A STUDY OF EXPERIMENTAL GONOCCOCAL INFECTION IN ANIMALS

BY

HARRY CULVER

B. S. University of Wisconsin

1910

THESIS

Submitted in Partial Fulfillment of the requirements for the Degree of

MASTER OF SCIENCE

IN PATHOLOGY AND BACTERIOLOGY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1918
I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Harry Culver ENTITLED A Study of Experimental Encephalitic Infection in Animals BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF M.S.

David E. Davis
In Charge of Thesis

Head of Department

Recommendation concurred in:

Committee on Final Examination*

*Required for doctor's degree but not for master's.
TABLE OF CONTENTS

1. Introduction .................................................. 1.
2. Literature .................................................... 2.
5. Comment ...................................................... 12.
6. Conclusions .................................................. 15.
A STUDY OF EXPERIMENTAL GONOCOCCAL INFECTION
OF ANIMALS.

The recognition of a microorganism as the probable cause of specific human disease leads in the natural course of events to attempts to cultivate the organism and to reproduce the disease by the inoculation of the germ into animals. When the organism is cultivatable and capable of producing specific disease in susceptible animals a careful study of the question of immunity and pathogenesis may be made and valuable information on both the specific and non-specific therapeutics of the infection may be obtained. Our present understanding of such important diseases as syphilis and tuberculosis is due to a considerable degree to this method of investigation and many other equally striking examples might be cited in connection with other infectious diseases.

Gonorrhoea has been recognized for centuries, there being indeed biblical descriptions made with sufficient accuracy to warrant present day recognition of this disease. Notwithstanding innumerable clinical reports appearing in early medical publications with reference to gonorrhoea, the beginning of modern conceptions of this infection dates back to the recognition of the causative organism by Neisser (1) in 1870. At once there were numerous attempts made to cultivate and to produce experimental infection by animal inoculation. Neisser (2) failing to get the organisms to grow on artificial mediums inoculated the conjunctivae of dogs with infectious pus with entirely negative results. Sternberg (3) inoculated the eyes and urethrae of dogs and
gave subcutaneous injections of gonorrheal pus to rabbits also without results. He reported inability to produce infection by intraurethral inoculation of cultures in volunteer male students. The use of plain broth as a culture medium, no doubt explains why the infection was not reproduced.

It remained for Bumm (4) in 1885 to demonstrate that for the isolation and artificial cultivation of the gonococcus blood serum must be present in the medium. Since this time practically all attempts at animal infection were made with suspensions or artificially cultivated gonococci. By this method it has been repeatedly shown that intraurethral inoculations of pure cultures in man almost invariably cause typical infections.

Loeffler and Leistikow (5) were unsuccessful in producing infection in rabbits by inoculating the abraded conjunctivae with pure cultures of gonococci. Similarly Finger, Gohn and Schlagenhaufer (6) failed to infect the urethrae or rectums of dogs or the peritoneum of white mice; but they produced an acute inflammatory condition within the knee joint of a dog by injecting pure cultures from serum agar; however, they failed to recover the injected microorganisms from the lesions produced. This was also the experience of Nicolaysen (7).

In 1896 Heller (8) reported the production of gonorrheal ophthalmia in young rabbits but his work was not confirmed by Nicolaysen (7) and de Christmas (9). Wertheim (10) first treated gonococci by growing them on mouse serum agar before injecting them intraperitoneally into mice and reports a true peritonitis from the exudate
of which the organisms were isolated in pure culture on the fifth day; but his results have not been confirmed by other investigators. Two thirds of Nicolaysen’s (7) white mice died within 24 hours after being injected intraperitoneally with gonococcus suspensions. The peritoneum showed only a clear exudate containing a few round cells and a variable number of gonococci seen in smears. The organisms could usually be cultivated from this exudate, occasionally from the heart’s blood but never from the spleen. He is of the opinion that the organisms had not multiplied within the experimental animals hence death was not caused by infection proper but probably to the action of toxins liberated by autolysis. This he apparently demonstrated by producing similar results on the introduction of either dead or living bacteria.

The presence of organisms in the blood stream after intraperitoneal injection has also been shown by Wilbolz (11), Jundell (12), Morax (13) and Scholtz (14). The latter author claims that only by giving very heavy doses will there be a real increase with the appearance of organisms in the blood and other organs. This increase he believes appears during the agonal stage or even after death, while the organisms in the blood are rendered passive by the leucocytes. Pleuritis of a serofibrinous character was produced by Pizzini (15) which caused the death of the animals in three days at which time the gonococci could not be demonstrated. Subeutaneous injections were made in animals by Stenischneider and Schaeffer (16) without result while Maslogsky (17) by similar methods produced sterile abscesses. Pompeani (18) inoculated animals intravenously, recovering the organisms from the blood in 48 hours. Endocardial, pericardial and meningeal
inoculations have been made by F. Meyer (19) and Jundell (13) with negative results. Hewes (20) claims to have produced genital gonorrhea in female dogs and Colombini (21) claims the production of similar infections in dogs and rabbits, however it has been impossible to confirm this work or that of Sorrentino (22) who reports the production of a genuine arthritis in dogs and rabbits.

De Christmas (9) attempted to produce local gonococcal infection by first paralyzing leucocytes and causing local edema by the injection of lactic acid, iodine or guiacol before the bacterial injection but he was unsuccessful, the failure in his opinion being due to the normally high temperature of rabbits and guinea pigs. Heat fast organisms have been used under similar conditions without success (23). De Christmas was successful in obtaining positive blood cultures in rabbits 48 hours after an intravenous injection of gonococci, but after three days the blood was sterile. Scholtz (14) caused the death of mice in 36 hours by injecting the culture medium containing gonococci intraperitoneally and found the spleen and peritoneum hyperaemic and swollen, the peritoneal exudate was sterile after twenty hours. He was unable to enhance the virulence of the gonococci by inoculating the peritoneum of one animal directly from the peritoneal exudate of another. A similar lack of increased virulence was noted by de Christmas after successive passages through the blood stream of animals.

Wildbolz (11) believes that the cultures are not only toxic but may be infectious, since he injected a 35 day old culture into the peritoneal cavity of a guinea pig which caused death in 36 hours. There
were 8cc of fluid in the abdominal cavity which contained many leukocytes, many of which were filled with typical gonococci. Reinoculations gave a pure culture. Since the microscopic examination of the old culture showed only a few whole organisms the remainder partially disintegrated he feels justified in concluding that the typical gonococcal clusters were the result of multiplication within the animal body. This finding occurred but once and has not been confirmed by others. Moskalew (24) believes that gonococci are pathogenic for white mice and rabbits since after intraperitoneal injection there is at first an increase in the number of organisms followed by death of the animals. This temporary increase in microorganisms, however, has been shown to be due to the presence of culture medium injected with the bacteria. The sudden fall of body temperature of the injected animal may also improve the possibilities of such an increase, this sudden fall of temperature being due to shock induced by the protein injection.

Anthropoid apes seem equally as resistant to gonococcal infection as other experimental animals since Bruck (25) was unable to produce any evidence of infection in them. Torrey has demonstrated gonococci in the heart's blood of guinea pigs five minutes after an intraperitoneal injection, the blood of these animals remained positive for 24 hours and gonococci could be isolated from the peritoneal exudate up to the twenty eighth hour after such injection. He believes that animal passage increases the virulence of gonococci, since by ten animal passages the minimal fatal dose was one fourth the original
minimal fatal dose. This he reasons is due to the adaptive mechanism of the gonococcus which renders it more resistant to the lytic agencies of the host.

From a search of the literature on this subject one is forced to conclude that gonococcal infection, in animals, in the sense that the organisms increase within the animals and invade their tissues, does not occur. The slight early increase in number of organisms seems to be development on artificial culture medium injected with the organism, together with the lowered body temperature immediately following such an inoculation. The many positive reports are due to lesions produced by the injection of gonococcal toxin and not the result of gonococcal infection. The variability of reported results undoubtedly is due to the difference in virulence and toxin production of the various strains of gonococci as well as to the varied susceptibility of different animals of the same or different species and to the great change in the immunity of a single animal during the different seasons of the year.

With the exception of Wertheim's experiments all endeavors to produce experimental gonococcal infection in animals were made by the use of organisms grown on ordinary serum media. He grew the organisms on a medium made from homologous serum, thus hoping to utilize the adaptive properties of these microorganisms, however no attempt was made to increase the concentration of this homologous serum for the bacterial cultures.

Danyz (26) found rat serum highly bastericidal for anthrax bacilli but by growing these organisms successively in rat serum broth tubes he noted that they grew in more and more concentrated serum.
The organisms thus immunized presented very different cultural and morphological characteristics. In a similar way he immunized anthrax bacilli to arsenical preparations.

With the above observations in mind I decided to grow gonococci under similar conditions previous to similar injection before concluding that the animals were absolutely immune to such infection. A laboratory strain of gonococcus was transferred from human blood agar to freshly made rabbit blood agar and this culture in turn was transferred in 48 hours to a second rabbit blood agar tube of the same blood concentration. 15 such transfers were made and the organisms were then tested to ascertain their resistance to fresh rabbit serum in the following manner: A rack was prepared of small, clean, sterile test tubes containing an unequal amount of phosphate broth to which were added varying amounts of fresh rabbit serum to make the serum concentrations $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1/8 to 1/128. The tubes now all contain the same volume. To each tube was added the same measured amount of gonococcal emulsion which had been made from a 24 hour growth. As soon as each tube was inoculated it was shaken and a standard platinum loop full of the contents was plated on blood agar. The tubes were incubated at 37°C and plates were made at intervals up to 24 hours. The plate cultures were then incubated for 48 hours and the colonies counted. By this method it was found that the bactericidal titre of rabbit serum for gonococci is about 1/75, that is, the organisms are killed in 30 minutes in one part of rabbit serum to 74 parts of broth. This titre varies somewhat for the sera of different rabbits. 1/75 represents the average of four sera tested.
Table I presents the relative effects of rabbit serum on a strain of gonococci transplanted 15 times on rabbit blood agar compared with the effect on a strain of gonococci grown on human blood agar. It is seen that a serum concentration 1/16 can be withstood by the rabbit serum strain for 38 minutes, while the bactericidal action of 1/32 concentration is withstood for four hours and thirty minutes. The control organism did not live in any concentration of rabbit serum after a 38 minute exposure.

Similar experiments made after the twenty sixth successive transplant on rabbit blood medium show a still further increase in tolerance, since a 2 hour exposure in a 1/3 rabbit serum failed to sterilize and a 19 hour exposure in a 1/6 concentration did not kill all the organisms. These experiments were repeated on the thirtieth and also thirty fifth transplants on rabbit medium, but the tolerance of these gonococci were the same as those studied after 26 transplants. It would seem that this method of treating gonococci increases the tolerance for rabbit serum up to the twenty sixth successive transplant but further transplanting neither increases nor decreases this tolerance.

Presuming that the natural immunity of rabbits to gonococcal infection is due, in part to the bactericidal substances in the blood, one may expect that neutralizing the effect of such substances might render animals injected with these organisms more susceptible to infection than animals injected with ordinary organisms.

To ascertain what effect if any such immunized organisms would have on rabbits, many animals were injected during the process of immunizing these gonococci and the protocols of a few representative ones
TABLE I. Showing the relative lytic action of fresh rabbit serum on two cultures of the same strain of gonococci.

Rabbit blood agar- 15th successive transplant. H blood agar- 6th successive transplant - same strain.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; &quot; 38 min.</td>
<td>0: 0: 0: 0: 20: 25: 0: 0: 0</td>
</tr>
<tr>
<td>&quot; &quot; 4 hr</td>
<td>0: 0: 0: 0: 0: 8: 0: 0: 0</td>
</tr>
<tr>
<td>&quot; &quot; 22 hrs.</td>
<td>0: 0: 0: 0: 0: 0: 0: 0: 0</td>
</tr>
<tr>
<td>&quot; &quot; 38 min.</td>
<td>0: 0: 0: 0: 0: 0: 0: 0: 0</td>
</tr>
<tr>
<td>&quot; &quot; 4 hr</td>
<td>0: 0: 0: 0: 0: 0: 0: 0: 0</td>
</tr>
<tr>
<td>&quot; &quot; 22 hrs.</td>
<td>0: 0: 0: 0: 0: 0: 0: 0: 0</td>
</tr>
</tbody>
</table>
are here presented. For animal inoculation young rabbits under 800 grams in weight were used.

Rabbit No. 4. Injected intravenously, in 2cc of medium, the sediment from 8cc of rabbit kidney broth in which gonococci (10th transplant on rabbit blood agar) had grown for 48 hours. At the same time injected a similar amount into the right wrist joint, and injected twice this amount intraperitoneally. In 5½ hours a blood culture was negative and the peritoneal cavity positive for gonococci. The animal died in 24 hours after the injection and both the heart's blood and peritoneal fluid were sterile as were also the liver, kidneys and spleen. There was no evidence of infection about the wrist joint and the fluid was sterile.

Rabbit No. 2. Injected intravenously in 3cc of medium the sediment from 30cc of rabbit broth in which gonococci (10th transplant on rabbit blood agar) had grown for 48 hours. 24 hours after injection, animal sick, refuses to eat. 3 days after injection; animal eats but is in very poor condition; lost 110 grams in weight; blood cultures negative. 5 days after injection; lost 200 grams in weight; blood culture positive for gonococci; animal died 8 hours after the blood culture was made and no gross lesions were found at post mortem. The blood, spleen, liver and kidneys were sterile. It is of considerable importance to note that on the third day after intravenous injection the blood was sterile while on the fifth day a similar culture was positive for gonococci, leading to the conclusion that gonococci were not constantly in the blood stream for five days but were intermittently cast into the blood from some protected focus. Post mortem findings and cultures, however, failed to reveal the focus.

Rabbit No. 5. Injected intravenously in 3cc of medium the 30 hours gonococcus growth from 30cc of rabbit heart broth. The gonococci were the nineteenth transplant on rabbit blood agar. 18 hours after injection; rabbit very sick. Does not eat. Diarrhea. Blood cultures sterile. 48 hours after injection; animal dead. No post mortem findings. Blood, liver, spleen, heart and kidneys sterile.

Rabbit No. 10 to 17 inclusive. Injected intravenously with doses varying from 3cc to 5cc of medium containing gonococci grown for 72 hours on rabbit serum broth 1-16. Each cubic centimeter contains the growth from 10cc of medium. Gonococci from 19th transplant on rabbit blood agar. Blood cultures were made on all of the animals 20 hours after the injection and but one (No. 15) was positive and it became sterile within 48 hours.

Rabbit No. 9. Injected intraperitoneally the sediment in 8cc of medium, 48 hour gonococcal growth from 50 cc of rabbit kidney broth. 5 hours after injection: blood cultures sterile; peritoneal culture positive for gonococci; peritoneal cavity distended; contains extra cellular and intracellular organisms. 20 hours after injection: blood
cultures sterile; peritoneal culture positive for gonococci. 72 hours after injection: blood cultures sterile; peritoneal cavity contains clear serous fluid which is sterile; animal made a complete recovery.

Rabbit No. 18. Injected intravenously 2cc of sediment containing the 48 hour growth of gonococci in 15cc of rabbit kidney broth. The gonococcus was the 33rd transplant on rabbit blood agar. 2½ hours after injection: blood cultures negative. 8 days later: injected intravenously 1cc medium containing the 48 hour growth of gonococci from 15cc of rabbit heart broth (35th transplant). 24 hours after second injection: left eye looks edematous and larger than other eye. Blood cultures sterile. 48 hours after second injection: left eye still closed; exudate not so profuse as the day before; moderate hyperaemia of conjunctival and iris blood vessels; cornea and iris are smoky; pupil contracted to ½ the size of iris fellow; blood culture sterile. 72 hours after second injection: eye looks better but still hyperaemic and smoky; pupil contracted. 5 days after second injection: eye looks normal. 8 days after second injection: animal died; cultures from anterior chamber of eye gave few colonies of typical gonococci by culture and smear, the organisms could not be grown on transplants.

It was thought essential to further increase the tolerance of gonococci for rabbit serum before attempting further animal inoculation, therefore a second series of experiments was made using different concentrations of rabbit serum in broth as follows:

Gonococci were grown for 48 hours in rabbit serum broth 1/80. From this culture tubes of greater concentration of rabbit serum were inoculated and incubated for 48 hours. These transplants were thus continued every 48 hours gradually increasing the strength of the serum. The order in which cultures were obtained is here outlined:

1. Gonococci were transplanted from blood agar to rabbit serum broth 1/80.
2. In 48 hours a transplant was made from the 1/80 culture to a 1-40 concentration.
3. In 48 hours transferred from the 1-40 culture to a 1-10, 1-20, and 1-40 concentrations of fresh rabbit serum.
4. In 48 hours there was good growth in all three tubes and a 1-5 concentration was inoculated from the 1-10 culture.
5. Fair growth in the 1-5 tube in 48 hours, and a second 1-5 tube was inoculated from the 1-5 culture.
6. In 48 hours there was a good growth in the 1-5 tube and a 1-2 tube was inoculated from it.
7. Only a slight growth in 48 hours in the 1-2 tube so transfers were made from it to a second one.
8. In 48 hours there was moderate growth only but by five successive transfers an organism was obtained that
grew readily on fresh rabbit serum 1-2.

(9) Tolerance could not be increased constantly beyond this point, although one strain of gonococci was obtained that eventually grew readily on whole rabbit serum.

It is apparent that gonococci readily develop a fastness for the gonococcidal substances contained in rabbit serum. This adaptive ability is not altogether unlike the tolerance acquired by typhoid bacilli for normal rabbit serum. Feiler (27) has found that these bacilli readily acquire fastness for immune serum and normal rabbit serum by growing the organisms on an active normal rabbit serum medium. This fastness is lost by one transplant on plain agar, but is present much longer when passed through bouillon.

The fastness developed by gonococci for normal rabbit serum is an acquired characteristic much more difficult to remove since it took 25 successive transplants of a gonococcus tolerant to 1-2 rabbit serum on human blood agar before this fastness was completely removed. There were no cultural or morphological changes noted between the serum fast and normal gonococcus.

Gay and Claypole (28) noted, as have many others that rabbits injected intravenously with typhoid bacilli would have positive blood cultures for a long time. The varying success of different observers undoubtedly means that all strains of typhoid bacilli are not equally resistant, that all rabbits are not equally susceptible, and that the organisms are not constantly in the blood stream but enter it periodically from a body focus proven to be the gall bladder. The real typhoid carrier state could be produced only occasionally in rabbits by Gay and Claypole until they injected typhoid bacilli previously grown on rabbit
blood agar where this condition would regularly follow. A strain of
typhoid bacilli was produced which when grown on rabbit blood agar
continuously caused the carrier state when injected into rabbits, while
the same strain injected from plain agar produced no such condition.

The more serum gonococci were injected into rabbits in a
manner similar to that previously described for the less tolerant orga-
nisms. The following protocol will suffice to explain the results:

Rabbit No. 33. Injected intravenously the sediment in 1 cc of
medium of a 48 hour growth of gonococci from 15 cc of rabbit serum broth
(1-2). 48 hours after injection the blood culture was sterile. Animal
made complete recovery.

The finding of a positive blood culture 48 hours after intra-
venous injection has been previously reported but could not be obtained
in these experiments by injecting other than the serum fast organisms
and then only in three instances out of thirty animals. The ordinary
strain of gonococci could rarely be obtained from the blood twenty hours
after injection and usually had disappeared within five hours. After
intraperitoneal injections of serum fast organisms it is usual to find
gonococci in the peritoneal cavity for 24 hours. Blood cultures made
at intervals between 5 minutes and 48 hours following intraperitoneal
injections have always yielded negative results.

As previously mentioned one strain (#48) of gonococci grew
readily in fresh whole rabbit serum and this was used to inject intra-
venously a series of fifty rabbits. The blood was cultured in most
animals but in none was a positive culture obtained more than 48 hours
following the injection and in most instances the blood was sterile
within 24 hours. Animals were injected with this organism intraperi-
toneally, intraarticularly and subcutaneously but no lesions were regularly produced; however many rabbits of this series developed a peculiar lesion of one or both kidneys not seen in other animals normal or experimental. Since this lesion has not been produced regularly under the same experimental conditions and the organism has not been recovered from the lesions more data must be obtained before it can be considered a gonococcal renal infection or even the result of such an infection.

In all 132 rabbits were injected in various ways with various strains of gonococci and other than the specific instances referred to there has been no evidence of animal infection; indeed many deaths resulted, some immediately following the injection, others at intervals up to five days. All these, however, can be explained as the effect of the gonococcal toxin.

Believing that the bactericidal activity of rabbit serum had been largely overcome by increasing the tolerance of the bacteria used I have thought that there must be other factors working to render rabbits immune to gonococcus infection. Tolerance to one serum in vitro does not necessarily mean tolerance to an homologous serum in vivo, since Peterson (29) has found that dog serum contains almost no bactericidal properties for anthrax bacilli yet dogs are highly resistant to this infection, whereas rabbits serum is very bactericidal for anthrax bacilli yet these animals are readily susceptible to infection.

The high body temperature of rabbits may have some influence on organisms as heat sensitive as the gonococcus. This factor no doubt plays an important part in the rapid destruction of gonococci as shown by negative blood cultures five minutes after an intravenous injection;
on the other hand there is no such rapid death of the organisms when injected intraperitoneally. Organisms thus injected can regularly be recovered after 5 hours and usually remain present 24 hours. If the body heat were the only inhibiting agent then those organisms found in the blood stream and peritoneal cavity two and five days after an injection should be so adapted to this temperature that infection would result.

It must be conceded that gonococcal infection in rabbits has not been produced. The inconstancy of blood culture findings may be explained by the individual differences in the inhibitory activity of the various animals used whether this resistance to infection is due to the bactericidal activity of the rabbit serum, to the deleterious effect of the body heat of rabbits or to both it is impossible to say. It may be that either the mechanical or chemical action of fixed tissues acting alone or in conjunction with the previously mentioned factors render the rabbit non-susceptible to gonococcal infection.

CONCLUSIONS.

1. Rabbit serum has a marked bactericidal action on gonococcal cultures. The titre varies for different rabbits but averages approximately 1-75.

2. Gonococci readily adapt themselves to such bactericidal substances and by growing them on gradually increasing strengths of fresh rabbit serum broth gonococci will readily grow on serum broth 1-2.

3. One strain of gonococci adapted itself to whole rabbit serum in which it readily grew.
4. Successive transplantation of gonococci on rabbit blood agar increases the tolerance of these organisms for rabbit serum up to the twenty sixth transfer. Further transplantation neither increases nor decreases the tolerance already acquired.

5. This serum fastness of gonococci seems highly persistent since it took 25 successive transplants on human blood agar to cause its complete disappearance.

6. The serum fast gonococci remain longer in the bloodstream of a rabbit than an ordinary strain of gonococci. Positive blood cultures were obtained 48 hours after intravenous injection on three occasions and in one animal five days after injection. Ordinary gonococci does not so behave.

7. Positive blood cultures were not obtained after intraperitoneal injections of gonococci. The organisms could usually be recovered from the peritoneal cavity up to 24 hours but rarely later.

8. There is no conclusive proof of gonococcal infection having taken place in any of the 132 rabbits injected. Typical iritis developed in one animal 24 hours after intravenous injection. Gono-coccus like organisms were recovered but not completely identified. This lesion could not be reproduced by exactly similar methods.
References.


7. Centralbl fur Bakt 1897-Bd 22-305.


17. Arm. de gyn. 1899 "Le role de la toxine du gonocoque."

18. These de Paris 1898.

25. Kolle u. Wasserman Handbuch usw. 1912 Bd. 4-S 721.