

THESES

BACILLUS ANTHRACIS.

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B2a

Bacillus anthracis (Cohn)

A special interest in the pathogenic relations of bacteria prompted the choice of this subject. In casually studying the species under consideration, I found it impossible to fully understand many important points which were often not explained with sufficient exactness and besides some of the best authorities differed considerably in prominent particulars. My object then was to see for myself more fully than I was able in my regular work, the effect of the micro-organism upon various animals and their exact location in the tissues of the body and the organs most severely attacked.

In the work a Leitz microscope with objectives numbers 3 and 9 and a $\frac{1}{2}$ inch homogeneous immersion lens were used. Number 9 and $\frac{1}{2}$ inch homogeneous immersion objective together with eye-pieces used magnify 688 diameters each with draw-tube closed.

The methods of staining were for free organisms an alcoholic solution of methyl-violet; and for organisms in tissues Gram's method of staining with auriline-gruitian-violet, with eosin as the second stain. Staining in auriline-gruitian-violet occupied 30 minutes where best results were obtained. All sections were mounted in Canada balsam.

Bacillus anthracis occurs in rods or as ~~in~~ single individuals. The single micro-organisms as measured by Klein are between 0.005 - 0.02 m.m. in length and 0.001 to 0.0012 m.m. in

thickness. The length of the rods vary as to length according to the circumstances under which they grow; they often appear many times longer than the microscopic field is wide under a power of 688 diameters. When the nutriment for growth is, ^{nearly} exhausted the micro-organism passes into the spore stage.

The spores as measured by Klein are 0.001 mm thick and 0.002-0.003 mm. long, they may be distinguished by their bright clear shining oval appearance. one spore under ordinary conditions is formed in each rod. This varies somewhat in size, being some-times much larger in one rod than in another.

The age of a growth in which spores may be found varies with the media used and the free access of air

In my experiments I found spores plentiful upon an Agar-Agar culture in 24 hrs, and at the end of 48 hours spores were very abundant; no rods were found from spores if culture had been subjected to a temperature of from 35-38°C. When culture was left in room at ordinary temperatures the growth reached its perfection more slowly, often requiring 48 hrs for the growth to give evidence of its nature.

On Agar-Agar *Bacillus anthracis* varies somewhat according to the kind of inoculation, if growth starts from a wire point the development is in the form of radiating filaments from $\frac{1}{8}$ to $\frac{1}{2}$ inch in length and very fine. In some cultures the growth was massive but still showed something of the filamentous character about the edges of the colonies.

In beef-broth the growth is always characteristic being always a white clouded growth projecting from the bottom of tube upward when growth is not disturbed and is filamentous in appearance. When tube is shaken the cloud is broken up into floating filamentous masses. After a growth of a few days the cloud becomes disintegrated and sinks to the bottom of the tube as a mass of sediment which upon examination reveals a mass of spore-bearing rods. Koch asserts that that *Bacillus anthracis* is aerobic; that if the air be excluded no growth takes place and no spores can be formed. I made cultures both upon agar and in beef-broth and immediately sealed the tubes by fusing melted paraffin into the mouths of the tubes. These

tubes were then placed in incubator at Temp 38°C and left there for 48 hrs. upon removing them from incubator, no growth could be noticed. Tubes were then placed upon table at ordinary temperature of laboratory and were unnoticed for three days when upon examination an abundant characteristic growth was noticed in the beef broth culture and a very small growth in one spot on Agar. They may be accounted for by the ^{theory} ~~arabic~~, by the fact that a sufficient amount of air was sealed up in the tube to allow the bacillus to obtain a sufficient supply of oxygen.

In trying the effects of *Bacillus anthracis* upon animals a mouse, rat and two rabbits were experimented upon. The microscopical details were shown in about 100 sections cut

and mounted from the organs most affected. In the first experiment made upon rabbit No 1 the inoculating material used was a beef broth culture made from stored material in bacteriological laboratory 6 years old. Inoculation was made Mar 11th in the hind leg by clipping the skin with sterilized scissors and putting material under skin with a sterilized pipette. Rabbit was in poor condition and but very small amount of material was placed under skin. Animal did not seem affected and Mar 18th was reinoculated with 217-199-172 - the original culture. 217 was pure *Bacillus Anthracis*, former place of inoculation was not yet properly healed there being still some evidence of suppuration. Rabbit did not afterward show any symptoms of illness except

possibly for one or two days. The last inoculation was not made under the best possible conditions as when skin was cut the blood flowed slightly thus lessening the chance of the inoculation being successful, otherwise the necessary precautions were taken during inoculation. The above experiment seemed to show that the number of injected organisms may be so small as to be ineffective or that they failed to reach places where conditions were favorable for development. Rabbit improved in condition after second inoculation owing to better care. A mouse was also inoculated Mar 11th with the same culture and at the same time as the first inoculation of the rabbit. The culture at time of inoculation was 24 hrs old

and had been in incubator 24 hrs Temperature 35°C.

Inoculation was made in two places on the inside of hind leg and at base of tail by cutting skin with sterilized scissors and thrusting a sterilized platinum wire into urethra and quickly transferring to cut. Mouse was inoculated Mar 11th 3 P.M. did not notice any particular symptoms of disease until time of death which occurred some time on the morning of Mar 13th and was found dead at 8 A.M. was dissected soon after found dead. The body seemed more or less swollen when found and showed some inflammation around liver in mesentery. Liver was dark colored and was slightly enlarged. Spleen dark color and much enlarged. Kidney seemed about normal or but

slightly enlarged. made three cover-glass smears from the kidney which after staining with methyl-violet made excellent slides of pure *Bacillus anthracis* pure organisms being numerous and containing no spores. Made three cultures from lung one in beef-broth and two on Agar-Agar. All were made by puncturing lung with sterilized needles and transferring to media, subjected to sterilize surface of lung before making cultures and as a consequence obtained a growth of micrococci along with an abundant growth of *Bacillus anthracis*. Also made cultures from the kidneys, liver and fluid from peritoneal cavity. Precaution to prevent contamination was neglected same as in culture from the lung. Abundant growths of *Bacillus*

anthracis were obtained together with some colonies of micrococci. The microscopical examination showed almost pure Bacillus and in great numbers from the liver, also Bacillus anthracis in great numbers from the kidney with a considerable number of micrococci and culture almost pure micrococci with but few Bacilli. The liver, heart, lungs, stomach and spleen were placed in 50% C_2H_5OH and after remaining 24 hrs were placed in 95% and after 24 hrs were placed in absolute C_2H_5OH . where the liver, kidneys and stomach were allowed to remain 48 hrs and after imbedding in paraffin were ready for section cutting. The examination of the sections show organisms in more or less abundance. The sections of the lung show Bacillus anthracis in enormous numbers.

The micro-organisms present in the sections are four Bacillus anthracis singly and in rods, the rods being composed of from 1-7 individual organisms joined together. The long rods are few in number and rods composed of four individuals are the most common.

The Bacilli generally are found between the cells of the tissues and are rarely or never found in the cells. Some few micro-organisms seem to be scattered indiscriminately throughout the section. Bacilli are also found in the tissues between the air cells in the sections of the lungs examined. In a small section perhaps only a part of it contains numerous organisms while the remainder has but few and those widely scattered. In some local spots the organisms are collected in enormous numbers while

in tissues adjoining those but few or none so that it would seem that they had been able to overcome the vigor of certain tissues and had there developed with unusual rapidity. There were no external developments on the lungs indicative of the disease. No spores could be detected in the tissues. No organisms are found in the muscular layer surrounding the alveoli; one section showed a few Bacilli in the epithelial tissues of the air cavities but it seemed like foreign matter dragged in by the knife while cutting and did not appear to be in the tissues themselves. In many instances the epithelial tissue is so abnormally developed as to almost entirely fill the air sacs.

The microscopical examination of the sputum showed great numbers of *Bacillus anthracis*

present. In five slides containing eleven sections there is shown a great variety in the number of organisms and their distribution. The Bacilli are never massed in such numbers as in the lungs, but are more generally distributed over the affected area. In the sections examined the Bacilli are most plentiful almost without exception in the part of the section next to the external surface of the spleen. The organisms are situated between the cells in the tissues without exception. no spores can be detected. Some sections show but few organisms while some parts of a section will contain great numbers and be entirely absent in other localities. The microscopical examination of the kidneys revealed just Bacillus anthracis.

but in smaller numbers than in either the lung or spleen. The organisms are distributed in the sections unlike those of the lungs or spleen. A few clusters of Bacilli can be seen which are situated near the external covering of the kidney. These local spots are almost if not quite as well filled with Bacilli as similar spots in the spleen and lungs. All Bacilli present are found in or near the external tissue of the kidney. They are situated as in the other sections examined in the cell walls but could detect none in the cells themselves. organisms are single or in short rods and contain no spores. The examination of the sections of the wall of stomach. contained few Bacillus anthracis in great abundance but are only found between the follicles of

The inner coat never being found in the muscular coats. In the ~~thin~~ follicles the organisms are found lying longitudinally with the follicles and are found between the cells. organisms are found in larger vots in the stomach than in any other sections examined. no spore could be found. The sections of the liver vary in appearance, some showing abundance of Bacilli and others being almost destitute of organisms. organisms are scattered throughout section, are not collected in masses and are found in the cell wall of the tissues and in small blood-vessels. Bacilli are short and do not contain spores. Slides containing sections of the muscles of the heart were examined but no Bacilli were visible sections of the muscle where inoculation was made

also showed no organisms.

Experiments were also made with *Bacillus anthracis* upon a rat. Rat was inoculated with a pure culture in beef-broth. Inoculation was made at base of tail by clipping of hair and skin with sterilized and infecting material with a sterilized syringe. Inoculation was made Mar 24th 2 P.M. Rat was half grown and exceedingly vigorous. The following day the effects of the disease were plainly visible and the animal being in a good place for observation, it was possible to observe the effects of the disease more fully than was possible in the other animals experimented upon. The first effect noticed was a disinclination to move about or eat this was evident the next morning after inoculation. Towards noon the animal scarcely be made to move and its breathing

was hard and heavy; breathing became more difficult until 3 P.M. when it scarcely showed any signs of life beyond a slight gasping for breath, by 5 P.M. breathing was so difficult that breath only came in violent gasps and as it was plainly evident that the animal could live only a few minutes at most it was killed with chloroform at 6.15 P.M.

27 hrs after inoculation. body was opened and blood drawn and animal injected. cultures from blood were pure *Bacillus anthracis*. sections of the spleen, liver and lungs do not reveal any Bacilli as far as I could determine. The death of the animal was evidently caused by asphyxiation as the organisms were very numerous in the blood. No organisms were given an opportunity to enter the tissues after death as an injection was made and the body placed

in 95% C_2H_5OH . Rabbit No 2 was inoculated
April 12th 2 P.M. with a pure prof both cultures of
Bacillus anthracis. Inoculation was made with
the necessary precautions and under the best
conditions as the animal was in good condition
and material was injected with ^{out} loss of blood.
After inoculation rabbit was well fed and
supplied with water. Animal found dead
April 13th 8 A.M. body upon dissection seemed
swollen, liver and spleen much enlarged, lungs
not normal color. Pleurae of liver and spleen
filled with blood non-coagulated and tissues
easily torn. seemed much more tender than
when in normal condition. Cultures made
from the liver and spleen showed pure Bacillus
anthracis. mounted a slide of blood of rabbit
before inoculation which stained with methyl-violet

which contained no Bacilli. The sections of the lungs show but few organisms when compared with the sections of lung of mouse. The epithelial tissue is abnormally developed but contains no Bacilli, many air cells being completely filled by it. The organisms visible are in the tissue between the air cells. The sections of the spleen showed few Bacillus anthracis present in considerable numbers situated same as described in sections of spleen of mouse but not present in quite such large numbers. The sections of the liver show organisms to be not very numerous and distributed through-out the section and are situated between the cell-walls. Sections of the stomach show few Bacillus anthracis in great numbers and are between the follicles in the middle coat of the stomach.

the muscular coats are free from organisms. The epithelial tissues contain numerous organisms between the cell walls.

The conclusions reached are 1st that *Bacillus anthracis* was the cause of the death of the animals experimented upon according to the postulates of Dr Koch which are that a disease germ must be taken from a diseased animal and carried through successive cultures until pure, then the pure organism must be placed in a healthy animal by inoculation, and must, after the death or severe illness of the inoculated animal, be found in its blood or tissues. The present experiments have substantiated these postulates in every case and more especially in the case of rabbit II whose blood

I examined before inoculation and found it free from organisms, then after the injection of a very small amount of the culture the animal sickened and died. The organisms introduced were those found in vast numbers in the blood, showing that they must have multiplied with great rapidity and at the same time have been carried far from the seat of inoculation. Their occurrence in such enormous numbers in the capillaries of the vital organs must take much nutriment from the blood and when in sufficient numbers much interfere with circulation of the blood. The presence of so many organisms about the air cells of the lungs must consume much oxygen which would otherwise aid in building up the system and in purifying the blood, for

it has been shown that Bacillus anthracis is
aerobic and cannot grow when deprived of
oxygen. The examinations of sections has
shown that Bacillus anthracis attacks most
seriously the most important organs of the
body, and that the effect is therefore most
likely to be damaging to the affected animal
or man, for the history of Bacillus anthracis
shows it to be the cause of diseases com-
mon ^{to man} as well as animals. The extreme
rapidity with which the Bacilli multiply and
the organs which it attacks being difficult
of access, render it a dangerous disease
producing organism and one difficult
to eradicate. The ease with which inoculation
may be effected renders the disease
produced exceedingly dangerous to animals

and man; there being instances recorded where it has been inoculated in animals by flies and the same being equally liable to man. The historical record shows that *Bacillus anthracis* is exceedingly difficult to eradicate from an infected territory because of its spore stage which protects the organism fully from adverse influences and on this account renders it a dangerous organism.

Bacillus anthracis used in the experiments recorded in this thesis had been standing for 6 years in some thoroughly dry agar-agar so that when the work first began it appeared that no results could be gotten from it; but with a little attention it gave abundant growths. So we may say that in the spore stage the organism would

or exceedingly difficult to destroy.

The investigation proves conclusively that Bacillus anthracis is an organism of which it is highly important that we should have a complete knowledge both from a financial and physiological point of view as its existence is a constant menace to our welfare and the well-being of the animals in our care.

Finis.