USE OF SODIUM BIFLUORIDE AND SODIUM SILICOFLUORIDE IN THE DISINFECTION OF HIDES [1]

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INTRODUCTION

The importation of hides and skins presents a hazard to the livestock industry of the United States because of the possible introduction of various disease germs or viruses that may adhere to or be incorporated in these materials. Such maladies as foot-and-mouth disease, rinderpest, and anthrax can be spread in this manner. Federal regulations covering the disinfection of hides and skins imported from countries in which such communicable diseases are known to exist, therefore, are rigidly enforced.

Research has been continuous in an effort to find some chemical substance or compound that would be effective in rendering all infected hides and skins safe for importation, without injuring them for tanning purposes. Various chemical substances have been found to be effective germicides or virucides when used with only the etiological agents of various diseases, but in the presence of animal tissue the disinfectants in many instances have been ineffective. This condition is due to the inability of some disinfectants to penetrate the tissues or to the formation of an insoluble substance by the combination of the chemical with the tissue proteins. Another equally important factor in hide disinfection is the effect of the disinfectants on the tanning properties of the hides. Some disinfectants have been found to be effective in destroying the contaminating germs or viruses, but they damaged the hides.

REVIEW OF LITERATURE

Most of the early work on hide disinfection was connected with anthrax. Extensive research was necessary to find methods of disinfection that would destroy both the anthrax bacilli and the spores. The most widely accepted methods were those recommended by Seymour-Jones (*11*) [2] and Schattenfroh (*10*). Work was done by Ponder (*9*), Smyth (*12*), Tilley (*13*), and O'Flaherty and Doherty (*7*) to determine the efficiency of these methods.

The prevention of foot-and-mouth disease in the United States is extremely important because of the enormous losses to livestock owners and the cost to the State and Federal Governments caused by the drastic methods of eradication. Much work has been done by various investigators toward the control of foot-and-mouth disease. The British Foot-and-Mouth Disease Research Committee of the Ministry of Agriculture and Fisheries, in its first (*2*), second (*3*), third (*4*), fourth (*5*), and fifth (*6*) progress reports, and the United States Foot-and-Mouth Disease Commission (*8*) found that agents which coagulate protein are ineffective against the virus in the presence of exudate or tissue. As a result, various noncoagulating protein chemicals were employed and found to be virucidal when used in the proper dilution.

[1] Received for publication January 18, 1941.

[2] Numbers in parentheses refer to Literature Cited, p. 47.

Among the chemicals mentioned are sodium hydroxide, sodium bisulfate, sodium bisulfite, antiformin, sulfoliquid, and formalin. Results obtained by Trautwein and Reppin (*14*), as well as Winkel (*15*), showed that 1 percent of sodium hydroxide killed the virus readily, and the latter worker used 0.1 to 2 percent of sulfuric acid with equal effectiveness. In the 1928 outbreak of foot-and-mouth disease in Bavaria, Germany, 1 percent of sodium hydroxide in conjunction with 5 percent of calcium hydroxide was successfully used to disinfect premises. Helm and Curtze (*1*) demonstrated that the efficiency of sulfoliquid and caustic soda was greatly increased when applied hot (100° C.).

In the third report of the British Foot-and-Mouth Disease Research Committee (*4*), mention is made of the use of formalin in the disinfection of hides heavily infected with foot-and-mouth-disease virus. In such hides soaked in 1-percent formalin solution for 48 hours, the virus was destroyed but the formalin had a detrimental effect on the hides. In the fourth report of that Committee (*5*), the statement is made that when infected hides were soaked in sodium bisulfate solution (1 to 10,000) for 5 hours or sodium bifluoride (1 to 20,000) for 2 hours, the virus was destroyed and no damage was done to the hides from the standpoint of leather production. No further mention was made of this method of hide treatment in subsequent publications nor were the protocols of the experiment given in that report.

To confirm this work, O'Flaherty and Doherty (*7*) used sodium bisulfate and sodium bifluoride in hides contaminated with the virus of vesicular stomatitis rather than foot-and-mouth disease, in accordance with the policy of the Bureau of Animal Industry of the United States Department of Agriculture not to experiment with the virus of foot-and-mouth disease in the United States. This has been a long-established policy because of danger to the livestock industry of the presence of foot-and-mouth disease virus in the country even for experimental purposes. These authors found that sodium bifluoride, in a solution of 1 to 10,000, destroyed the virus in 24 hours, whereas sodium bisulfate, in a solution of 1 to 400, failed to kill in 24 hours. No undesirable influence on the skin and subsequent leather was noticed by several tanneries that used the sodium bifluoride process of treatment.

PURPOSE AND EXPERIMENTAL PROCEDURE

To test the virucidal action of sodium bifluoride and sodium silicofluoride on hides impregnated with the virus of vesicular stomatitis, experiments were conducted in 1939 at the United States Department of Agriculture Animal Disease Station, Beltsville, Md. Virus of vesicular stomatitis was used because of its similarity to the virus of foot-and-mouth disease. The use of sodium bifluoride was suggested by the report of the British Foot-and-Mouth Disease Research Committee showing its value in destroying the virus of foot-and-mouth disease. Sodium silicofluoride was tested because it had been reported to be the most efficient of the fluorine compounds for curing or preserving hides.[3]

The vesicular virucidal virus was of the New Jersey type and was obtained from artificially infected cattle and horses. The lesions from these animals were dried, sealed in vacuo, and held at 4° C.

[3] Test made at the suggestion of C. E. Senseman, Industrial Farm Products Research Division, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

This dried virus was inoculated on scarified metatarsal pads of guinea pigs. Only freshly harvested virus from guinea pigs with high temperatures at 24 to 28 hours and good lesions at 48 hours after inoculation was used in the experiments. The use of a virus after only a single passage through guinea pigs eliminated as much as possible any changes in virulence or structure of the virus that might occur from continued passage in laboratory animals or live-tissue culture.

The tests included two methods of determining the virucidal action of the disinfectants: Use of (1) infected guinea pig pads placed with calfskin in the various soaks, and (2) calfskin artificially impregnated with the virus and placed in the various soaks. In each method, soak solutions were prepared of sodium bifluoride and sodium silicofluoride. Untreated tap water from a well was used as a control. The tests were repeated twice and identical results were obtained unless otherwise stated. The entire experiment was carried out at room temperature (20° to 24° C.).

In the first method, sodium bifluoride and sodium silicofluoride were dissolved in tap water in dilutions of 1 to 5,000, 1 to 10,000, and 1 to 20,000. The three dilutions for each of the two chemicals were placed in hard rubber vats, which were used instead of glass jars to eliminate any possibility of chemical reaction between the fluorine and the elements in glass. During the soaking periods, the vats were covered to prevent the admittance of light, thus eliminating any possible harmful effect on the virus. Hydrogen-ion determinations were made on each dilution before soaking and after 24 and 48 hours of soaking. Infected metatarsal pads from 6 guinea pigs and 10 pieces weighing 50 gm. each of salt-cured calfskin were placed in 2,500 gm. of each dilution of test solution and held at room temperature for 24- and 48-hour periods. At the end of each holding period, half of the guinea pig pads were removed from each dilution of test solution, washed in 0.85-percent saline solution, and ground in a sterile mortar. Then an 0.85-percent saline solution was added in sufficient quantity to make a 10-percent suspension. These suspensions were shaken thoroughly and then allowed to settle. The supernatant fluid from each suspension was inoculated on scarified metatarsal pads of guinea pigs; all pads were covered with sterile gauze for 24 hours. Temperatures were taken on the guinea pigs at 24 to 28 hours after inoculation, and the metatarsal pads were examined for lesions after 48 hours. These guinea pigs were saved for 13 to 21 days and then reinoculated with fresh virus to determine whether any immunity had been acquired as well as to eliminate the possibility of false clinical manifestations that may have been caused by the test solutions. In addition to the control experiments involving the placing of affected guinea pig pads in tap water, control experiments were also carried out with normal guinea pigs to determine the virulence of the virus used in all the soak solutions as well as that used to test the animals for immunity.

In the second method, conditions were the same as in the first except that 1 gm. of infected pads from guinea pigs was ground in a mortar with 5 cc. of 0.85-percent saline solution, and 0.25 cc. of this suspension of virus was then injected intradermically into circular pieces of calfskin, each 1 inch in diameter and weighing approximately 1 gm. Six inoculated pieces were placed between 50-gm. pieces of calfskin in each of the dilutions of sodium bifluoride and sodium sili-

cofluoride. The proportion of the 50-gm. pieces of skin to soak solution was 1 to 5 by weight as in the first method. Half of the impregnated circular pieces of calfskin were removed from the test solutions at 24 hours and the remaining half at 48 hours. In each instance they were washed in 0.85-percent saline solution, cut into very small pieces with sterile scissors, placed in a mortar, and ground to a pulpy consistency. This material was inoculated on guinea pig pads, the remainder of the procedure being the same as described in the first method. The purpose of this type of experiment was to determine the penetrating ability of the soak solution and to duplicate naturally infected skins as nearly as possible.

RESULTS

The results obtained by the first method are shown in table 1. The sodium bifluoride, in the three dilutions used, destroyed the virus of vesicular stomatitis in the guinea pig pads after 24 hours of soaking. Sodium silicofluoride, in dilutions of 1 to 10,000 and 1 to 5,000, destroyed the virus in 24 hours. The 1 to 20,000 dilution killed the virus only after 48 hours of soaking. The virus was still viable after 48 hours of soaking in tap water at room temperature. Results with the normal control guinea pigs demonstrated the virulence of the virus used in all the soak solutions as well as that used to test guinea pigs for immunity.

TABLE 1.—*Effect of sodium bifluoride and sodium silicofluoride on virus of vesicular stomatitis in guinea pig pads, in the presence of salt-cured calfskins, 1939*

Disinfectant		Length of soak	Guinea pig No.	Guinea pig data [1] on—					
				Test of virulence				Test of immunity [2]		
Kind	Dilution			Date of inoculation	Temperature at 24 hours	Lesions, at 48 hours, on—		Temperature at 24 hours	Lesions, at 48 hours, on—	
						Left pad	Right pad		Left pad	Right pad
		Hours			°F.			°F.		
Sodium bifluoride [3]	1- 5,000	24	664	Oct. 10	101.8	o	o	104.6	d	d
	1-10,000		670		101.7	o	o	105.4	d	d
	1-20,000		667		103.2	o	o	105.0	d	d
	1- 5,000	48	825	Oct. 11	103.2	o	o	105.4	c	d
	1-10,000		816		101.6	o	o	105.6	d	d
	1-20,000		818		102.2	o	o	105.9	d	d
Sodium silicofluoride [3]	1- 5,000	24	663	Oct. 10	102.5	o	o	105.6	d	d
	1-10,000		699		101.8	o	o	105.3	d	d
	1-20,000		674		102.9	a	o	104.6	b	d
	1- 5,000	48	822	Oct. 11	102.0	o	o	105.3	d	d
	1-10,000		801		102.4	o	o	105.1	d	d
	1-20,000		815		103.0	o	o	106.1	d	d
Tap water (controls) [4]		24	1078	Oct. 10	105.2	d	d	101.6	o	o
			1087		105.1	d	d	102.4	o	o
			1092		105.0	d	d	102.1	o	o
			1098		105.8	d	d	101.5	o	o
		48	1084	Oct. 11	106.0	d	d	103.0	o	o
			1089		106.0	d	d	102.1	o	o
			1090		105.8	d	d	102.5	o	o
			1093		106.4	d	d	102.6	o	o
None (normal controls)			1	Oct. 9	105.4	d	d			
			2		105.1	d	d			
			3		105.6	d	d			
			4		106.0	d	d			
			5					106.2	d	d
			6					105.0	d	d
			7					105.2	d	d
			8					105.7	d	d

[1] Key: o, none; a, slight (swelling and tenderness); b, moderate (swelling and tenderness plus a few small vesicles along the hair line); c, good (pad loosened, tenderness plus incomplete vesicle formation around and under entire pad); d, severe (pad loosened, complete vesicle formation under and around pad and sloughing).

[2] Fresh virus of vesicular stomatitis from guinea pig pads was used; date of inoculation in all instances, Oct. 28,1939.

[3] 12 affected guinea pig pads used in each dilution of the soak solution.

[4] 12 affected guinea pig pads used.

The results obtained by the second method are shown in table 2. With sodium bifluoride, in dilutions of 1 to 10,000 and 1 to 5,000, the virus was destroyed after 24 hours of soaking. In the 1 to 20,000 dilution, the virus was destroyed in 50 percent of the cases in 24 hours, but the destruction of virus was complete only after 48 hours of soaking.

With sodium silicofluoride, 1 to 10,000 and lower dilutions destroyed the virus in 24 hours, whereas the 1 to 20,000 dilution completely destroyed the virus in only 50 percent of the cases after 48 hours of soaking.

TABLE 2.—*Effect of sodium bifluoride and sodium silicofluoride on virus of vesicular stomatitis inoculated into salt-cured calfskins, 1939*

Disinfectant				Guinea pig data[1] on—						
				Test of virulence				Test of immunity[2]		
Kind	Dilution	Length of soak	Guinea pig No	Date of inoculation	Temperature at 24 hours	Lesions, at 48 hours, on—		Temperature at 24 hours	Lesions, at 48 hours, on—	
						Left pad	Right pad		Left pad	Right pad
		Hours			°*F.*			°*F.*		
Sodium bifluoride[3]	1- 5,000	24	975	Oct. 18	102.0	o	o	104.4	d	d
	1- 5,000		755		102.7	o	o	105.1	d	d
	1-10,000		974		102.9	o	o	105.4	d	d
	1-10,000		874		103.0	o	o	104.8	d	d
	1-20,000		858		103.4	o	o	105.0	d	d
	1-20,000		964		103.6	b	a	103.0	a	a
	1- 5,000	48	948	Oct 19	102.8	o	o	104 4	d	d
	1- 5,000		932		102.7	o	o	105.5	c	c
	1-10,000		968		102.0	o	o	105.3	d	d
	1-10,000		788		102.0	o	o	104.8	d	d
	1-20,000		970		102.0	o	o	105.1	d	d
	1-20,000		928		103.0	o	o	105.0	d	d
Sodium silicofluoride.[3]	1- 5,000	24	865	Oct. 18	102.4	o	o	104.7	d	d
	1- 5,000		867		103.1	o	o	104.4	d	d
	1-10,000		871		103.0	o	o	103.8	c	c
	1-10,000		866		102.6	o	o	104.6	d	d
	1-20,000		851		102.8	o	a	104.6	d	c
	1-20,000		870		103.3	a	o	102.6	b	b
	1- 5,000	48	936	Oct. 19	101.7	o	o	104.5	d	d
	1- 5,000		937		101.9	o	o	105.8	d	d
	1-10,000		942		103.0	o	o	105.3	d	d
	1-10,000		945		102.5	o	o	104.8	d	d
	1-20,000		927		103.0	o	a	103.6	b	a
	1-20,000		949		102.8	o	o	104.6	d	d
Tap water (controls)[4]		24	1082	Oct. 18	105.2	d	d	101.8	o	o
			943		105.5	d	d	102.4	o	o
			1097		104.8	d	d	102.2	o	o
			1069		106.2	d	d	101.4	o	o
		48	1001	Oct. 19	104.5	d	d	102.0	o	o
			1071		105.6	d	d	101.8	o	o
			1076		105.0	d	d	102.6	o	o
			1080		104.8	d	d	102.2	o	o
None (normal controls)			9	Oct. 17	105.2	d	d			
			10		105.0	d	d			
			11		105.6	d	d			
			12		105.4	d	d			
			956					105.1	d	d
			910					104.9	d	d
			955					105.4	d	d
			777					104.6	d	d

[1] Key: o, none; a, slight (swelling and tenderness); b, moderate (swelling and tenderness plus a few small vesicles along the hair line); c, good (pad loosened, tenderness plus incomplete vesicle formation around and under entire pad); d, severe (pad loosened, complete vesicle formation under and around pad and sloughing).

[2] Fresh virus of vesicular stomatitis from guinea pig pads was used; date of inoculation in all instances, Oct. 31, 1939.

[3] 6 1-gm. pieces of calfskin, each injected with 0.25 cc. of affected guinea-pig-pad emulsion, used in each dilution of soak solution.

[4] 6 1-gm. pieces of calfskin, each injected with 0.25 cc. of affected guinea-pig-pad emulsion, used.

The virus was still viable after 48 hours of soaking in tap water. Similar results were obtained with the control guinea pigs as in the first method.

The hydrogen-ion concentration of tap water and the various solutions is shown in table 3. All the soaks tended to be less acid with increase in length of time, but none of the solutions reached alkalinity. However, the virucidal action of these solutions was not comparable with the degree of acidity as sodium silicofluoride in the comparative dilutions had in almost every instance a higher acidity than sodium bifluoride, whereas sodium bifluoride, in the virulence tests, was the more virucidal.

TABLE 3.—*Average hydrogen-ion concentration of the soak solutions at various periods in all experiments*

Soak solution	pH value—		
	Before soaking	After 24 hours	After 48 hours
Sodium bifluoride:			
1–5,000	3.7	4.7	5.4
1–10,000	3.75	5.6	5.9
1–20,000	3.8	6.0	6.2
Sodium silicofluoride:			
1–5,000	3.5	4.6	5.0
1–10,000	3.55	5.2	5.7
1–20,000	3.6	5.7	6.2
Tap water	6.6	6.85	7.2

DISCUSSION

Results obtained from the studies indicate that both sodium bifluoride and sodium silicofluoride are virucidal to the virus of vesicular stomatitis. Sodium bifluoride, in a dilution of 1 to 20,000, was slightly more effective than sodium silicofluoride under like conditions.

A longer time was required for the higher dilutions of the disinfectants to kill the virus injected intradermically in hides than to kill the virus in the guinea pig pads. This fact was due, no doubt, to the extra time required by the disinfectants to penetrate the skin before coming in contact with the virus. After the skins had been in the various soak solutions for 24 to 48 hours, a greater thickness was noted in the skins soaked in the two disinfectant solutions than in those soaked in tap water.

It appears that the sodium chloride in the cured skins had little influence on the effectiveness of sodium bifluoride or sodium silicofluoride, as its presence on the skins in tap water failed to affect the virulence of the virus.

The importance of sodium bifluoride and sodium silicofluoride for use in hide and skin disinfection depends on their effectiveness against foot-and-mouth disease virus. The present experiments showed that these disinfectants destroyed vesicular stomatitis virus after 24 hours of soaking in dilutions of 1 to 10,000. The British Foot-and-Mouth Disease Research Committee in its fourth report (*5*) states that a dilution of 1 to 20,000 of sodium bifluoride killed foot-and-mouth disease virus in 2 hours, and O'Flaherty and Doherty (*7*) found that

a dilution of 1 to 10,000 of sodium bifluoride destroyed vesicular stomatitis virus after 24 hours.

From these data it may be assumed that the use of either sodium bifluoride or sodium silicofluoride, in a solution of 1 to 10,000 for 24 hours at room temperature, would be effective in the disinfection of hides or skins infected with foot-and-mouth disease when the ratio of hide or skin to soak solution is 1 to 5 by weight.

SUMMARY AND CONCLUSION

The efficiency of sodium bifluoride and sodium silicofluoride as hide disinfectants was studied, the virus of vesicular stomatitis being used as the contaminant. This work was carried on at the United States Department of Agriculture Animal Disease Station, Beltsville, Md., in 1939.

Two methods of approach were used: (1) Infected guinea-pig pads soaked for 24 and 48 hours in solutions of 1 to 5,000, 1 to 10,000, and 1 to 20,000 of the two fluorine compounds, in the presence of salt-cured calfskins; (2) sections of salt-cured calfskin injected intradermically with vesicular stomatitis virus in the aforementioned soak solutions. In both types of experiments, tap water was used as a control. The proportion of salt-cured skin to the quantity of soak solution in all experiments was 1 to 5 by weight. All experiments were conducted at room temperature.

The results were as follows:

In the first experiment, sodium bifluoride killed the virus in guinea-pig pads in all three dilutions in 24 hours. Sodium silicofluoride killed the virus in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours and in all dilutions in 48 hours.

In the second experiment, sodium bifluoride killed the virus in artificially inoculated calfskins in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours and in all dilutions in 48 hours. Sodium silicofluoride killed the virus in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours but was not completely effective in the dilution of 1 to 20,000 in 48 hours.

Tap water did not affect the virus in either guinea-pig pads or calfskins after 24 or 48 hours of soaking.

The hydrogen-ion concentration of the various soak solutions was determined at the beginning and after 24 and 48 hours of soaking. Sodium silicofluoride, at equal dilutions, had a higher hydrogen-ion concentration than sodium bifluoride, both decreasing with length of time. However, a high hydrogen-ion concentration does not necessarily indicate greater virucidal powers. The hydrogen-ion concentration of tap water also decreased with length of time.

By analogy with similar research by the British Foot-and-Mouth Disease Research Committee, it is a logical assumption that sodium bifluoride and sodium silicofluoride are also effective in destroying the virus of foot-and-mouth disease.

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