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THE S I S  
THE ACTION OF WHITEWASH  
UPON BACTERIA

J. W. RIGTERINK

1897.



THESIS  
ON  
THE ACTION OF WHITEMUSH UPON BACTERIA.

By Jno. W. Rietorink.

Michigan Agricultural College, 1897.

**THESIS**

## TITLES

### THE ACTION OF WHITEWASH UPON BACTERIA.

The object of this investigation was to determine, if possible, whether whitewash ( $\text{Ca}(\text{OH})_2$ ) possesses any disinfecting or germicidal properties. Whitewash was prepared by placing a quantity of calcium oxide ( $\text{CaO}$ ) in an Esmarch dish. This was then thoroughly slacked by direct application of boiling water which had been previously sterilized. The object of using boiling water was to facilitate the slacking of the lime. Calcium oxide brought in contact with water forms calcium hydrate ( $\text{Ca}(\text{OH})_2$ ). Calcium hydrate exposed to air will take up carbon dioxide ( $\text{CO}_2$ ) and change to calcium carbonate ( $\text{CaCO}_3$ ). But in all the following experiments whitewash ( $\text{Ca}(\text{OH})_2$ ) was used before any change to a carbonate had taken place.

A number of cover-glasses were cut into halves and sterilized in a hot-air oven at 150 °C. for one hour. Also white silk thread was cut into sections of one inch each and sterilized as above. A bouillon culture was made from a gelatin culture of *Bacillus Anthracis*. This was placed in an incubator for twenty-four hours. A hanging drop from the culture revealed the vegetative form of *Bacillus Anthracis*. No spore form of the above bacillus was observed.

Thirty of the sterilized cover glasses were now coated with an even film of this culture, leaving the extreme margins of the cover glasses free from germs. This was done in order to make sure that the entire

film could be covered when whitewash was applied. These were allowed to dry, and then, as nearly as possible, an even coat of whitewash was applied to the entire surface of the cover glasses by means of a glass rod having a rounded end. Thirty cover glasses were given a coating of the whitewash only to serve as controls.

Also thirty of the sterilized silk threads were thoroughly soaked in the Bouillon culture, and after being partly dried, they were thoroughly covered with whitewash. This was done by placing the threads in the Esmarch dish and covering them completely with whitewash, after which each thread was removed separately by means of sterilized forceps. Threads for control were prepared in a similar manner using whitewash only.

The whitewash formed an adhesive film on the cover glass, and in order to overcome the possibility of keeping the germ in confinement, one-half of all the cover glasses used had the white wash carefully removed at the time they were put into a bouillon tube. This was done by holding the cover glass with sterilized forceps over the mouth of the bouillon tube, and scraping the whitewash away with another sterilized forceps. Care was taken to get the entire contents into the tube. The cover glasses and controls were prepared on March 11th. The following table shows the result of treating the vegetative form of anthrax with whitewash. The object of inoculating tubes at different times in the following experiment was to see whether whitewash would produce any different result in varying lengths of time it was applied to germs.

Time of inoculation  
of Bouillon tube.

Mar.12. Mar.13. Mar.15. Mar.17. Mar.24. Total.

No. of tubes inoculated

with coverglasses 2 2 2dx 2ex 2 10

undisturbed.

Result. - - - - -

Number of controls. 2 2 1 1 1 5

Result. N o G r o w t h -----

No. of tubes inoculated

with line scraped off 2ax 2bx 2 2 2xb 10

coverglasses.

Result. - - - - -

Number of controls. 2 2ex 1 1 1 5

Result. No growth. Contam- N o G r o w t h .  
inated.

x indicates growth of some kind. With the exception of the controls,  
each of the above tubes was plated.

ax Contaminated in one tube, due to moulds.

bx " " " " " " micrococci.

cx " " " " " " a short bacillus.

dx " " " " " " micrococci.

ex " " " " " " moulds.

Number of coverglasses treated with Bacillus Anthracis 20.

" " contaminations of above 4.

" " controls treated with whitewash only 10

" " contaminations of above. 1.

Time of inoculation of Bouillon tube.	Mar.12.	Mar.13.	Mar.15.	Mar.17.	Total
No. of sections of thread with germs.	4	3	2	2	11
Result.	-	-	-	-	-
Number of controls.	2	2	2	2	8
Result.	W o G r o w t h .				

All of the tubes were kept in the incubator for ten days before examination was made. With the exception of the controls, plates were made from each tube in the above tables.

Result: Whitewash prevented the growth of the vegetative <sup>form</sup> of anthrax in thirty-one experiments, where whitewash was brought in direct contact.

It was determined to see whether whitewash would prevent the growth of the vegetative form of Bacillus Anthracis if added to a bouillon tube that was inoculated with the above bacillus instead of bringing the whitewash in direct contact with the bacillus. Five bouillon tubes were inoculated with the vegetative form of Bacillus Anthracis, to which were added respectively, one, two, three, four, and five drops, each dropful being about the size of a grain of wheat. These were placed in the incubator for ten days. Plates made from these cultures revealed typical anthrax colonies in each case.

The next part of the investigation was to determine whether whitewash would kill the spore forms of Bacillus Anthracis. A number of cover slips or glass threads with their controls were prepared in the same manner in the case of the vegetative form. The material used in this



case was taken from an agar culture to which has been added a drop of calcium hypochlorite which is supposed to facilitate the growth of spores. A hanging drop made from this culture revealed abundant spores. The coverglasses and their controls were made on March 22d; the threads on March 24th. The following table indicates the result.

Time of inoculation of Bacillus tube.	Mar. 23.	Mar. 25.	Mar. 27.	Apr. 1.	May 4.	Total.
No. of tubes inoculated						
with coverglasses	2	2	2	2	2	10
indicator of.						
Result.	x	x	x	x	x	
No. of controls.	1	1ax	1	1	1dx	5
Result.	No growth. Contam. No growth -- Contaminated.					
No. of tubes inoculated						
with line scraped off	2	2	2	2	2	10
cover glasses.						
Result.	x	x	x	x	x	
Number of controls.	1	1xb	1	1cx	1ex	5
Result.	No growth. Contamin- No growth. Contaminated. sted					

Plates were made of each of the above tubes, not including the controls. Five of the controls (ax, bx, cx, dx, ex) were contaminated. Contamination of the controls was due in each instance to some short bacillus. The plates revealed typical anthrax colonies. Hanging drops made from these colonies, further revealed typical Bacilli Anthracis. The next table indicates work with sections of threads.

Time of inoculation

of Rouillon tube.	Mar.24.	Mar.25.	Mar.27.	Apr.21.	May 4.
No. of sections of thread with germs.	4	2	2x	2	2
Result.	x	x	?	x	x
Control.	2	2	2	2	2
Result.	Contaminated. No growth.				

Plates of these cultures show typical anthrax colonies, with the exception of the cultures made on March 27th. These cultures were contaminated by some actively motile bacillus which liquified gelatin before any anthrax colonies appeared. A hanging drop did not reveal any anthrax bacilli. The contamination of the controls was due to some actively motile bacillus.

Result: Thirty cultures gave positive result of growth of anthrax. In two cultures, growth of anthrax was not established.

#### Experiment with Bacillus Tuberculosis.

On April 4th a number of silk threads were infected by bringing them in contact with the tuberculous material. The material was taken from a guinea pig having tuberculosis. An incision was made into the infected glands, one end of the thread was held by sterilized forceps, and then by means of rotation, every part of the thread was brought into direct contact with the material. It had been previously established by microscopical examination that the tubercle bacillus was present.

The threads were then treated with whitewash in the usual manner.

A number of threads were not treated with whitewash to serve as controls. On April 22d, two guinea-pigs were inoculated on the inside of the thigh with threads treated with whitewash. A third guinea pig was inoculated in a similar manner with a control thread. These pigs were weighed every week.

	1st week.	2d week.	3d week.	4th week.	5th week.	6thw.
Guinea-pig #1	609 <i>grams</i>	577 <i>grams</i>	614 "	592 "	634 "	630 "
" " #2	536 "	572 "	593 "	572 "	537 "	613 "
" " #3	630 "	673 "	722 "	717 "	716 "	730 "

On June 3d they were killed and examined. Not a trace of tuberculosis was discovered. The three guinea-pigs were apparently perfectly healthy. It is a difficult matter to account for such a result. There is a possibility that none of the threads used had any tubercle bacilli present. It may also be possible that the tubercle bacillus was destroyed by use of whitewash, and that it was not present in the control.

#### Experiments with Bacillus of Hog Cholera.

Cover glasses and threads with their controls were made from a bouillon culture 24 hours old in the usual manner.

Time of inoculation of Bouillon tube.	Apr.23.	Apr.30.	May 3.	May 5.	May 20.				
No. of tubes inoculated with cover glasses undisturbed.	2	2	2	2	4				
Result.	-	-	-	-	-				
Number of controls.	1	1	1	1	2				
Results.	N	O	G	r	o	w	t	h	.
No. of cover glasses with lime removed.	2	2	2	2	4				
Result.	-	-	-	-	-				
Control.	1	1	1	1	2				
Result.	N o G r o w t h. ---- Contaminated.No growth								

Time of inoculation of Bouillon tube.	Apr.27.	Apr.29.	May 3.	May 5.	May 20.				
No. of sections of thread with germ.	2	2	2	2	4				
Result.	-	-	-	-	-				
Control.	1	1	1	1	1				
Result.	N	O	G	r	o	w	t	h	.-

These cultures were allowed to remain in the incubator for ten days. No growth could be discovered, and in the place of plating, new bouillon cultures were made from each tube. Of the thirty-six cultures not a single one had growth. Of the seventeen controls, one was contaminated. It was thought that the Bacillus of Hog Cholera might be more highly resistant if taken from an old culture. To determine this, cover glasses and threads were prepared from a culture thirteen days old. These were

used on May 5th and 20th in the above experiment. The result did not differ from the tubes inoculated with cover glasses and threads prepared from the culture twenty-four hours old.

#### Experiment with *Staphylococcus Pyogenes Aureus*.

Cover glasses and threads with their controls were made from a bouillon culture twenty-four hours old in the usual manner.

Time of inoculation of Bouillon tube.	May 10.	May 11.	May 12.	May 20.
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No. of tubes inoculated with cover glasses undisturbed.	2	2	2	5
---	---	---	---	---

Result.	-	-	x	-
---------	---	---	---	---

Number of control.	1	1	1	3
--------------------	---	---	---	---

Result.	N	o	G	r	o	w	t	h	.
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No. of tubes inoculated with line of cover - glasses <sup>line</sup> removed.	2	2	2	5ax
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Result.	-	-	-	-
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Number of Controls.	1	1	1	2
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Result.	N	o	G	r	o	w	t	h	.
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Time of inoculation of Bouillon tube.	May 10.	May 11.	May. 12.	May 20.
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No. of sections of threads with germs.	2	2	2	7
---	---	---	---	---

Result	-	-	-	-
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Control.	1	1	1	4
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Result.	N	o	G	r	o	w	t	h.	-
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These tubes were allowed to remain in the incubator for ten days.

Bouillon tubes were then made from each culture. One tube was contaminated. The two cultures made on May 12th had growth. Plates were made from these and developed characteristic colonies of golden pus. Hanging drop showed presence of micrococci. Everything indicated that they were the germ of golden pus. This may probably be due to the fact that there is a possibility that some germs at the extreme margin of the cover glass were not covered with white oil.

#### Experiment with bacillus typhi abdominalis.

Cover glasses and threads with their controls were prepared from a Bouillon culture twenty-four hours old in the usual manner.

Time of inoculation of Bouillon tube.	May 14.	May 21.
No. of tubes inoculated with cover glasses undisturbed.	2bx	8
Result.	-	-
No. of controls.	1	4
Result.	G r o w t h .	
No. of tubes inoculated with line removed from cover glasses.	2	7bx
Result.	-	-
No. of controls.	1	4
Result.	N o G r o w t h .	
No. of sections of thread with germs.	May 14.	May 21.
Result.	2	12
Control threads.	2	6
Result.	-	-

Contamination in one tube ax due to a micrococcus.

" " " " bx " " " thread forming bacillus.

Plates from these two colonies did not reveal colonies of *Bacillus Typhi Abdominalis*.

Effect of adding whitewash to bouillon tubes after being inoculated with germs.

Experiments were performed, as in case of vegetative anthrax, to see whether whitewash added to a bouillon tube after it was inoculated would prevent growth of *Bacillus of Hog Cholera*, *Staphylococcus Pyogenes Aureus*, and *Bacillus Typhi Abdominalis*. To each of five bouillon tubes inoculated with the above germs were added, respectively, one, two, three, four, and five loopfuls of whitewash.

In case of *Bacillus of Hog Cholera* and *Staphylococcus Pyogenes Aureus*, typical growth was found in the tubes to which was added one, two, and three loop-fuls of whitewash. In case of *Typhi Abdominalis*, growth was found in the tubes to which was added one and two loopfuls. New bouillon tubes were made from the different cultures which gave the same result as the above. These tubes were taken out of the incubator at the end of four days. The above experiments may not conclusively show that the addition of whitewash prevented growth in some tubes, because we failed to preserve the necessary precaution of leaving the tubes in the incubator ten days.

Conclusion: Whitewash prevented the growth of the vegetative form of *Bacillus Anthracis*, *Bacillus of Hog Cholera*, *Staphylococcus Pyogenes Aureus*, except in two cultures as noted, *Bacillus Abdominalis* where

Whitewash was brought in direct contact with the germs. Whitewash did not prevent the growth of spore forms of *Bacillus anthracis*.





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