THE DISINFECTION OF ANTHRAX-INFECTED DRIED HIDES IN THE DRY CONDITION BY MEANS OF HYDROGEN SULPHIDE.

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Work on the disinfection of anthrax-infected dried hides has been going on for some three years in the laboratories of the British Leather Manufacturers' Research Association at the Lister Institute. Two methods of disinfection have had to be considered:

(1) "Wet"—suitable for use in tanneries in the first stage of the conversion of hides into leather.

(2) "Dry"—such as might be applied to hides in the dry condition before they reach the tanneries.

As the problem of "wet" disinfection seemed more hopeful of solution than that of "dry," and wet methods the more likely to be capable of immediate practical application, most attention was given at first to the subject of disinfection in the tanneries. Modifications of the ordinary liming process (the first stage in the conversion of hides into leather), based on the known toxicity of sodium sulphide towards anthrax, involving certain conditions of temperature and proportion of sodium sulphide in the lime liquors, have been devised, by means of which anthrax disinfection has been secured in both laboratory and large-scale tannery work and the disinfected hides have been in good condition for leather making. This work has already been reported (Jordan Lloyd, Marriott and Robertson, 1930; Jordan Lloyd and Robertson, 1930), and is being continued.

Although a satisfactory method of disinfection of anthrax-infected hides during the liming process will greatly reduce the risk of anthrax in tanneries, danger to those handling such hides in the dry state, both before and after their arrival at the tannery, is not by this means eliminated. Recent work has, therefore, been directed towards discovering a method of disinfection that could be applied to hides in the dry state.

While seeking to determine why anthrax tends to die out in putrefying carcases, Andrjewski (1928) concluded that the principal anthrax-destructive agent at work was the hydrogen sulphide evolved during the process of putrefaction.

This work and the efficacy of sodium sulphide in anthrax disinfection already demonstrated in "wet" disinfection experiments suggested the possi-

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bility of utilising hydrogen sulphide as a disinfecting agent for dried hides, and a number of experiments with it, using both anthrax-infected threads and dried hides as test material, have been carried out with promising results. These are described below.

Experimental methods.

(*a*) *Test material used.*

(α) *Anthrax-infected threads.* Sterile surgical silk threads, steeped from 1 to 3 days in a heavy emulsion of anthrax spores, of a strain sufficiently resistant to withstand boiling for 4 min., dried in a desiccator over $CaCl_2$ (not longer than 12 days) and stored in sterile bottles at room temperature until required for use. (In none of the experiments to be described had the threads been kept for more than 3 months; in most they had been kept for a month to 6 weeks.)

(β) *Dried china hide naturally infected with anthrax.* (Proved to be infected by guinea-pig inoculation.)

(*b*) *Methods of dealing with the specimens after treatment.*

The threads, after treatment with the hydrogen sulphide by the methods described below (*c*), were steeped in sterile water, laid on agar Petri plates and incubated at 37° C., control threads from the same batch steeped similarly in sterile water being incubated on the same plates.

The preparation of a suspension for inoculation from the hide specimens was the same in every case, whether control or treated specimen, except that the "control" suspensions were heated to 70° C. in a water bath for 15 min. to destroy any of the ordinary pathogenic non-sporing organisms that might be present, whereas the treated specimens were not. The samples were steeped for 2–18 hours in sterile water (15 c.c. to 1 grm. of hide), cut into small pieces and rubbed up with sand in a mortar. The steeping water was then added and well mixed with the hide and sand, and the resulting suspension pipetted into a centrifuge tube. The control specimens were heated at 70° C. for 15 min. in a water bath. Finally, all specimens were centrifuged. Most of the supernatant fluid was then pipetted off and the residuum used for guinea-pig inoculation.

All the guinea-pigs used were given anti-gas-gangrene serum 24 hours before the test inoculation in order to defend them as far as possible against organisms of the gas-gangrene group (not so susceptible as anthrax to the action of sulphides) with which most dried hides are infected and which were liable to confuse the issue of experiments by causing the death of the experimental animals from gas gangrene.

(*c*) *Methods of disinfection employed.*

After one or two preliminary trials, the use of glass jars adapted for the growth of anaerobic organisms was decided upon; air was drawn off from the jar as completely as possible by means of a filter pump and hydrogen sulphide

then run in from a Kipp's apparatus, first being passed through 1 per cent. sodium hydroxide to remove any strong acid that might be present. This technique was adopted simply as a convenient method of bringing the hydrogen sulphide into contact with the experimental material. It was also considered that the method might be serviceable in ensuring rapid penetration of the disinfecting gas into the tightly packed bales in which dry hides are usually imported. The object was not to ensure anaerobic conditions. This point is discussed below (see also Table VIII).

EXPERIMENTS.

(*a*) *The disinfection of anthrax-infected threads in Petri dishes.*

Exp. 1. Anthrax-infected threads (3 months old), contained in a Petri dish, were placed in the disinfecting jar and exposed to hydrogen sulphide as above described. Of four threads exposed for 4 days at 20° C., only one showed a small growth after 48 hours' incubation at 37° C. Four control threads not exposed to hydrogen sulphide all showed a heavy growth of anthrax. This experiment shows that hydrogen sulphide exerts a definite destructive effect on anthrax spores.

(*b*) *The disinfection of anthrax-infected hide pieces in Petri dishes or cotton-wool stoppered bottles.*

Exp. 2. In view of the results of Exp. 1, further experiments were undertaken with pieces of infected hide placed in Petri dishes or cotton-wool stoppered bottles. The pieces weighed 1·52 grm. The hide sample from which they were cut had been wrapped in a damp cloth overnight to facilitate cutting and the cut pieces were still damp when used. The disinfection procedure was the same as that employed in Exp. 1.

The results of this experiment are given in Table I.

Disinfection having apparently been satisfactory in the case of damp and rather soft test-hide pieces, the next experiment was designed to see whether equally good results could be secured with extremely dry and hard test-hide pieces. The specimens used were from the same hide as that used in Exp. 2, but had been dried over $CaCl_2$ for $4\frac{1}{2}$ months.

Exp. 3. In this experiment the disinfecting period was extended from 4 to 10 days and the disinfection results were excellent. This experiment is tabulated in Table II.

The hide pieces used in this experiment weighed approximately 1·2–1·5 grm. each.

(*c*) *The disinfection of anthrax-infected hide pieces in leather pockets (to test penetration of gas) and of anthrax-infected threads in a cotton-wool stoppered bottle.*

In all the experiments reported thus far, the test specimens, whether infected threads or hide pieces, had been kept in the disinfecting jars either in Petri dishes or wide-mouthed cotton-wool stoppered bottles, easily accessible to the disinfecting gas.

The next point to be investigated was whether the hydrogen sulphide in experimental conditions such as those described in Exps. 1–3 would have much power of penetration, and the experiments now to be described were designed to test this and to study also the influence of temperature upon the rate of disinfection.

Table I.

Control exp.			No. of guinea-pigs inoculated	Inoculation dose (c.c.)	Results
Hide 18 (anthrax-infected dried hide) *A* *B*	No disinfection		2	1·5	*A*. Guinea-pig died 3rd day, typical anthrax. *B*. Guinea-pig died 2nd day. Post-mortem appearances, mixed infection, gas gangrene and anthrax. Anthrax isolated in culture from site of inoculation
Specimens	No. of days' disinfection	Temperature of disinfection (° C.)	No. of guinea-pigs inoculated	Inoculation dose (c.c.)	Results
Hide 18 (1)	4	20	1	1·5	Guinea-pig survived*
,, 18 (2)	4	20	1	1·5	Guinea-pig died in 2 days. Post-mortem appearances those of gas gangrene. No anthrax obtained in cultures
,, 18 (3)	4	20	1	1·5	Guinea-pig survived

* In this case the centrifuged steeping water only was used for inoculation; in (2) and (3) the preparation for inoculation was as already described (page 368).

Table II.

Control exp.		No. of guinea-pigs inoculated	Inoculation dose (c.c.)	Result
Hide 18 *A*	No disinfection	2	1	Died in 2 days, typical anthrax
B			1·5	Died in 3 days, typical anthrax

Treated specimens	No. of days' disinfection	Temperature of disinfection (° C.)	No. of guinea-pigs inoculated	Inoculation dose (c.c.)	Result
Hide 18 (1)	10	20	2	1	All survived
,, 18 (2)	10	20	2	1	
,, 18 (3)	10	20	2	*A*, 1·5 *B*, 1	

Exp. 4. Three sets of four pockets of increasing sizes, the one to fit into the other, were made from thinnish chrome-tanned leather. A piece of anthrax-infected hide (1–1·5 grm.) was put in the smallest pocket in each series of four. The smallest pocket was then fitted inside the next size, its open end against the closed end of the second pocket and so on until a packet of four pockets was produced. This was then doubled over along its long axis and each end secured by copper wire twisted round, the resulting little bundle with its

infected hide piece in the middle providing conditions somewhat analogous to those obtaining in a bale of infected hides. The pieces of infected hide used were extremely dry and hard. The bundles were then placed in the experimental jar, along with an open vessel containing water and a cotton-wool stoppered bottle containing anthrax-infected threads. The air from the jar was exhausted by a filter pump as before and hydrogen sulphide run in from a Kipp's apparatus, passing first through 1 per cent. sodium hydroxide. The jar was then kept at 20° C. and an infected hide piece and some threads were removed after 5 days, 7 days and 10 days, the jar being exhausted and refilled with hydrogen sulphide after each opening. The hide pieces after removal were steeped overnight in fifteen times their weight of water and emulsions prepared from them for guinea-pig inoculation, as in the previous experiments. The threads were soaked for a few minutes in sterile water and incubated on agar plates.

Table III. *Disinfection of infected hide specimens in leather pockets.*

Control exp.				No. of guinea-pigs inoculated	Inoculation dose (c.c.)	Result
Hide 7 (anthrax-infected dried hide)		*A* *B*	No disinfection	2	1	Both died in 2 days. Typical anthrax
Treated specimens	Period of disinfection (days)	Temperature of disinfection (° C.)	No. of guinea-pigs inoculated	Inoculation dose (c.c.)		Result
Hide 7 (1)	5	20	2	*A*, 1·5 *B*, 1		*A* died in 3 days. Post-mortem appearances suggestive of mixed infection gas gangrene and anthrax. Anthrax recovered in culture from spleen and heart's blood. *B* survived
„ 7 (2)	7	20	2	*A*, 1·5 *B*, 1		*A* survived *B* died in 6 days, not from anthrax
„ 7 (3)	10	20	2	*A*, 1·5 *B*, 1		*A* survived *B* died in 11 days, not from anthrax

Gradual disinfection of the threads took place and, after they had been exposed for 10 days to hydrogen sulphide, no growth could be obtained from them on incubating for 48 hours on agar. A few small points of growth, however, developed on eight out of ten in the course of a further 10 days' incubation. Six control threads, not exposed to hydrogen sulphide, all showed good growth of anthrax after 24 hours' incubation.

The results obtained in the case of the hide specimens are recorded in Table III. They show that penetration of the gas appears to have taken place and that after 7 days in the hydrogen sulphide atmosphere the anthrax spores have been destroyed or at least rendered so little virulent as not to infect guinea-pigs.

(*d*) *The disinfection of anthrax-infected threads and doubly infected dry hide samples in leather pockets at varying temperatures.*

Exp. 5. As experiments on other lines with the particular hide used in Exp. 4 had shown it to possess rather slight resistance to disinfection, another set of tests was carried out in which anthrax-infected threads, resistant to 4 min. boiling, and doubly infected hide specimens were the test material. The threads were wrapped in sterile lint and placed in the innermost leather pocket of a series of four prepared as already described.

The method of disinfection was the same as before, but three different temperatures were tried, 20° C., 37° C. and 42° C. After disinfection, in this set of experiments, the threads were soaked in sterile, distilled water overnight, as it was thought that the short period of soaking given in the earlier experiments did not moisten and soften them sufficiently and growth of any organisms left alive tended to be feeble and slow.

Disinfection was very slow at 20° C.; after 32 days in hydrogen sulphide at this temperature two out of four threads were not entirely cleared of anthrax, but after 14 days at 37° C., four out of five, and after 10 days at 42° C., one out of four threads were sterilised, whilst the remainder showed only a few very small points of growth after prolonged incubation. The control threads (2) both gave good growth of anthrax after 24 hours' incubation. The expansion of the disinfecting gas at temperatures of 37° and 42° C., with consequent increase of pressure in the experimental jar leading to better penetration of the leather pockets, would probably be a factor in the increased rapidity of disinfection at the higher temperatures.

Exp. 6. One more confirmatory test of this method of disinfection on sample hide pieces was carried out, and in order to make it as drastic and conclusive as possible, the hide samples used (naturally infected hide tested by guinea-pig inoculation) were steeped for half an hour in a heavy emulsion of virulent anthrax spores, dried for 10 days over calcium chloride and then kept for a further 3 days in ordinary atmospheric conditions. They were hard and dry when used for the experiment. Specimen pieces thus prepared were placed in the innermost of a series of four chrome-leather pockets, fitted one inside the other as in former experiments. The resulting packet was then rolled up and tied at each end. A further covering of thick but rather porous vegetable-tanned leather was then rolled round it and tied tightly at the ends and in the middle. The packet thus formed was placed in an experimental jar, the air exhausted by a filter pump and hydrogen sulphide run in as before. The jar was kept at 37° C. for 16 days. The results are shown in Table IV.

(*e*) *A comparison of the effect on anthrax spores of atmospheres of hydrogen sulphide and hydrogen.*

Exp. 7. As *B. anthracis* is a facultative anaerobe, it was realised that the disinfection in the experiments described was not likely to be due purely to lack of oxygen, but in the next experiment this point is made clear. Anthrax-

infected threads, of a different batch but infected in the same way and by the same anthrax strain as before, were completely disinfected in 8 days at 37° C. in an atmosphere of hydrogen sulphide, whereas similar threads in an atmosphere of hydrogen were still highly infected after 16 days at 37° C.

(*f*) *The effect on anthrax spores of a mixture of hydrogen sulphide and air.*

Exp. 8. The effect of hydrogen sulphide in the undiluted state having been demonstrated by the foregoing experiments, the result of diluting the gas with an equal quantity of air was next tried. The anaerobic jar used in disinfection was evacuated to half atmospheric pressure and hydrogen sulphide then run in from a Kipp's apparatus as before. The threads used were from the same batch as those in Exp. 7 and disinfection was carried out at temperatures of 37 and 60° C.

Table IV. *Disinfection of dry anthrax-infected hide specimens by hydrogen sulphide in* 16 *days at* 37° C.

Control exp.			No. of guinea-pigs inoculated	Inoculation dose (c.c.)	Result
Hide 3—naturally and artificially infected	No disinfection		4	*A*, 1·5 *B*, 1 *C*, 1 *D*, 0·5	*A* died in 3 days, typical anthrax. *B* and *C* died in 4 days, typical anthrax. *D* died in 5 days, typical anthrax
Treated specimens	Period of disinfection (days)	Temperature of disinfection (° C.)	No. of guinea-pigs inoculated	Inoculation dose (c.c.)	Results
Hide 3, as above	16	37	4	*A*, 1·5 *B*, 1·5 *C*, 1 *D*, 1	All survived

The threads were tested for growth at the end of 3 and 10 days. Sterilisation was complete after 3 days at 60° C. and after 10 days at 37° C.

A certain number of China hide samples treated for 16 days at 37° C. in an atmosphere of hydrogen sulphide and afterwards limed in a sulphide-lime liquor (1 per cent. $Na_2S.9H_2O$ in 1·2 per cent. CaO) have been examined microscopically and do not seem to have suffered any damage. More work on this point is being carried out, and the effect on dried hide specimens highly infected with anthrax of mixtures of hydrogen sulphide and air at different temperatures is also being investigated.

DISCUSSION.

The results obtained by the "dry" method of anthrax disinfection described confirm the conclusions reached in earlier "wet" disinfection work with sodium sulphide as to the toxicity of sulphides for anthrax spores. The minimum amount of hydrogen sulphide in the atmosphere necessary to destroy dry anthrax spores on hides has yet to be determined and work thereon is in

progress, but that about 50 per cent. would be sufficient is indicated by the results of thread Exp. 8 (see p. 373). More rapid penetration of the disinfecting gas into bales of hides would perhaps be secured by exhaustion of the air from the disinfecting chamber before running in the hydrogen sulphide than by merely displacing the air by hydrogen sulphide, but it would seem that good diffusion of the gas can be secured by either method.

Rapidity and completeness of penetration could be assisted by raising the temperature of the disinfecting chamber and so increasing the volume of gas present. The relation of temperature to the rate of disinfection is under further investigation.

Whether the disinfective gas actually penetrates into dried hides is doubtful, but if even good surface disinfection could be secured by means of hydrogen sulphide, the risk in handling dry hides would be greatly diminished, and if they were afterwards subjected to a suitable sulphide-lime wet disinfection process in the tanneries, danger to tannery workers would be practically eliminated.

Summary.

1. Hydrogen sulphide has been found to have a destructive action on dry anthrax spores.

2. Disinfection of hard, dry, very resistant infected hide specimens, in conditions both of ease and difficulty of access of the disinfecting gas, has been obtained by treatment with hydrogen sulphide at 20° and at 37° C. in periods of 7–16 days.

3. The fact that hydrogen sulphide, in the experimental conditions described, penetrates to the middle of a bundle of leather pockets has been verified by its action in disinfecting (sometimes partially—sometimes completely) anthrax-infected threads placed in the middle of such a bundle.

4. Increase of the temperature at which treatment is carried out has been found to hasten disinfection.

5. Disinfection of anthrax-infected threads has been obtained by treatment with equal parts of hydrogen sulphide and air in 3 days at 60° C.

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