 per cent)+sodium chlorid (20 per cent)	24	No growth.
16	Mercuric chlorid (1:500) alone	24	Growth.
17	Mercuric chlorid (1:1,000) alone	24	Do.
18	Mercuric chlorid (1:1,000)+acetic acid (1 per cent)	24	Do.
19	Mercuric chlorid (1:2,000)+acetic acid (1 per cent)	24	Do.
20	Mercuric chlorid (1:3,000)+acetic acid (1 per cent)	24	Do.
21	Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	No growth.
22	Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	Do.
23	Mercuric chlorid (1:3,000)+formic acid (1 per cent)	24	Growth.
24	Control rod	(a)	Do.

[a] Not exposed.

A similar experiment (Table X, experiment 25) was made with rods infected by a spore suspension of about the same density as a 24-hour bouillon culture of *Bacillus typhosus*.

In experiments 26 and 27 (Table XI) are shown a comparison of hydrochloric acid and common salt with several other disinfectants, all with 24-hour exposure. Three rods were used with each dilution, showing the result, respectively, when no defibrinated blood was added, with ½ c. c. of blood added to each tube and with 1 c. c. of blood added to each tube. The hydrochloric-acid rods were washed with a 2 per cent sodium-carbonate solution, the mercuric-chlorid rods with a saturated hydrogen-sulphid solution, and the formalin and carbolic-acid rods with distilled water.

TABLE XI.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid, formalin, phenol, and mercuric chlorid, with and without formic acid, by the rod method*

EXPERIMENT 26

Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.		
		No blood added.	½ c. c. blood added.	1 c. c. blood added.
	Hours.			
Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent).	24	No growth.	No growth.	No growth.
Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	24	.....do.....	.....do.....	Do.
Hydrochloric acid (3 per cent)+sodium chlorid (10 per cent).	24	.....do.....	.....do.....	Do.
Mercuric chlorid (1:1,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Growth.
Mercuric chlorid (1:2,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Do.
Mercuric chlorid (1:4,000)+formic acid (1 per cent).	24	.....do.....	Growth.....	Do.
Mercuric chlorid (1:8,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Do.
Mercuric chlorid (1:16,000)+formic acid (1 per cent).	24	.....do.....	.....do.....	Do.
Formalin (1:50)	24	.....do.....	No growth.	Do.
Formalin (1:100)	24	.....do.....	Growth.....	Do.
Formalin (1:250)	24	Growth.....	.....do.....	Do.
Formalin (1:1,000)	24	.....do.....	.....do.....	Do.
Phenol (5 per cent)	24	.....do.....	.....do.....	Do.

TABLE XI.—*Germicidal efficiency of hydrochloric acid plus sodium chlorid, formalin, phenol, and mercuric chlorid, with and without formic acid, by rod method*—Continued

EXPERIMENT 27

Disinfectant (10 c. c.) and dilution.	Time of exposure.	Result.	
		½ c. c. blood added.	1 c. c. blood added.
	Hours.		
Hydrochloric acid (1 per cent)+sodium chlorid (10 per cent)	24	No growth	No growth.
Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent)	24	do	Do.
Mercuric chlorid (1:1,000)+formic acid (1 per cent)	24	do	Do.
Mercuric chlorid (1:2,000)+formic acid (1 per cent)	24	do	Growth.
Mercuric chlorid (1:4,000)+formic acid (1 per cent)	24	Growth	Do.
Mercuric chlorid (1:6,000)+formic acid (1 per cent)	24	do	Do.
Mercuric chlorid (1:8,000)+formic acid (1 per cent)	24	do	Do.
Formalin (1:50)	24	No growth	Do.
Formalin (1:100)	24	Growth	Do.
Formalin (1:200)	24	do	Do.

The technique of experiment 27 was the same as that of No. 26. In this case two rods were used with each dilution, showing results with ½ c. c. and 1 c. c. of defibrinated blood.

II. EXPERIMENTS UPON PIECES OF HIDE INFECTED WITH SPORES OF BACILLUS ANTHRACIS

In experiment 28 (Table XII) a 2 per cent hydrochloric-acid solution plus 10 per cent of sodium chlorid was used with a 48-hour exposure, 25 c. c. of the disinfectant being used for each piece of hide. After exposure the pieces of hide were soaked for 15 minutes in a 3 per cent solution of sodium carbonate (25 c. c. for each). The pieces of hide used were prepared by the method given by Ponder (9, 10) and were part of the same lot as the pieces used in experiment 14. After disinfection the clots were scraped off and inoculated subcutaneously into guinea pigs.

TABLE XII.—*Inoculation of guinea pigs with clots scraped from pieces of hide*

EXPERIMENT 28

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Number of clots used.	Result of inoculation.
		Hours.		
25556	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	48	2	Lived.
25557	Do	48	2	Do.
25558	Do	48	1	Do.
25559	Do	48	1	Do.
25560	Do	48	1	Do.
25561	Do	48	1	Do.
25562	Do	48	1	Do.
25563	Do	48	1	Do.
25554	No disinfectant	(a)	.....	Died. Anthrax.
25555	Do	(a)	.....	Do.

a Not exposed.

Experiment 29 (Table XIII) was made as follows: Pieces of hide were prepared by soaking in spore suspension and then drying in an electric oven. Details given in connection with experiment 15 will apply to this experiment. The technique otherwise was the same as that of experiment 28. Material was scraped from the surface of each piece and inoculated subcutaneously into guinea pigs.

TABLE XIII.—*Inoculation of guinea pigs with material scraped from pieces of hide*

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Time of exposure.	Result of inoculation.
	EXPERIMENT 29		
		Hours.	
25710	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
25711	Do	48	Do.
25712	Do	48	Do.
25713	Do	48	Do.
25714	Do	48	Do.
25715	No disinfectant	(a)	Died in 3¾ days. Anthrax.
25716	Do	(a)	Died in 6 days. Anthrax.
	EXPERIMENT 30		
25733	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
25734	Do	48	Do.
25735	Do	48	Do.
25736	Do	48	Do.
25737	Do	48	Do.
25738	Do	48	Do.
25739	Do	48	Do.
25740	Do	48	Do.
25741	Do	48	Do.
24742	Do	48	Do.
25729	No disinfectant	(a)	Died. Mixed infection.
25730	Do	(a)	Died after 5 days. Anthrax.
	EXPERIMENT 31		
27593	Hydrochloric acid (2 per cent) + sodium chlorid (10 per cent).	48	Lived.
27594	Do	48	Do.
27595	Do	48	Do.
27596	Do	48	Do.
27597	Do	48	Do.
27598	Do	48	Do.
27599	Do	48	Do.
28000	Do	48	Do.
28001	No disinfectant	(a)	Died after 4 days. Anthrax.
28002	Do	(a)	Died after 3 days. Anthrax.

[a] Not exposed.

Experiment 30 (Table XIII) was made upon pieces of hide prepared in the same way but infected with a different culture.

In experiment 31 (Table XIII) the pieces of hide were prepared by soaking in spore suspension and drying them over sulphuric acid in a vacuum at 10° C. for 48 hours. As before, each piece of hide after disinfection was immersed for 15 minutes in 25 c. c. of a 3 per cent sodium-carbonate solution.

In connection with experiment 31 an attempt was made to determine the efficiency of disinfection by plating out material from the piece of

hide. The plates showed no growth even after three days' incubation; hence, it seemed that the hydrochloric acid and sodium chlorid had destroyed the anthrax spores and all other organisms as well.

Experiments 32 and 33 (Table XIV) show comparative tests of the Seymour-Jones and Schattenfroh methods upon pieces of hide of the same lot. These were prepared by the method described under experiment 31. The greatest possible care was taken to neutralize the disinfectant, so far as the Schattenfroh method was concerned. Sodium sulphid was used both for Seymour-Jones and Schattenfroh pieces, because it seemed possible that the depilatory action of the sodium sulphid might bring up undisinfected spores from the depths of the hair follicles. A number of pieces of disinfected hide were kept several days and then treated with the neutralizing agent.

TABLE XIV.—*Comparison of Seymour-Jones and Schattenfroh methods of disinfecting hides*

EXPERIMENT 32

Guinea pig No.	Disinfectant (25 c. c.) and dilution.	Neutralizing solution and time required.	Time of exposure.	Result of inoculation.
			Hours.	
28556	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	Sodium carbonate (2 per cent), ½ hour.	48	Lived.
28557	Do	do	48	Do.
28558	Do	Potassium hydroxid (0.5 per cent), 2 hours.	48	Do.
28559	Do	do	48	Do.
28560	Do	Sodium sulphid (1 per cent), 2 hours.	48	Do.
28561	Do	do	48	Do.
28562	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	do	24	Do.
28563	Do	do	24	Do.
	Neutralization 4 days later.			
28588	Mercuric chlorid (1:2,500)+formic acid (1 per cent).	Sodium sulphid (1 per cent), 2 hours.	24	Died. Anthrax.
28589	Do	do	24	Do.
28590	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	do	48	Lived.
28591	Do	do	48	Do.

EXPERIMENT 33

28735	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	Sodium carbonate (2 per cent), ½ hour.	48	Lived.
28736	Do	do	48	Do.
28737	Do	Potassium hydroxid (0.5 per cent), 2 hours.	48	Do.
28738	Do	do	48	Do.
28739	Do	Sodium sulphid (1 per cent), 2 hours.	48	Do.
28740	Do	do	48	Do.
28729	Mercuric chlorid (1: 2,500)+formic acid (1 per cent).	do	24	Do.
28730	Do	do	24	Died. Anthrax.
	Neutralization 4 days later.			
28773	Mercuric chlorid (1: 2,500)+formic acid (1 per cent).	Sodium sulphid (1 per cent), 2 hours.	24	Died. Anthrax.
28774	Do	do	24	Lived.
28771	Hydrochloric acid (2 per cent)+sodium chlorid (10 per cent).	do	48	Do.
28772	Do	do	48	Do.

As a part of experiment 32, plates were made from the material scraped off the pieces of hide. In every instance the plates made from material treated by 2 per cent of hydrochloric acid and 10 per cent of sodium chlorid were sterile. On the other hand, growth was observed on all the plates from material exposed to mercuric chlorid and formic acid.

In this experiment, as in several of the last few experiments described in the previous discussion of the Seymour-Jones method, it will be noted that material from pieces of hide exposed to mercuric chlorid and formic acid and treated shortly after completion of the disinfection with sodium sulphid failed to kill guinea pigs into which it was inoculated. On the other hand, material from pieces of hide allowed to stand for several days before using sodium sulphid caused guinea pigs to die from anthrax. It was noted that the depilatory action of the sodium sulphid was far more complete in the case of the pieces of hide which had been kept for several days after disinfection before treatment with the sulphid. The results of plating, as before mentioned, showed that disinfection was not complete; therefore it seems probable that the more extensive depilatory action of the sodium sulphid upon pieces which had stood for some time brought up from the depths of the hair follicles spores which had been practically untouched by the disinfectant. It also seems possible that there had been some development and multiplication of these uninjured organisms during the period of waiting.

It should be noted that in the preparation of the pieces of hide used in all the above-mentioned experiments particular care was taken to secure penetration of the spores into the pieces of hide. In order to accomplish this, the pieces of hide after being infected by spore suspensions were placed in closed Petri dishes and kept in the ice box for four or five hours before the drying process was begun.

As will be seen by reference to Table XIII, the Schattenfroh method was entirely successful in every instance, and the results of plating showed that actual sterilization was accomplished.

Experiment 33 (Table XIV) was exactly similar to the preceding experiment except that the pieces of hide used were infected by spores derived from a different culture. The method of preparation was the same as that described under experiment 31.

In this experiment, as in the preceding one, the efficiency of the disinfectants was tested by plating out material from the pieces of hide. The results obtained varied from the results of experiment 32 in that a few colonies were found on two plates from material treated with hydrochloric acid and salt, while all other plates from similar material were sterile. One plate from material neutralized by 0.5 per cent of potassium hydrate showed two colonies, while the other, from material neutralized by sodium carbonate, showed one colony. In none of the three was *Bacillus anthracis* the organism present. Therefore, although hydro-

chloric acid and salt did not accomplish actual sterilization in every instance, it did destroy anthrax spores in every instance.

Several pieces of hide about 50 gm. weight each were exposed to 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid for 48 hours and thoroughly washed with 3 per cent sodium-carbonate solution. They were then examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry, along with pieces of hide which had been treated by other disinfectants. This work was in charge of Mr. F. P. Veitch, and the result is shown in his memorandum on page 91.

OTHER DISINFECTANTS

Bacteriological tests were made with formalin and phenol, and pieces of hide treated by these disinfectants were examined and tanned in the Leather and Paper Laboratory of the Bureau of Chemistry. Without going into details it may be stated that, so far as could be determined by the limited number of tests, 2½ per cent of formalin is efficient bacteriologically both against anthrax spores and against other organisms, while 5 per cent of phenol is fairly efficient against non-spore-bearing organisms, but is practically useless against anthrax spores. It should be noted also that pieces of hide disinfected by formalin in 2½ per cent solution were so seriously affected by the disinfectant that it was almost impossible to tan them, while pieces treated with carbolic acid were uninjured.

A few tests were made of the germicidal efficiency of mercuric-chlorid solutions saturated with sodium chlorid. It was found that this combination is, if anything, not as efficient as mercuric chlorid alone. This is presumably due to interference of the salt with the ionization of the mercuric chlorid, as the work of Krönig and Paul (6) quite clearly indicates.

During the course of the investigations herein recorded, the writer noted considerable variations in the vitality and virulence of anthrax spores from different sources. It was also noted that the processes employed in infecting and drying test preparations exercised a variable influence upon the vitality of the spores. In view of these variations, it was found to be necessary to repeat the tests many times, and in order to test the various methods as thoroughly as possible, every effort was made to maintain at the highest possible point the vitality and virulence of the spores used in test preparations and to make sure of the presence of a considerable number of such spores upon each test preparation.

It seems likely that anthrax spores occurring upon naturally infected hides might in many cases be present in much smaller numbers and possess far less vitality and virulence than those used in the experiments. However, in view of the results obtained by Ševčík (14) and

others working with naturally infected hides, it is evident that the spores upon such hides frequently possess very high vitality and virulence. Therefore it seems that the only safe rule to follow is to use only such disinfectants and such methods of disinfection as have been found efficient against spores of maximum vitality and virulence.

SUMMARY AND CONCLUSIONS

(1) THE SEYMOUR-JONES METHOD.—The strength of disinfectant originally recommended by Seymour-Jones (mercuric chlorid, 1 to 5,000, plus 1 per cent of formic acid) was not found to be efficient, even without neutralization of the disinfectant. A stronger dilution, 1 to 2,500, plus 1 per cent of formic acid, was found to be efficient where no neutralization was attempted. The latter strength was not sufficient, however, to prevent fatal infection of guinea pigs by disinfected material when the disinfectant was neutralized by a 1 per cent sodium-sulphid solution three or four days after the completion of the process of disinfection. No infection was caused by the inoculation of material which had been kept a week or more after disinfection. It seems, therefore, that the Seymour-Jones method might be employed with dilutions of mercuric chlorid, 1 to 2,500, plus 1 per cent of formic acid, provided the treated hides are not to be subjected within a week or two to the action of any substance which will neutralize the disinfectant. This would be the case, for instance, if hides were disinfected at foreign ports before shipment to this country.

(2) THE SCHATTENFROH METHOD.—Hydrochloric acid and sodium chlorid in the proportions of 2 per cent of the acid and 10 per cent of the salt and with 48 hours' exposure have proved efficient in every instance. Consequently from the bacteriological standpoint the Schattenfroh method seems to be entirely satisfactory. This conclusion is supported not only by this work but by the exhaustive researches of Gegenbauer and Reichel (3) and Hilgermann and Marmann (4). The recently published work of Ševčík(15) is not so favorable to the Schattenfroh method as that of the investigators previously mentioned. He finds that complete disinfection can be accomplished when the hides worked with are thin. But when the hides are thick and heavily infected, he was able, after very thorough neutralization, to extract from pieces of the treated hides anthrax spores which were virulent for mice, and in some instances for guinea pigs, even after exposure to a solution of 2 per cent of hydrochloric acid plus 10 per cent of sodium chlorid for 7 days.

Although in view of the above-mentioned results the Schattenfroh method can not be regarded as perfect, it nevertheless seems to be far superior to other methods and well worth a trial as a standard method for the disinfection of hides.

(3) EFFECT OF DISINFECTION UPON HIDES AS REGARDS TANNING.—Mr. F. P. Veitch, Chemist in Charge of the Leather and Paper Laboratory

of the Bureau of Chemistry, has been kind enough to furnish the following memorandum in regard to the tanning of small pieces of normal hide treated by the Seymour-Jones and Schattenfroh processes of disinfection.

No marked differences in color were noted among the various pieces of tanned leather. Slight differences, due to difference in thickness, were noted in pliability, but these did not appear to be connected with the disinfecting treatment. No marked difference could be detected in the appearance of the grain of the leather. All the pieces cracked when severely bent, owing probably to excessive tannin in the grain of the leathers. The treated leathers did not display more pronounced cracking than those which were not treated. Microscopical examination of the hide fibers after deliming and of the leather fibers after tanning shows no marked differences among the several pieces of hide.

The results in general seem to indicate that the several treatments have not injured the hides. The evidence, however, is not sufficient to permit of definite conclusions being drawn at this time. More extended work in commercial tannery, using whole hides, has been planned to determine definitely whether any of the disinfectants result in the production of inferior leather. Since tanning is a slow process, it will require from nine months to a year to secure these data.

Mr. Veitch also states that all the leathers gave reactions for chlorids, but that the leathers treated with disinfectants apparently contained larger amounts of chlorids than the other leathers.

It seems, then, so far as the evidence at hand permits any conclusion at all, that neither the Seymour-Jones method nor the Schattenfroh method exerts any injurious effect upon hides or leather.

LITERATURE CITED

(1) Anthrax Investigation Board for Bradford [England] and District.
[1908.] Third Annual Report. [1907]/08, 15 p.

(2) Eurich, F. W.
1912. The prevention of "woolsorters' disease" (anthrax). *In* Jour. Roy. Sanit. Inst. [London], v. 33, no. 10, p. 507–514.

(3) Gegenbauer, Viktor, and Reichel, Heinrich.
1913. Die Desinfektion milzbrandiger Häute und Felle in Salzsäure-Kochsalzgemischen. *In* Arch. Hyg., Bd. 78, Heft 1/3, p. 1–128. Literatur, p. 123–126.

(4) Hilgermann, R., and Marmann, J.
1913. Untersuchungen über die durch Gerbereien verursachten Milzbrandgefahren und ihre Bekämpfung . . . *In* Arch. Hyg., Bd. 79, Heft 4/5, p. 168–258. Literaturverzeichnis, p. 256–258.

(5) Hill, H. W.
1898. A method of preparing test objects for disinfection experiments. *In* Pub. Health Papers and Rpts. Amer. Pub. Health Assoc., v. 24, p. 246–249, 1 pl.

(6) Krönig, B., and Paul, Th.
1897. Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. *In* Ztschr. Hyg. u. Infektionskrank., Bd. 25, Heft 1, p. 1–112, 2 fig., pl. 1.

(7) Moegle, Erich.
1912. Zur Desinfektion milzbrandsporenhaltiger Häute und Felle. *In* Centbl. Bakt. [etc.], Abt. 1, Orig., Bd. 66, Heft 5/6, p. 442–462. Literatur, p. 462.

(8) Otsuki, Ukichi.
1900. Untersuchungen über den Einfluss der Unterlage auf die Wirksamkeit von Desinfektionsmitteln gegenüber Milzbrandsporen. *In* Hyg. Rundschau, Jahrg. 10, No. 4, p. 153–174.

(9) Ponder, C. W.
1911. The prevention of anthrax infection due to imported hides and skins. (The Seymour-Jones formic-mercury process.) *In* Lancet, v. 181, no. 4601, p. 1260–1262.

(10) ———
1911. A Report to the Worshipful Company of Leathersellers on the Incidence of Anthrax amongst Those Engaged in the Hide, Skin, and Leather Industries, with an Inquiry into Certain Measures Aiming at its Prevention. 88 p., illus., 111 diagr. London. Bibliography, p. 69–72.

(11) Roos, Otto.
1912. Ueber die Einwirkung von Salvarsan auf Milzbrandbacillen. *In* Ztschr. Immunitätsforsch. u. Exp. Ther., T. 1, Orig., Bd. 15, Heft 6, p. 487–505.

(12) Schattenfroh, A.
1911. Ein unschädliches Desinfektionsverfahren für milzbrandinfizierte Häute und Felle. *In* Wiener Klin. Wchnschr., Bd. 24, No. 21, p. 735–736.

(13) Schnürer, J.
1911. Zur Frage der Häutedesinfektion. *In* Tierärztl. Zentbl., Jahrg. 34, No. 29, p. 443–444.

(14) Ševčík, Franz.
1913. Experimentelle Beiträge zur Frage der Desinfektion milzbrandsporenhaltiger Häute und Felle. *In* Ztschr. Infektionskrank. [etc.], Bd. 13, Heft 6, p. 323–348; Heft 7, p. 439–452.

(15) ———
1914. Zur Desinfektion von Milzbrandhäuten. *In* Wiener Tierärztl. Monatsschr., Jahrg. 1, Heft 3, p. 127–131.

(16) Seymour-Jones, Alfred.
1910. The Seymour-Jones Anthrax Sterilization Method. 31 p. London.