A NEW DIPHTHERIA VACCINE.

by

C.B. Weld, B.A.

April 25th, 1924.
John Ridington, Esq.,
Librarian,
University of B. C.,

Dear Sir:

I beg to advise that the attached thesis, entitled "A New Diphtheria Vaccine" by C. Beecher Weld (No. 949) has been approved.

Yours truly,

[Signature]

Head of Department.
A NEW VACCINE FOR USE IN
TREATING DIPHTHERIA CARRIERS.

by

Charles Beecher Weld.

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A Thesis submitted for the Degree of
MASTER OF ARTS
in the Department of
BACTERIOLOGY.

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THE UNIVERSITY OF BRITISH COLUMBIA.

APRIL, 1924.
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A NEW DIPHTHERIA VACCINE.

The Diphtheria Carrier.

It is well known that many Diphtheria patients, who have recovered from the disease, carry virulent Diphtheria bacilli in their throats for weeks and even months after they have recovered. There are also other normal people apparently immune to the disease, who harbor, without symptoms, in their throats and nasal cavities virulent organisms. Both these types of carriers are capable of conveying these organisms to other and less immune persons, who may promptly contract Diphtheria, and they constitute a serious problem among Public Health workers, and in Epidemiology. The only way, which is sure of success, of treating the problem is to rigidly isolate and quarantine all carriers, until they have been shown to be free of bacilli. If at the same time all susceptible people are immunised against Diphtheria, the disease will be completely wiped out once and for all. This however is impossible at the present time, even though the methods for testing one's susceptibility are simple and accurate, and the immunising process effective and not as a rule uncomfortable. Even the possibility of isolating all carriers is remote, as it is impos-
sible to tell how long the quarantine will have to last. The carrier would have to be segregated in an institution, and not only would this be a great expense to the state, but the personal inconvenience to the carrier would be so great that it is improbable that all would submit to the isolation.

However if some method could be devised which should ensure the "clearing up" of the carrier within a reasonable time, some definite policy could be arrived at. At present there is no method of treatment whereby a persistent carrier can be freed of the bacilli with any degree of certainty.

Dreyer (1) has recently developed a new method of preparing vaccines which he claims is of general application. His work has been chiefly with the Tubercle Bacillus, and the early results are encouraging, though as yet they have not been thoroughly confirmed. Is it possible to use Dreyer's method, or some modification of it, and produce a vaccine which will aid in the clearing up of carriers?

Dreyer's Reasoning and outlines of Method and Work.

In brief, Dreyer states that successful vaccines are made from Gram Negative Organisms, while in general Gram Positive organisms do not make good vaccines. He
allows many exceptions to this rule but claims it to hold in general. Gram negative bacteria differ chiefly from gram positive ones in that they contain less of the lipoidal class of substances. "Acid Fast" organisms contain even more of these lipoids. Is there then some connection between the lipoids and the antigenic value of the bacterial suspension? To answer this question he developed a method of extracting these fatty substances, which consists of treating a mass of bacteriae with formalin and then extracting with acetone. After this treatment, Gram positive, and even acid fast organisms are rendered Gram Negative. Then, using "Defatted" tubercle bacilli he showed by animal inoculation that the antigenic qualities of the bacterial suspension were not destroyed. Indeed, judging from the antibody response they seemed rather to be increased. Human cases, treated with this new tuberculin have given very encouraging results, but of course as it usual in dealing with Tuberculosis, considerable time will have to elapse before early indications can be confirmed.

**Immunology of Diphtheria.**

The clinical symptoms of Diphtheria are due for the most part to the production, during the growth of the organism, of a soluble toxin which profoundly affects the
local tissue. This toxin diffuses through to the bloodstream, whence it is carried throughout the body, and a general toxemia produced. In addition to this however it stimulates the body to produce an antitoxin which neutralizes the toxin free in the blood, and when present in adequate quantity it has neutralized all the free toxin in the body, and is combining with all fresh toxin as it is liberated. Thus the disease is checked, though the damage which the toxin has already done is not righted. After this necessary amount of antitoxin is obtained, either by natural production, or by artificial administration, the Diphtheria bacilli usually but not always disappear from the throat.

Why does the organism disappear, when this antitoxin is obtained? The three following explanations suggest themselves:

(1) That the bacillus is unable to grow in the absence of toxin or in the presence of an excess of antitoxin.
(2) That in the presence of an excess of antitoxin it is more readily overgrown and smothered out by other organisms.
(3) That when its toxin is neutralized it is more susceptible to the body defenses than before.

The first two possibilities do not seem reasonable
when one considers that Diphtheroids, such as B. hoffmanni, B. xerosis and a virulent but otherwise typical B. Diphtheria are frequently found in the nose and throat. Virulent diphtheria bacilli too are quite often found present even when the patient has in his blood more than 1/30 unit antitoxin per cc., which is the amount considered necessary for a person to be immune. The carriers referred to belong to this latter class. In any event, it has been shown (x) that virulent Diphtheria bacilli grow readily in vitro in a glucose broth medium containing 100 units of antitoxin per cc., and that in such a medium the bacilli will not be outgrown by contaminants any more readily than in the same medium containing no antitoxin.

The third possibility however opens up a question which is far too large to be discussed completely here, though a few details may be considered. In the first place, what are the body defences, and in the second place how do they act in the removing of the bacilli from the nose and throat?

If the Diphtheria organisms are present in the throat or nasal cavities, some will be on the surface of the

(x) - See Appendix I for details of experiments.
membranes, and others will be more or less embedded in the tissues. Those on the surface, are out of reach of the phagocytes, and antibodies in the circulation and the tissues, but are readily reached by the mouth fluids. These, and the nasal secretions do not seem to have any harmful effect on the bacteriae normally. However the possibility of specific antibodies being present in these secretions must be thought of, and probably many organisms are washed away to the stomach and intestines, whose secretions may exert a bactericidal effect on the foreign organisms. Those bacilli, which are embedded in the tissues however are in a position to stimulate the production of antibacterial antibodies, and are also within reach of these substances. Phagocytosis must play an important part in the removal of these organisms from the body. Heque (2) has shown that the Diphtheria bacillus is very susceptible to phagocytosis; Turnic lip (3) states that the phagocytic power of the blood of Diphtheria carriers is increased, but that at the same time the nose and throat may contain large numbers of leucocytes not engaged in phagocytosis. These findings indicate that while Phagocytosis does play an important part in the removal of the bacilli, other factors must be involved. Some antibodies
or substances are probably present in the convalescing case which is not produced in the carrier, and which either exert a bactericidal action themselves or aid the Phagocytes in their task. If some means could be found to stimulate the production of these antibody substances, the time required for the clearing up of carriers would be very materially shortened.

Park (4) mentioned that the ordinary immunisation procedure, using a Toxin-Antitoxin mixture has no apparent effect on clearing up carriers. Park and Zingher (5) attempted to remedy this by adding 1000 million killed Diphtheria bacilli to each cc. of Toxin-Antitoxin mixture, but at the time of writing of their article, sufficient time had not elapsed to determine its efficacy. Seebohm (6) discusses the use of a Diphtheria vaccine to clear up carriers, and reports that in two weeks, 53.5% are clear; that in three weeks, 77%; and that in four weeks, 89.5% are clear. His results however are exceptional and the general opinion is that Diphtheria vaccines are not successful.

This, according to Dreyer's (1) hypothesis is only what is to be expected, as the bacilli are Gram positive organisms; but if they are "defatted" by his process, they might prove capable of producing the necessary antibodies.
An attempt was made to investigate this possibility in practice.

**Preparation of the Vaccine.**

The Diphtheria bacilli are grown on Loeffler's serum medium for forty-eight hours. In order to obtain as large a surface for growth as possible, the serum is solidified on the flat side of eight ounce medicine bottles. Five or six of these will produce sufficient growth for convenient handling. The mass of bacilli is washed off with sterile physiological salt solution containing 0.9% sodium chloride, into sterile centrifuge tubes. To free the suspension of traces of liquid serum, which are present in the condensation liquid, and which will give an undesired precipitate when formalin is added, it is washed three times with saline and then once with formalin. The washed bacterial mass is ground up in a sterile agate mortar with pure formalin, (40% formaldehyde) using 20-30 ccs. of formalin to every gram of bacilli weighed wet; and the resulting suspension is poured into a sterile flask, connected to a "reflux-condenser" and heated in a water bath at 100° Centigrade for four hours. At this stage Dreyer filters the suspension through calcium free filter paper and washes it with acetone. Using ordinary filter paper it was found very difficult even when using a Buchner funnel and suction to force the
solution through the paper, and that when the filtering was finally complete, it was impossible to scrape the precipitate from the paper. To avoid this difficulty, the suspension is centrifuged and the sediment washed three times with acetone. It is then suspended in acetone, poured into a coarse alundum thimble and placed in a Soxhlet extractor with ground glass joints. Acetone is the extracting fluid used, and the whole apparatus is immersed in a water bath to the level of the top of the thimble and kept at 65-70°C. for 24 hours. The thimble is now removed from the apparatus, and the extracted bacterial mass dried. If the bacilli on staining prove to be completely Gram negative, the extraction has been effective, and they need only to be emulsified in saline and the suspension standardized. If however they are not definitely negative to Gram, the whole process from grinding in formalin to extracting in acetone must be repeated.

Dreyer's method of standardizing is to weigh out exactly 0.1 gram of the dried extracted mass, and grind it up thoroughly in an agate mortar with 10 cc's. saline. He then centrifuges down the coarser particles and determines the weight of bacteriae left in suspension by subtracting from the original 0.1 gram, the weight of the centrifuged sediment, not forgetting to allow for the
weight of sodium chloride which will be dried with it. The chances of error with such a method are great, and working with Diphtheria it was found impossible to obtain an accurate standardization. For this reason, and also because grinding in a mortar is a tedious process and one where proper aseptic precautions are difficult to maintain, the following procedure was adopted. The dried extracted mass is weighed out carefully into a sterile vaccine shaker bottle containing a dozen or so glass beads, and 10 ccs. sterile saline added for every 0.1 gram of the defatted bacilli. The whole is now shaken in a mechanical shaker for six or seven hours and immediately pipetted into a weighed sterile centrifuge tube and centrifuged at 3000 revolutions per minute for about five minutes. The supernatant fluid, which is somewhat turbid but must contain no granulation visible to a 6X lens, is the vaccine and is diluted as desired. From this point, there are three convenient methods of standardizing the vaccine.

1) That involving the use of the weighing described above. The shaken emulsion contains exactly 0.01 grams defatted bacilli per cc. The weight of the centrifuge tube is known, and the weight of the residue in the tube after centrifuging can easily be determined by weighing
the tube with its contents after dessicating, and subtracting the former from the latter. The weight of salt (0.9% of the volume in cc. of solution left in the tube after centrifuging and decanting) must be calculated and allowed for. The weight of solid still in suspension will be then the difference between the original 0.1 gram per cc. and the corrected weight of residue. This method however gives us the concentration of the vaccine by weight, and the normal method is to speak of the number of organisms present.

(2) The individual bacteriae not having been broken up by the shaking, it is possible to count the number present. A "Levy" counting chamber, ruled in squares 1/20 mm. square, and with a chamber 1/50 mm. deep is satisfactory, if a reinforced coverslip, thin enough to allow the use of the oil immersion lens, is used.

(3) A standard having once been prepared by either of the above methods, future preparations may be matched to it by the opacity method. This is a very simple procedure and is sufficiently accurate.

Animal Experiments.

It is impossible to give a vaccine prepared in such a radically new way to human patients without first proving its harmlessness to animals, and without obtain-
ing at least suggestive evidence that the giving of the vaccine would be beneficial to the patient. In this series of experiments, Guinea Pigs were first used. Six healthy guinea pigs were selected, weighing between 350 and 400 grams. Two were given a polyvalent Diphtheria vaccine, consisting of equal parts of three known virulent type strains, "Park," "Durand," and "American" and three local avirulent strains from the Vancouver General Hospital, of unknown agglutinative type. The polyvalent vaccine was used in order to ensure as far as possible the production of antibodies against the particular type concerned in the particular case. For use in animals however it has no advantage, and was later dropped in favor of the monotype vaccine, in order that results could be more closely compared. Two guinea pigs were inoculated with Diphtheria strain Park and two with Durand strain. The suspensions given these four animals were of living virulent organisms washed repeatedly with saline in the manner suggested by Fitz Gerald (7) who found that by using washed living organisms a serum could be obtained with an agglutinating titre of from 1/1200 to 1/4000 in three or four weeks. Bell (8), using a suspension of bacilli killed by heat succeeded in obtaining a titre of only 1/300 to 1/400 after many weeks of
immunising. After a month or more of treatment (see Appendix), no evidence of the presence of antibodies could be shown, judging by examinations for agglutinins. On this account it was decided that Guinea Pigs were of no use for Diphtheria bacterial antibody production, and Rabbits were selected as being the most likely to prove useful.

Three young rabbits were chosen, not full grown at the beginning but after the course of immunisation they had attained normal weight. It was thought that young animals might react more strongly to the injections than old ones. One was given strain number 100 from the General Hospital, which was known to be very virulent. One was given strain "Park," a standard laboratory strain of known virulence. Both these suspensions consisted of living organisms, washed 10 x with saline. The third was given Park strain, defatted suspension, prepared according to the technic described above. The two rabbits given Park, were twins, identical in weight, and were given precisely the same dosage throughout. All three were inoculated on three successive days, weekly, as is advised by Gay and Fitz Gerald (9). At no time did any of the animals become sick, nor did they appear to suffer any ill effects from the vaccination. For full details as to inoculations and
and dosage, refer to the individual animal charts in the appendix.

From time to time blood was taken from the animals and the titre of agglutinins determined. This is a comparatively simple test and, it was thought that if the antigen was stimulating the production of agglutinins the production of other, perhaps more important, and possibly unknown, antibodies would be stimulated simultaneously.

**Examination of Sera.**

**Technic of Agglutination Tests.**

Blood is taken from animals, and the serum separated from the cells. This serum keeps well in the ice-box and is diluted just before use with sterile saline. As there is no way of foretelling the titre of the serum to be tested, a series of dilutions must be used. In an animal in the early stages of immunisation a series of dilutions ranging from 1 part in 10 to 1 in 200 will probably indicate the titre present, but animals well immunised may give a serum which will agglutinate in a dilution anywhere between 1 in 1000 to 1 in 10,000. In detail the test is as follows: A suspension of the strain under consideration is made by washing off the growth of one or two Loeffler's slopes in saline. This is shaken
free of clumps and is standardized by the opacity method
to about 500 million organisms per cc. A series of
small, clear glass test tubes is placed in a rack and
0.1 cc. of suspension put in each. To each tube is
then added 0.9 cc. of a serum dilution already prepar­
ed. A control tube is set up which contains suspension
and saline but no serum. The rack is then shaken, incu­
bated at 56°C. for two hours, and placed in the ice-box
overnight. The results are read in the morning, and
the tube containing the highest serum dilution which
shows agglutination indicates the titre of the serum. As
a rule there is no difficulty in reading the tests, those
tubes in which complete agglutination has taken place
will be perfectly clear, while the others, and the con­
trol tube remain turbid. If there is only partial agg­
lutination the sediment must be shaken up, and the coarse­ness of the granulation as compared to that of the con­
trol will determine the result. The tubes must not be
shaken vigorously as the flocculi that are formed are
easily broken up, especially the small ones that are hard
to see. One or two light flicks of the tube will suffice
to throw up the sediment and allow the granulation to be
seen.
Technic of Complement Fixation Tests.

The technic is that described in detail by Dreyer (1). He titrates the complement against sensitized sheep’s red blood corpuscles, and dilutes it so that two minimal haemolytic doses are contained in five drops. He then titrates the antigen against the complement and sheep cells, (sensitised cells are used throughout - 0.25 ccs. of a 5% suspension) and dilutes it so that the largest dose which is not anticomplementary is contained in 15 drops. The test then consists in adding graded amounts of the unknown serum to fixed amounts of complement (5 drops) and antigen (15 drops) and incubating for one hour at 37.5°C. and then adding 0.25 ccs. of sensitised sheep cells to each tube and incubating another hour. The smallest quantity of serum being tested which will give complete fixation of the complement can thus be readily determined.

Observations on Results of Sera Examinations.

For all details refer to the animal records in the Appendix.

No evidence of the presence of antibodies was obtained from any of the Guinea Pigs.

The Rabbits however, all show good response to the
antigenic stimuli. That one receiving #100 had in twenty days developed a serum showing an agglutinating titre of more than 1/720 and in four weeks a titre of about 1/1800. The one receiving live Park bacilli had in fifteen days, a titre of more than 1/240; in twenty four days, a titre of about 1/2000; and in thirty days, using glycerinated serum, a titre of about 1/6000. The Rabbit receiving the "Defatted" Park bacilli seemed to have developed no agglutinins in 15 days but in 24 days the titre was 1/2000, the same as that given by the other Park rabbit. This serum also showed the presence of Complement Fixation antibodies in considerable quantities.

An attempt was made to preserve these sera with glycerine, but unexpected results occurred. On adding the glycerine, the agglutinating power of serum #100 and Defatted Park serum seemed to be immediately destroyed, and that of the live Park serum while not affected at first was completely lost after a few weeks. The glycerine was added as a preservative and its action is rather puzzling, unless one assumes the presence of certain unknown impurities in the glycerine.

The agglutinating titre of sera from all three rabbits is high and was rapidly formed. The Park serum and the Defatted Park serum in 24 days both showed the same
Unfortunately, no agglutinations were done with the 30 day serum from the Defatted Park animal, before glycerine was added. However the differences of the sera are so small that they might readily be explained as due to individual animal idiocyncrasies.

It would appear then that Defatted Diphtheria bacilli have antigenic powers equal to that of live organisms. This is noteworthy because Fitzgerald (7) has shown that live Diphtheria bacilli make a better antigenic stimulus for the production of agglutinins than dead organisms. Judging from this then it would seem that Defatted bacilli make a better antibody producing antigen than do Diphtheria bacilli killed by heat as is done in making ordinary vaccines.
Conclusions.

(1) The question of the carrier of virulent Diphtheria bacilli is becoming very important, and new methods of treatment are necessary to hasten the removal of the bacilli from these carriers.

(2) A vaccine which would effectually produce antibodies either bactericidal in themselves or with power to aid the Phagocytes would be useful.

(3) A Diphtheria vaccine has been made by defatting the bacilli as suggested by Dreyer.

(4) The vaccine has proved harmless to Guinea Pigs and to Rabbits.

(5) The antibodies produced by rabbits, as evidenced by agglutinins, are stimulated by the Defatted bacilli equally well as by washed living organisms, and therefore better than by dead organisms.

(6) As the vaccine has proved in animals to be an efficient and harmless antigen, it would be safe and advisable to try its efficacy in human cases.
APPENDIX I.

Comparison of Growth of B. Diphtheria in media containing Antitoxin with that in media containing no Antitoxin.

16/4/24.

To two of four similar tubes of Glucose broth containing 5 ccs. each, 500 units of Diphtheria antitoxin were added to each. Then all four tubes were inoculated with one loopful of a suspension of Diphtheria bacilli, strain # 77 from the General Hospital.

17/4/24.

The growth in each of the four tubes, judging by the opacity, is equal to that of any other.

18/4/24.

A tube of glucose broth + 500 units Diphtheria Antitoxin was sown with a field culture containing a few Diphtheria bacilli. A normal tube of glucose broth was similarly sown.

22/4/24.

The opacity of the growth in both tubes is similar. In a stained smear the proportion of Diphtheria bacilli to the other organisms is about 50% in each culture.
APPENDIX II.

Animal # 1.

Guinea Pig. Curly Hair, Black, White and Tan.

Inoculated with living "Park" organisms, washed toxin free by 14 washings. 1 cc. = 250 million.

On Nov. 26 with 0.25 ccs. subcutaneously.

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</tr>
<tr>
<td>Nov. 26</td>
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</table>

Bled Dec. 18. Agglutinations against live Park strain were all negative.

Bled Dec. 27. Agglutinations - negative.

Animal # 2.

Guinea Pig. Curly Hair, Black, White and Tan.

Inoculated with living "Park" strain, washed 14 X.

1 cc = 250 million.

Inoculations.

On Nov. 30 with 0.25 ccs. intracardially

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</tr>
</tbody>
</table>

Bled Dec. 18. Agglutinations against Park strain, in dilutions from 1/50 up - negative.

Bled Dec. 27. Agglutinations - negative.
APPENDIX III.

Animal # 3.

**Guinea Pig.** Grey, Light Brown.

Animal # 4.

**Guinea Pig.** Black, White and Brown.

Both inoculated with Defatted Diphtheria Bacilli,
Polyvalent mixture containing equal parts of "Park","Durand"
and "American" strains, and of three local avirulent strains.

### # 3. Inoculations. 1 cc. = 250 million.

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<tr>
<td>Dec. 5</td>
<td>0.25 cc. intracardially</td>
</tr>
</tbody>
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Bled Dec. 21, and 27. With live Park bacilli as antigen all agglutinations were negative in dilutions from 1/50 up.

### # 4. Inoculations. 1 cc. = 250 million.

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<tbody>
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<td>Dec. 5</td>
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</tbody>
</table>

Bled Dec. 27. Using live Park suspension as antigen - agglutinations were negative in dilutions as low as 1/50.
APPENDIX IV.

Animal # 5.

Guinea Pig. Brown and Fawn.

Animal # 6.

Guinea Pig. White, Tan and Yellow.

Both are inoculated with a suspension of live Durand Strain Diphtheria bacilli, washed 10 X.

# 5. Inoculations. 1 cc = 250 million.

On Nov. 30 with 0.25 cc. intracardially.

" Dec. 1 " 0.25 " "
" 2 " 0.25 " "
" 5 " 0.4 " "
" 7 " 0.5 " "
" 11 " 1.0 " subcutaneously.
" 15 " 1.0 " "
" 18 " 1.0 " "
" 21 " 1.0 " "
" 25 " 1.0 " "

Bled Dec. 27. Using live "Durand" strain bacilli as antigen, there were no agglutinations in any dilution from 1/50 up.

# 6. Inoculations. 1 cc. = 250 million.

On Nov. 30 with 0.5 cc. subcutaneously.

" Dec. 1 " 0.5 " "
" 2 " 0.5 " "
" 5 " 0.5 " "
" 7 " 1.0 " "
" 11 " 1.0 " "
" 15 " 1.0 " "
" 18 " 1.0 " "
" 21 " 1.0 " "
" 25 " 1.0 " "

Bled Dec. 27. Using live "Durand" strain bacilli as antigen, there were no agglutinations in any dilution from 1/50 up.
APPENDIX V.

Animal #7.

Rabbit. Young, grey, white ring around neck.

Injected with a living suspension of strain B.C. 100 washed 10 X and proved toxin free by intracutaneous injection into a normal Guinea Pig.

Inoculations.

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</table>

Bled Jan. 10. With strain # 100 as antigen, agglutinations were + in all dilutions made up to 1/720.

Bled Jan. 18. With strain # 100 as antigen agglutinations were strongly + up to 1/1200 and weakly + in 1/1800. Using strains Park, Durand and American as antigens there was no agglutination.

The animal was bled out and killed Jan. 29. The serum was separated and an equal volume of glycerine added.

On Feb. 7 with #100 as antigen, agglutinations were negative in dilutions 1/2000-1/6000.

On Feb. 12 " " " " " " " " were + up to 1/1600 and - from 1/2400-1/3200.

On April 16 " " " " " " " " - in dilutions from 1/100 - 1/900.
APPENDIX VI.

Animal 8.

Rabbit. Young, grey with white left front foot.

Injected with a living suspension of "Park" strain washed 10 X and proved toxin free by inoculation intracutaneously into a normal Guinea Pig. 1 cc. = 250 million.

Inoculations.

On Dec. 26 with 0.25 cc, intravenously.

<table>
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</table>

Bled Jan. 10. Against live Park, agglutinations were + in all dilutions made, up to 1/240.

Bled Jan. 18. Against live Park, agglutinations were + up to 1/1920 and - in 1/2160.

The animal was bled out and killed Jan. 31 and an equal volume of glycerine added to the serum.

On Feb. 4 against live Park, there was + agglutination in all dilutions made up to 1/4000.

On Feb. 7 " " " was strong + agglutination up to 1/5000 and a weak + agglutination in 1/6000.

On Apr. 10 " live Park there was + agglutination up to 1/200 and - agglutination in 1/400."
APPENDIX VII.

Animal # 9.

Rabbit. Young, grey, white hind feet. Injected with a suspension of Defatted "Park" bacilli. 1 cc. = 250 million.

Inoculations.

On Dec. 26 with 0.25 cc. intravenously.

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</table>

Bled Jan. 10. Agglutinations against live Park and Defatted Park were negative in dilutions from 1/60 up.

Bled Jan. 16. 

The animal was bled and killed Jan. 31, and an equal volume of glycerine added to the serum.

On Feb. 4 and 7 against live Park there was no agglutination in dilutions 1/200 - 1/4000.

On Feb. 12 there was no agglutination against live or Defatted Park in dilutions 1/200 - 1/3200.

On Feb. 7. Using Dreyers Technic a complement fixation test showed complement to be fixed in tube # 4 containing 10 drops of a 1/20 dilution of serum. The Antigen consisted of a heavy suspension of live Park bacilli and was not anticomplementary in any concentration.

On April 16 against Defatted Park bacilli agglutination was + in 1/200 and 1/400 and - in 1/600.
APPENDIX VIII.

BIBLIOGRAPHY.


