STUDIES ON PNEUMOCOCCUS INFECTION IN ANIMALS.

SECOND PAPER: ACTION OF IMMUNE SERA ON PNEUMOCOCCUS INFECTION.*

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In my first paper on "Studies on Pneumococcus Infection in Animals" I summarized in the introduction the significant results of previous work and then recorded my experimental studies on the action of the pneumococcus on animal tissues and of immune sera on the pneumococcus. The present paper deals with the action of immune sera on pneumococcus infection and with the mechanism of recovery in animals.

The earliest observers, Foa and Carbone, Emmerich and Fowitsky, Kruse and Pansini, G. and F. Klemperer, and many others mentioned in the introductory summary of the first paper, record experiments showing the effect of immune serum on pneumococcus infections of animals and human beings. G. and F. Klemperer (1) treated inoculated mice with immune serum which they obtained from animals immunized with culture precipitates. In these experiments and in a limited study of the treatment of pneumonia in human beings they found this serum effective and considered its action antitoxic. Although these results were considered favorable by the Klemperers, they have recorded no other experiments. Similarly, on the basis of favorable effects in studies on animals several observers, notably Pane (2), Römer (3), and Neufeld (4), have since urged the use of immune sera in pneumococcus infection of man. But in the hands of others, these sera have failed to yield convincing results. Thus, previous attempts to secure an efficient serum for the treatment of pneumonia in man

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have failed. It is evident, however, from all this study that immune sera have a definite though limited action on pneumococcus infection.

In animals this is manifest under certain conditions of experiment. Immune sera mixed with the virus, or given before or simultaneously with the introduction of the virus, exert marked protective properties. Infection does not develop, or if it does, it is evanescent and not fatal, the result depending on the activity or dosage of the serum and the strength of the virus. Experiments of this kind have given uniformly definite results and form the basis of many of the recommendations for the treatment of man.

When immune sera have been given after infection has become established they have been found to possess little curative action. If treated very early, many animals are saved, but this action is more protective than curative. Occasionally the infection is prolonged and a few animals are saved. The results of experiments of this character are conflicting. Comparatively few observers claim significant practical results, and the great majority acknowledge failure.

In order to determine why immune sera possess marked protective, but slight curative action, the following study was made of the action of immune sera on pneumococcus infection in the rabbit.

STUDIES ON THE CURATIVE ACTION OF IMMUNE SERA ON PNEUMOCOCCUS INFECTION OF THE RABBIT.

From the study of the action of dead pneumococcus cells and of culture filtrates recorded in the first paper, it was found that although this culture material did not contain the active poisons formed by the organisms in infection, substances of a similar or a related nature were present. These gave rise to immunity in animals. In the following experiments the serum of these animals was tested as to its curative value.

Experiments with Antibacterial Sera.—In order to exclude all soluble products of the pneumococcus cell metabolism, it was necessary to use, not only washed pneumococcus cells, but dead cells to prevent the development of soluble products in the body tissues.
after inoculation. Broth cultures after growing for twenty-four hours were centrifugalized, the sediment shaken in salt solution, and again centrifugalized. This sediment after being taken up in a small quantity of salt solution made a dense, cloudy suspension, representing a concentration of the culture to one tenth of its original volume or less. To kill the pneumococci these suspensions were exposed in a bath at 52° to 55° C. for twenty minutes.

With this material rabbits were immunized by intravenous inoculation. Two weeks after they had fully recovered from the last inoculation, they were bled and the curative value of the serum was tested by treating rabbits that had been inoculated with virulent pneumococci.

These experiments need not be cited in detail because in none was there definite evidence that this serum possessed greater curative action than normal rabbit serum. In one experiment a few of the treated animals died before the untreated controls.

**Experiments with Antitoxic Sera.—** In the previous study of dead culture material, filtrates of the cultures incited an immunity that was proportionately greater or more active against virulent infection than that obtained with concentrated cultures or pneumococcus cells in salt solution. Since antibacterial or endotoxic sera failed to prove of any practical value, attention was turned to the study of the antitoxic serum obtained from animals immunized with culture filtrates.

In order to avoid, as far as possible, the liberation into the culture fluid of endotoxins from the pneumococcus cells, twenty-four hour broth cultures were filtered through a Berkefeld filter.\(^1\) These filtrates were tested by subculture in broth to prove their sterility. They were then used for immunization by intravenous inoculation and the curative action of the serum from these animals was tested as in the previous experiments with antibacterial sera.

Many of these experiments, like the previous ones, need not be

\(^1\) The pneumococcus undergoes early autolysis in culture even under the most favorable conditions for growth, but this is not apparent until the growth is on the wane. Estimates of the rate of pneumococcus growth in broth suggest that this point is reached shortly after twenty-four hours, depending on the conditions present. Autolysis is apparent after the second and third days, depending also on the conditions present.
cited in detail for the results failed to show that these antitoxic sera had much practical curative value. The course of the infection in the treated animals, however, was occasionally materially prolonged. There were also signs of improvement in the condition of the animals and occasionally a critical fall in the temperature following the serum treatment. As studied from the records, these signs were not convincing. But as seen in the animals they were striking enough to suggest the presence of antitoxic immunity in pneumococcus infection.

For an instance of this the following experiment is cited.

Experiment 1.—June 6, 1911. Two rabbits were inoculated intravenously with 1.5 c.c. of a culture of pneumococcus $R$, which was exceedingly virulent, and similarly, two rabbits were inoculated with cultures of pneumococcus $H$, which was less virulent. One of each set, seemingly the sickest, was chosen for treatment with serum $A$ obtained from an animal highly immunized with culture filtrates of pneumococcus $R$.

5 c.c. of this serum was given six and a quarter hours after inoculation,—too late to have any material effect on the infections with culture $R$, but not too late to affect the infections with culture $H$.

Of the two animals inoculated with the homologous culture pneumococcus $R$, the control died in eight hours and the treated animal in less than twenty-four hours. Taking the control as a criterion, the serum was given ante mortem.

Of the two animals inoculated with the heterologous culture $H$, the control died in less than twenty-four hours, but the treated animal lived. The bacteriemia persisted and on the third day the animal was given a second dose of the serum. Signs of infection were slight and the temperature fluctuations evanescent after the first day.

As shown by the blood cultures this antitoxic serum had little or no effect on the bacteriemia.

Other sera of this type were less active or the infections were too virulent to show a curative action. In order to secure more definite experimental evidence of the presence of antitoxic immunity in pneumococcus infection, a study was made of it in its active form.

Animals were partially immunized by two doses of culture filtrates, and for purposes of comparison culture sediment in relatively larger quantities was similarly inoculated into animals. After an interval the immunity of these animals was tested by inoculations of virulent organisms. The experiment is recorded in detail in table I.

Although the curative action of these sera was slight the protective action, when the serum was mixed with the culture before inoculation, was well marked.
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Experiment 2.—February 9, 1911. After twenty-four hours' incubation, 300 c.c. of a pneumococcus R broth culture were centrifugaled. The supernatant clear culture fluid was decanted and filtered free of bacteria for immunizing purposes (A). The sediment or concentrated culture was divided in two portions: 5 c.c. was heated to 52° to 55° C. for twenty minutes to kill the bacteria before using them for immunization (B); 10 c.c. was precipitated with 60 c.c. of alcohol and centrifugaled, and the precipitate was partly dissolved in 20 c.c. of distilled water and again centrifugaled. The clear solution (C) and the sediment (D) shaken with water were used. Thus, for the purposes of this experiment, A represents immunization to soluble products; B, concentrated culture killed by heat; C and D, concentrated culture killed and precipitated by alcohol. Thirteen days after immunization the eight animals and two fresh normal animals received intravenously virulent cultures of the pneumococcus R.

In the immunization procedure of this experiment as recorded in table I, material A (culture filtrate) and B (culture sediment heated to 52° C.) failed to cause a toxic reaction in the pneumococcus immunization without living organisms. Material C and D, each derived from culture sediment precipitated with alcohol, gave rise to unusually severe toxic reaction, but without elevation of temperature. The immunization attained by these two inoculations varied in degree and kind. When compared with their controls this immunization was definitely marked in all the animals. The two controls died in less than twenty hours after inoculation. The immunization by culture filtrate differed, being less effective in degree and yet sufficient to neutralize the products of the infectious agents for a time. In one instance this was inadequate, the animal dying in forty-eight hours, but showing modified fever reaction; in the other instance it was adequate, the animal having no fever or signs of infection and ultimately recovering. The infectious agents persisted for nine days as harmless parasites.

The blood of the other animals at this time proved sterile, and it is also interesting to note that although the immunity produced by culture filtrate A failed to prove as effective against the infection, the disease reactions on the whole, as compared with the others, especially with the immunity obtained with culture sediment B, were more completely neutralized. The immunity of C and D for present purposes was quite similar to that of B.

The presence of an antitoxic immunity was thus established. The culture fluid of the pneumococcus after growth for twenty-four hours contains substances capable of inciting an immunity in the tissues which will so neutralize the products of the pneumococci present in the tissues that they give rise to few or no signs of disease. Yet neither with culture filtrates nor with dead pneumo-

1In this experiment the animals immunized with culture sediment B, C, and D, received, as above recorded, proportionately greater quantities of material.

4This action and reaction between soluble pneumococcus poison and the immunity suggests immediately the toxin and antitoxin reaction of diphtheria. The two are doubtless similar if not identical in nature, but the toxin of diph-
coccus cells could this immunity be sufficiently exalted to yield sera of any definite practical value in the treatment of infection in animals.

In cultures the pneumococcus does not develop the very active poisons it forms in infection; hence my study turned to that of sera derived from immunization with living cultures.

*Experiments with Sera Obtained by Immunization with Living Cultures.*—Since the intravenous inoculation of culture filtrates had proved so effective in immunization, rabbits were first thoroughly immunized with these; then, in increasing doses, the living virulent cultures were substituted until the animals withstood the inoculation of large quantities without giving any signs of reaction. The blood serum was tested as in the previous experiments with antitoxic and antibacterial sera.

For convenience and to show in detail the effect of these sera the results of certain typical sets of experiments are tabulated. In table II (experiment 3) is shown the effect of repeated treatment. Three one cubic centimeter doses of immune serum and three of normal serum were administered at intervals during the first twenty-nine hours of the infection. In table III (experiment 4) is shown the effect of a single dose of two cubic centimeters given to different animals at varying intervals after the inoculation of five times the quantity of virus used in table II. And table IV shows the curative action of this serum on homologous and heterologous infections.

*Experiment 3.*—June 13, 1911. Of ten normal rabbits, each received 0.05 c.c. of an extremely virulent culture of pneumococcus R. Two rabbits were untreated and two were treated with normal rabbit serum. These four animals served for control. The other six were treated with serum B (from rabbits highly immunized with living cultures).

In table II the virulence of the organisms is well shown in the two controls which died eighteen and twenty-one hours respectively after inoculation. The slight but inadequate protective value of normal sera is also well shown by the death in forty-eight hours of each of the two treated animals.

chemically, is a powerful poison that is fatal for animals, and this poison may be titrated accurately against its antitoxin, whereas the soluble substances of the pneumococcus are not fatal and are recognized only by the immunity incited by their presence. Moreover the immune products can be measured only by their activity against infection.
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The curative value of the immune serum is clearly demonstrated by the complete recovery of four of the six animals receiving this treatment. The two that died lived five and seven days, i.e., two and a half, and three and a half times as long as the controls similarly treated with normal serum. Although this serum was administered in these small doses, it never failed to produce marked betterment in the animals' condition, and the temperatures taken after the serum treatment are lower than those in the animals treated with normal serum.

It is therefore evident that both normal rabbit sera and immune sera exert a beneficial action when used in the treatment of infected animals. The effect of the serum from the normal rabbit is slight and evanescent. But in the serum from the rabbit highly immunized with living cultures this action may be greatly exalted, and may modify and prolong the course of fatal infections, or speedily effect permanent cures.

In order to determine the effectiveness of a single dose of serum administered at varying intervals after infection was established, in the following experiment (table III) were employed double the dose (two cubic centimeters) of immune serum, and five times as much of the culture for inoculation, as was used in table II.

Experiment 4.—June 10, 1911. Ten rabbits were inoculated with 0.25 c.c. of this virulent culture of pneumococcus R, and subsequently at intervals of a half hour, one hour, three hours, and seven hours; all but two of these rabbits received 2 c.c. injections of serum B obtained from rabbits which had been highly immunized with living cultures of pneumococcus R.

In table III the results of this experiment show very clearly the effect of 2 c.c. doses of immune serum, each given at varying intervals after the virulent inoculation of 0.25 c.c. of pneumococcus culture. The fall in temperature of the treated animals was often marked, and strikingly uniform. The controls died in less than twenty hours. The animals treated seven hours after inoculation died in forty-three and forty-four hours. Of those treated three hours after inoculation one lived and one died in seventy-two hours. All those treated half an hour and one hour after inoculation recovered.

Under the conditions recorded in table III, and with the same quantity of virus and the same dosage of serum it is evident that three hours after the injection of the virus is the limit of delay in treatment which will allow of a cure by a single dose of the serum.

If serum treatment is to be effective, it must be given within a certain interval after the inoculation of virulent organisms; after this interval has elapsed, treatment is ineffective. This is true alike in infection and in toxemia without infection.

Soon after the toxins of diphtheria and tetanus are injected, they are so fixed by the animal cells, or the animal cells are so in-
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juried, that recovery is no longer possible and antitoxin fails to cure. In the toxemia caused by the tetanus and diphtheria toxins, the time required for the fixation of the poison or for the injury to the cells is definite, but depends within certain limits on the quantity of toxin injected and on the susceptibility of the animal tissues to the toxin. In generalized pneumococcus infection of the rabbit, the development of the bacteria and their virulence complicate still further the conditions present in toxemia without infection.

In order to determine the effect on homologous and heterologous infections of serum from animals immunized with living pneumococci the following experiments were carried out with the homologous organism R and the heterologous organism H.

**Experiment 5.**—Four animals were inoculated intravenously with 0.5 of a cubic centimeter of an extremely virulent culture of R, and three with a less virulent culture of H. Immune sera derived from immunization with living cultures of R were given intravenously three hours after inoculation, each dose being one cubic centimeter. The records of this experiment appear in table IV.

In experiment R, recorded in table IV the controls died in less than eighteen hours. Of the treated animals one died on the third day, the other recovered. Against 0.5 c.c. of this virulent homologous culture a single dose of 1 c.c. of this serum given three hours after inoculation cured one animal and caused the infection in the other to be nearly four times as long as that in the control.

In experiment H the control died on the fifth day. Against 0.5 c.c. of this heterologous culture a single dose of 1 c.c. of this serum given three hours after inoculation induced crisis and effected a complete cure in both animals. Animal III died from an accident. At autopsy, despite extensive hemorrhage into the muscles of the neck, no pneumococci were present.

It is evident from these experiments that immune serum obtained by immunization with living cultures may be, like the antitoxic serum (see page 81), active on both heterologous and homologous infections, but the action here is more marked than that of the antitoxic serum.

**SUMMARY OF THE STUDIES ON THE CURATIVE ACTION OF IMMUNE SERA ON PNEUMOCOCCUS INFECTION.**

In one set of experiments pneumococcus cells washed free of soluble products were killed by heat at 52° C. and injected into animals. In another set of experiments culture filtrates freed of pneumococcus cells that had been growing for twenty-four hours
were employed. Both sets of experiments gave rise to an immunity in the body tissues. This immunity in the tissues was well marked in each instance, but in certain respects the immunity produced by the injection of the dead cells differed from that induced by the injection of the cell-free filtrates. After immunization with culture filtrates the tissues acquired an immunity which was distinctly selective and antitoxic, for in animals immunized in this way the toxic products of the pneumococci were neutralized and the surviving parasites became harmless. On the other hand, after immunization with pneumococcus cells, the tissues acquired an immunity which was effective in its active form but one which was stronger in its bactericidal action on the pneumococci in infection and weaker in its antitoxic action than the immunity induced by the culture filtrates. Nevertheless, as the pneumococcus cell contains some of the substances present in the culture filtrates, the immunity produced by dead cells may be, to a certain degree, antitoxic.

The immunity to the pneumococcus cells and to the culture filtrates was thus readily studied in its active form, but it was never sufficiently exalted by immunization with this culture material to yield potent sera. The antibacterial sera had little or no curative action, the antitoxic sera proved only slightly better; and neither gave promise of practical usefulness.

The serum obtained with living virulent cultures, however, proved by contrast definitely curative. Although no attempt was made in these experiments to measure the extreme limits of its curative action, the results leave no doubt of its activity against both homologous and heterologous infections.

These sera varied greatly in their curative action: some proved efficient, others of little value. With improved methods of immunization with pneumococcus R this variation was less marked. Other strains of the pneumococcus were apparently less serviceable. Whether this was due to differences in the methods used or to differences in the strains was not clearly determined.

It was thought that certain strains of the pneumococcus were more toxic than others. Certainly some virulent organisms gave rise to extensive hemorrhagic lesions not present in other fatal infections. The organism R, used so frequently in these experi-
ments, was of this toxic type. But, important as it is to solve these questions, they are scarcely within the scope of this paper.

Having clearly demonstrated the limitations in the usefulness of certain immune sera and the marked efficiency of others, an attempt was made to determine how these effected cure.

In the recovery from infection the protective mechanism acts in two ways. It neutralizes the products of the infectious agents, and destroys the bacterial cells. The toxins, the endotoxins, the aggressins and the leucocidins are neutralized in the specific reactions of immunity, and the bacterial cells are destroyed by lysis extracellularly or by phagocytosis intracellularly.

How much is to be gained by the present complex conceptions of the protective activities is not yet clear, but they have already contributed much valuable information to our knowledge of the manner in which the adaptive reactions of immunity take place. Nevertheless, how fundamental all these distinctions are, or how they will hold in the light of complete knowledge, is still to be determined. Although extremely important and of great theoretical interest, these finer distinctions of the minor, or secondary, phases of the protective mechanism have not only failed to yield practical results in the cure of disease, but have often proved misleading, and particularly so in the attempts to cure pneumonia in man.

The simpler conceptions of the early observers, now so long discarded, are far more practical. In these, toxin and antitoxin neutralization are dominant, but a convincing experimental demonstration of their presence has been lacking. And yet it is difficult to interpret immunity to culture filtrates in any other way. In the filtrates of cultures that have grown for twenty-four hours substances are present which give rise to immunity. This fact renders scarcely tenable the classification of these substances as endotoxin, as aggressin, or as antigen for the development of lytic or opsonic activities. It would be far more reasonable to classify in this way diphtheria toxin which is obtained from cultures after several days' growth. Failure of these substances to prove as markedly toxic as diphtheria or tetanus toxin does not exclude their being of a related or similar nature. Diphtheria toxin may be so altered through degen-
eration or under experimental conditions that it no longer is actively
toxic, but in the tissues it is still capable of producing antitoxin
which will neutralize active toxin. Similarly non-virulent strains
of the diphtheria bacillus give rise to substances not actively toxic
but capable of producing antitoxin. In fact it is by these reactions
that non-virulent and non-toxic strains are identified.

But in my experiments thus far recorded, toxin and antitoxin
reactions were clearly recognized only in the active phases of im-
munity. In passive phases the evidence was suggestive, but uncon-
vincing and of no practical moment. It was only with serum ob-
tained by immunization with the living pneumococci that the pro-
tective mechanism was manifested experimentally in passive phases.
With serum derived from living organisms as compared with that
obtained by culture filtrates the reactions are complex. In these
complex reactions, factors other than toxin and antitoxin neutrali-
zation might well be conceived as active or even dominant. In order
to determine this the mechanism of recovery was studied.

In studies of lysis and phagocytosis recorded in the first paper,
phagocytosis was practically absent in the rabbit. Typical pneu-
monic lesions do not develop in the rabbit, whereas they are readily
induced experimentally in the dog. Accordingly, in seeking light
on what actually takes place in the pneumonia of man, I studied the
mechanism of recovery in the dog instead of in the rabbit.

STUDIES ON THE MECHANISM OF RECOVERY IN THE DOG.

In an unpublished study, made by the author in 1905, of the con-
ditions underlying the development of organizing pneumonia, ex-
tensive lesions of lobar pneumonia were readily induced by insert-
ing a catheter into the trachea of etherized dogs and injecting
virulent cultures of pneumococci through it to any desired part of
the lung. This method was therefore adopted in these experiments
on dogs.

Experiments on Lung Infection of the Dog.—On February 20, 1911, two dogs
were etherized and 30 c.c. of a culture of pneumococcus R from a dead rabbit
were injected through the trachea. Dog 1 was not sick the first day after the
injection, but became sick on the second day, and on the third day had a
temperature of 104.6° F. Dog 2 was very sick at first with a temperature of
104.5° F., but made a quick recovery and was practically normal on the third
day after the injection, when both dogs were pithed and autopsied.
In dog 1 extensive lesions of hepatization in the lower right lobe were found; in dog 2, circumscribed areas of consolidation. The heart blood, and the exudates squeezed from the pneumonic lesions and from the pleura, were centrifugalized in citrate solution, the leucocytes pipetted off, again centrifugalized in salt solution, and the sediment smeared and examined by Jenner's stain for evidences of phagocytosis. No pneumococci were found inside or outside the leucocytes.

In cultures of the blood and of the lung exudate, pneumococci grew in small numbers, but plates made of small amounts of blood and exudate were sterile. The results of the study in the two dogs were similar despite the different stages of the infection. In dog 1 there were extensive lesions of red hepatization, in dog 2 circumscribed lesions.

The efficiency of the dog's protective processes was thus clearly demonstrated, but no clue was left to show the mechanism by which the infectious agents had been destroyed.

On March 15, 1911, two dogs were etherized, and 35 c.c. of broth culture from a rabbit dying from pneumococcus R infection were injected through the trachea. These dogs were prostrated, but recovered slightly by the third day, when one was treated intravenously with 15 c.c. of immune serum from an immunized dog, and two hours later both were pithed, autopsied, and studied as in the previous experiment. No signs of phagocytosis and in fact no bacteria were seen in the smears of leucocytes centrifugalized from the blood or in the exudates from either animal. In cultures made from the blood and exudate of the untreated animal, one pneumococcus colony was found in 2 c.c. of blood and 344 colonies in 1 c.c. of the exudate. This animal's right upper lobe was completely hepatized and was in the early red stage. In the other animal before treatment two pneumococci were present in 2 c.c. of blood, but two hours after treatment the blood culture of this dog was sterile. The lung exudate also was sterile. In this second animal, the right lower lobe was completely hepatized, and was similar to that of the first animal. Whether the results in this dog are to be attributed to the serum treatment or to a more efficient protective mechanism is not apparent.

In this, as in the previous experiment, the protective mechanism of the dog was efficient even in these extensive early lesions and by the third day evidences of the means by which the infectious agents were eliminated had practically disappeared. The quantity of culture inoculated was, therefore, increased and the study was made at the end of twenty-four hours instead of seventy-two hours.

On March 24, 1911, three dogs were etherized, and the tracheal injections of pneumococcus R culture were made proportional to the weight of the dogs, 50 c.c. being injected for each 10 kilos. One dog was untreated. Each of the other two received intravenously 18 c.c. of the serum from a highly immunized goat. In these dogs the temperatures rose to 104° F. and 105° F. In the two that were sickest, physical signs of pneumonia were easily made out, but in the other dog these were doubtful and it was accordingly selected as the control. All three dogs were pithed and autopsied twenty-four hours after inoculation.
In the untreated animal there was a small area of pneumonic consolidation in the lower right lobe. Cultures from the heart's blood and from the exudate were sterile. Although this exudate was sterile, by morphological study a few pneumococci were found inside the leucocytes. Both of the treated animals had extensive lesions of red hepatisation in the right and left lower lobes. The blood of one was sterile before the serum treatment and also two hours after it. In the blood of the other, six colonies per cubic centimeter were found before treatment, and twelve colonies two hours after treatment. No signs of phagocytosis were seen in the leucocytes of the blood, but in the lung exudate of both animals signs of phagocytosis were marked, and in culture innumerable colonies grew.

Again in this experiment the action of the serum, which in other tests both in rabbits and in dogs had proved efficient, was not clearly shown. But the protective mechanism and the activity of phagocytes was clearly demonstrated. Evidently the protective mechanism is so efficient and yet so variable that even under these experimental conditions, it is almost as difficult to measure the action of the serum in the recovery of the dog as it is in that of man.

Previous experience with experimental pneumonia in more than seventy-five dogs which were used in other studies on organizing pneumonia, had shown that, apart from the fact that recovery in dogs is so frequent, practically the rule, neither recovery nor death could be definitely foretold.

Meltzer and Lamar (5) note a mortality of 16 per cent. in their series of fifty dogs. It occurred to me that if the pneumonic infection of the dog were taken practically ante mortem the effect of serum treatment might be more apparent.

In two series of my experiments, each consisting of ten dogs, the animals were inoculated through the trachea and their temperatures were carefully watched. After an evanescent rise to 104° F. or 105° F. on the day following the inoculation the temperatures of the animals were practically normal. Although many were extremely sick, all but two recovered. This was attributed to lack of virulence for the dog in the cultures used.

This pneumococcus R had been kept in rabbits by continuous passage. A strain was accordingly passed through four dogs to exalt its virulence for this animal. But virulence is apparently not so readily increased in the dog as it is in the rabbit. Twenty-five
cubic centimeters inoculated intravenously gave rise to an infection that was fatal in three weeks, although in the dogs from which this culture had been isolated the same dose had killed in forty-eight hours. Dogs evidently vary in susceptibility far more than rabbits. The effort is now being made to exalt the virulence of pneumococcus R for dogs to such an extent that it will invariably prove fatal in these animals, but in this attempt I have not yet succeeded.

Experiments on Bacteriemic Infection of the Dog.—Since it was not possible with the cultures at hand to secure uniformly fatal lung infection of the dog a study was made of the bacteriemia after large doses of concentrated culture material.

I. On April 10, 1911, a normal dog received intravenously 10 c.c. of a dense suspension of centrifugalized pneumococcus culture. Before the inoculation of the pneumococci, the blood was sterile and the leucocytes free from phagocytosis. Twenty minutes later in 1 c.c. of blood 68,400 pneumococcus colonies were found, and in the leucocytes, definite signs of a moderately active phagocytosis. Fifty minutes after inoculation, the blood contained 6,000 pneumococci per cubic centimeter and signs of a more active phagocytosis were present in the smears of the leucocyte cream. In about forty hours the animal died of the infection. In the blood at autopsy very few pneumococci and no signs of phagocytosis were found. The leucocytes were comparatively few in number and in many of them signs of degeneration were marked.

II. On the 15th of March, 1911, a normal dog was etherized, the jugular vein was exposed and clamped, and into it in the direction toward the heart was inoculated 5 c.c. of a dense suspension of virulent pneumococcus cells obtained by centrifugalizing 250 c.c. of broth culture. Thirty minutes later blood was drawn from a place in the vein that was well above the site of inoculation. Part of this blood was diluted and plated. It contained 51,670 pneumococci in 1 c.c. The remainder was centrifugalized in citrate solution, the cream pipetted off, centrifugalized again, and then examined in smears for phagocytosis. No free bacteria were seen and very few were found inside of the leucocytes, none of which contained more than two cocci. Signs of phagocytosis were extremely scarce at this stage. Two and a half hours after inoculation the examination of the circulating blood was repeated. There were only 384 pneumococci per cubic centimeter, and relatively few signs of phagocytosis despite the enormous number of pneumococci that had been disposed of.

Evidently the activity of phagocytosis in normal dogs varies considerably, for in this animal, as compared with the previous one, the difference was marked. These, however, represent the extremes of the results of experimentation on the unimmunized dog.
On March 16, 1911, the day following the inoculation of the virus, this second dog was greatly prostrated, and although there was no fever, it was expected to die. On the third day, 35 c.c. of the serum of an immunized dog was injected intravenously. In the blood before the serum treatment nineteen pneumoccci were found per cubic centimeter, and after treatment, four. No evidence of phagocytosis was seen, but this might have been due to the small number of bacteria present. Following this treatment, however, the animal recovered rapidly and five days later was again the subject of an experiment.

On March 23, the pneumococci from 250 c.c. of broth culture suspended in 7 c.c. were inoculated intravenously into the dog used in the last experiment, and twenty-five minutes later the animal received intravenously 35 c.c. of immune goat serum. This serum caused slight hemolysis. Before the inoculation of the virus the blood was sterile. Fifteen minutes after inoculation and ten minutes before the serum treatment, 1 c.c. contained 436,350 pneumococci. Phagocytosis was marked. An hour after inoculation and a half hour after the serum treatment 1 c.c. of blood contained only 32 pneumococci, but phagocytosis was if anything still more marked.

On March 13, 1912, a year later, this animal was again studied, after having been left untreated for ten months. The pneumococci (R) in 200 c.c. of broth culture were concentrated, suspended in 20 c.c. of the culture fluid, and inoculated intravenously. One and a half hours after inoculation, blood was drawn from another vein. In a plate containing 1 c.c. of blood, 5,088 colonies of pneumococci were found, and in a plate containing 0.03 c.c. of blood, 3,600 per cubic centimeter. No signs of phagocytosis were seen in the leucocytes obtained by centrifugating 2.5 c.c. of blood in citrate solution. These leucocytes, however, gave evidence of the extent to which the animal was poisoned. Many stained faintly, many others appeared as shadow forms, and a few were granular and disintegrating. In short, the signs were those of the toxemia so frequently noted ante mortem in both dog and rabbit, or seen when these animals are seriously poisoned. This dog after inoculation was nauseated and greatly prostrated, but recovered.

By studying this one animal, first in the unimmunized state when phagocytosis was slight, then after an active immunization when phagocytosis was marked, and again a year later when, in the latent stage, phagocytosis was absent, not only is the relationship of phagocytosis to the immunity apparent, but it is evident that the immunity must be in an active state to prove effective in bringing about phagocytosis. When even the immunized dog is badly poisoned, phagocytosis is in abeyance, and the destruction of the pneumococci is then extracellular.

This is an excellent illustration of how variable the growth of the pneumococcus is in plates of agar. The two plates were inoculated and poured under identical conditions. The presence of the blood determined the difference in the results; in the plate containing 0.03 c.c. of blood, many of the pneumococci failed to develop.
A bull terrier that had been well immunized on March 8, 1911, was ether-
ized, 400 c.c. of blood were taken, and the jugular vein was tied. In the stump
toward the heart 22 c.c. of a dense suspension of the pneumococci centrifugal-
ized from 500 c.c. of broth were injected. In the blood drawn fifteen minutes
later, 385,416 pneumococci per cubic centimeter were found and there was an
active phagocytosis. No signs of degeneration were seen in the leucocytes and
nearly all contained bacteria. Some leucocytes enclosed as many as 50 pneu-
mococci, and 71 leucocytes taken at random gave a phagocytic index of 5. The
dog died in forty hours from the combined effects of bleeding and inoculation.

At autopsy the blood contained 230 pneumococci per cubic centimeter. Signs
of degeneration were marked in the leucocytes, and the number of these cells
was greatly diminished. In one leucocyte the shadow form of a diplococcus
was found. With this exception no signs of phagocytosis were seen in the
smears.

In this immunized animal phagocytosis was at first extremely
active. Then, as the toxemia developed, phagocytosis disappeared
and was absent ante mortem. Even after this overwhelming infec-
tion the bacteriemia at autopsy was slight compared with that ob-
tained in the rabbit.

**SUMMARY OF THE STUDIES ON THE MECHANISM OF RECOVERY IN
THE DOG.**

These experiments demonstrate the importance of phagocytosis
as a means of disposing of the pneumococci in the infection of the
dog. Both in the lesions of the lung and in the blood phagocytosis
plays an active part in the mechanism of recovery. The difference
in the activity of phagocytosis in the normal dog and in the immu-
nized animal is striking. In the normal dog phagocytosis may be
practically absent and in this respect the dog may resemble the
rabbit. But phagocytosis is greatly augmented in the actively, and
also in the passively immunized animal. Ante mortem, phagocy-
tosis is absent in both normal and immunized animals. It is also
absent in the highly immunized animal when the immunity has long
been dormant and the unneutralized poisons act on the body cells.

Thus it is evident that phagocytosis in the dog depends very
largely upon the extent to which the toxic products of the pneumo-

*If there are 10,000 leucocytes to 1 c.mm. of blood and each takes up as few
as five bacteria, 500,000,000 per c.c. are disposed of in this way. In the blood
culture fifteen minutes after the injection, 385,416 pneumococci were found in
1 c.c. of blood.
Studies on Pneumococcus Infection in Animals.

coccus are neutralized. Hence it is that although phagocytosis may be a dominant process in the disposal of the pneumococcus cells it is secondary to the specific reactions which take place between the protective substances of the body and the poisons of the bacteria.

As a means of studying phagocytosis in pneumococcus infection, these experiments were especially successful, but in many other respects the results were unsatisfactory. The dog’s protective mechanism was so extremely efficient that demonstrations of the action of immune sera were not convincing. Conditions present before treatment with sera and those present after, were not sharply contrasted in the dog. The differences noted were those of degree only and were not so definite as they were in the rabbit. After infection became established the bacteriemia was never so marked as in the rabbit. On the contrary, following the injection of the culture, the pneumococci were invariably destroyed in large numbers and relatively few survived in either treated or untreated animals. The serum simply hastened this destruction of the pneumococci. Even ante mortem the organisms were not numerous. Although the dog developed typical lung lesions, there was no systemic reaction with sustained temperature and no crisis. This was true also of the bacteriemic infection of the dog.

Resistant animals, such as the dog and man, react locally alike, but differ in their systemic reactions. In these reactions man and the rabbit resemble each other more closely.

STUDIES ON THE MECHANISM OF RECOVERY IN THE RABBIT.

In order to study in detail the effect of more active curative sera it was necessary to confine the experiment to a single animal. To control the results, however, the animals were taken in series. In these the control and the test animals alternated. Each rabbit was inoculated with cultures isolated from the one preceding it. An excessive inoculation, five cubic centimeters in each instance, was purposely used to secure exaggerated conditions of bacteriemia and toxemia. There were three controls, and three animals treated with immune sera.

Of the controls, one was left untreated, another was treated with
normal rabbit serum, and the third with leucocyte extract prepared according to the method of Hiss, but of double strength. In the studies of Hiss and in several of my experiments with pneumococci of moderate virulence, this leucocyte extract materially modified the course of infection in the rabbit. It was more active than normal serum, and in the experiments of Hiss it was more efficient than immune serum. But owing to the excessively virulent inoculation in this series of experiments the leucocyte extract caused no modification in the course of the infection. Hence, with normal serum, leucocyte extract made an excellent control.

Experiment of March 22, 1911.—Three animals were treated with immune sera. The supply of the most active serum, designated B and obtained by immunization with living cultures, was limited, and at the less important points in the treatment another serum, A, derived from immunization with culture filtrates, and thus purely antitoxic in action, was substituted for it. That this other serum had to be employed was unfortunate from the point of view of securing complete cures, but was fortunate for purposes of comparison.

Treatment was given intravenously three hours after inoculation in the controls and in the test animals alike except in animal IV. In this instance, on account of the extreme prostration of the animal, it was unwise to wait longer than two and one half hours. Before each serum treatment and again two hours afterward blood cultures were taken in all animals. In the case of the untreated control, they were taken two hours after inoculation. This blood was also centrifuged in citrate solution and the leucocytes were examined for signs of phagocytosis.

The records of the experiment appear in table V.

In the experiments recorded in table V the inoculation of 5 c.c. of the culture was excessive. In all the animals the effects of the poison were shown by immediate prostration, panting respiration, and very rapid heart action.

The controls, whether treated with normal serum, or with leucocyte extract, or left untreated, died in twelve to eighteen hours. The bacteremia, as shown by the blood cultures, developed immediately after inoculation and increased rapidly. At autopsy the pneumococci were numerous in all of the tissues and organs. The severity of the toxemia was manifest from the degree of parenchymatous degeneration and from the extent of the hemorrhagic lesions present in all these animals. In the animals treated with immune sera the course of the infection was greatly modified. In all three, crises were induced by the immune sera; the prostration disappeared and the pneumococci of the bacteremia were destroyed. Only one animal was completely cured. One died from thrombosis on the sixth day, but in the third animal the fatal issue was not delayed.

Infections of the rabbit with many strains of pneumococci, even when they are fatal as early as these, are often free from hemorrhagic lesions and there is comparatively little evidence of severe poisoning.
The two immune sera differed greatly in the extent and possibly also in the character of their action. Serum B was obtained from rabbits highly immunized with living cultures; serum A, from rabbits immunized with culture filtrates and precipitates.

The treatment of animal I with serum B induced crisis: the bacteriemia fell in an hour from 715,500 cells per cubic centimeter to 62, and then in two hours to 12. The fever continued despite the marked visible improvement, but fell sharply to normal the following noon. Recrudescence with moderate increase of the bacteriemia proved evanescent, and thereafter the animal was normal.

Similarly in animal IV, serum B induced crisis. The bacteriemia fell in an hour from 8,959,060 cells per cubic centimeter to 128; then serum A was substituted, but caused no further reduction. As in animal I, the fever continued despite a most marked visible change in the dog (which was able to sit up and eat) but by the next day the fever fell sharply. Recrudescence with marked return of the bacteriemia developed the following day. On the third day serum A caused a marked fall in the temperature, but the bacteriemia increased from 1,110 to 34,142 in an hour. This must have been due to the thrombosed condition of the veins found later at autopsy. By the intravenous injection of serum the pneumococci were washed into the blood stream from a focus of infection. Subsequent serum treatment was given subcutaneously and the blood was sterile before and after its use. The animal's general condition improved steadily from the first treatment, but the ears became swollen and, without inflammatory reaction, the edema increased, involving the eye and cornea. The edema continued to increase, but the animal appeared well and ate as usual until the sixth day, when it died.

At autopsy a general thrombosis was found which extended from the ear vein through all the larger veins to the heart and through all the organs and extremities. No signs of toxemia or infection were present. Morphological examination of the blood and viscera failed to show any pneumococci. One c.c. of pericardial fluid was sterile, but from two drops of blood a few colonies of the pneumococcus developed, an evidence of the harmless parasitism which had persisted in this animal.

Animal V received only one dose of serum B, yet this reduced the bacteriemia in an hour from 9,902,520 to 3,739, and two hours later, after a dose of serum A, to 2,567. It is, however, doubtful if serum A had any significant action on the infection in this animal. In about eighteen hours the animal died.

At autopsy the lesions of toxemia and bacteriemia were even more marked than in the controls; especially in the heart in which the hemorrhagic lesions involved two thirds of the auriculo-ventricular ring, a third of the right auricle, and a part of the right ventricle. On morphological examination very few pneumococci were found and these were in the peritoneum. In culture, however, the pneumococcus was recovered from all the organs.

The study of the blood was especially significant in this experiment. In the three controls the development of the pneumococci proceeded unchecked, whereas in all three of the animals treated with immune sera the pneumococci were immediately destroyed in large numbers. Despite this, signs of phagocytosis were not found. When numerous, pneumococci were seen in the smears, but these were extracellular. The degenerative changes in the leucocytes were
marked in all the controls, and in animals IV and V at the second examination, but not in animal I at any stage of the infection. These changes were evanescent in animal IV, and were not present at subsequent examinations of the blood. Evidently the treatment with immune sera prevented the development of these changes in the leucocytes and also favored the recovery of the leucocytes.

Despite the remarkable destruction of the pneumococci in the first hour or two after treatment with this immune serum a few invariably survived. These persisted for several days as harmless parasites. By the passive immunization with this serum treatment a condition was brought about which is comparable with that observed in the infection of animals actively immunized with culture filtrates. Clearly, in the recovery of the rabbit as in that of the dog, the neutralization of the infectious material was the dominant factor, the destruction of the bacteria being secondary.

SUMMARY OF THE STUDIES ON THE MECHANISM OF RECOVERY IN THE RABBIT.

From these experiments a clearer conception is gained of how certain phases of immunity modify the course of pneumococcus infection in the rabbit. The normal rabbit is so extremely susceptible to pneumococcus infection that the significance of the conditions of experiment and the effect of special treatment is clearly emphasized, a result not possible in experimenting on animals that are less susceptible to this infection and that possess normally a more complicated and efficient mechanism for recovery.

Phagocytosis played no part in the recovery of the rabbit from pneumococcus infection, yet crisis occurred spontaneously, and was induced experimentally by treatment with immune sera. In these crises there was a neutralization of the poison and a destruction of the bacteria which, according to modern conceptions of immunity, in the pleural infections of man similar conditions are not infrequently found. In the lung lesions it is perhaps less common, but it may persist for a long period as in Ewing's (6) case of unresolved pneumonia. Although the practical significance of this harmless parasitism is obvious it cannot be too strongly emphasized. Frequently this condition develops in animals immunized with living cultures and the danger of carrying over living pneumococci in serum therapy is a real one, against which the necessary precautions should be taken.
must be regarded as toxin and antitoxin neutralization and as bacteriolysis.

Bacteriolysis of the pneumococcus can be demonstrated in vitro and in vivo, but the presence of toxin is suggested only indirectly by the presence of substances which are separable from the bacterial cell and are capable of inducing an immunity in which protective substances neutralize the products of pneumococcus infection. This much seems to be definitely established, however narrowly we may limit our conceptions of toxin action.

The known toxins are recognized and measured only in terms of their action or their susceptibility to various influences. That toxin injures the body tissues is known. It is definitely recognized, moreover, that as they recover the injured tissues elaborate neutralizing antitoxins, and the amount of antitoxin formed may be precisely measured and demonstrated.

But it is not clear just how the tissues are poisoned. Toxin may act directly on the animal cell, as Ehrlich suggests; or, as now seems possible in the light of recent work on snake venin and on anaphylaxis, toxin may bring about more complicated changes from which arise substances of manifold potency.

GENERAL CONSIDERATIONS.

There is no experimental evidence that lobar pneumonia in man differs fundamentally from pneumococcus infection in animals. Conditions of susceptibility vary in different animals and these determine the form and course of the disease. Conditions underlying recovery also vary and with them the manifestations of the protective mechanism. But fundamentally these conditions are similar.

As compared with the dog or with man, the rabbit is extremely susceptible to infection. Virulent pneumococci develop without local tissue reaction. The protective mechanism of the normal rabbit is slight and inadequate, and the animals die quickly from

*According to Dellezeenne and Ledebt (7), constituents of cobra venin added to body fluids in vitro yield a substance 10,000 times as potent as the venin, thus suggesting that products of bacteria which form no toxin in the test-tube may give rise to very active poisons in the body.
the bacteriemia; but, when infection is prolonged, tissue adaptation develops rapidly and recovery may take place suddenly by crisis as in man. The sustained temperature of prolonged bacteriemia in the rabbit also resembles that of the lobar pneumonia of man.

Compared with the rabbit the dog is relatively insusceptible. Pneumococci develop and cause extensive local tissue reaction. The protective mechanism in the dog is efficient and infection fails to give rise to the sustained temperature, or to recovery by crisis comparable to that in man, despite the presence of characteristic and extensive lung lesions.

It may well be that in the lobar pneumonia of man the lung lesion plays a minor part in determining the disease as a whole, and that after all, even in man, it is the bacteriemia which accounts for many of the manifestations of the disease seen at the bedside. In both the dog and the rabbit, material that is infectious when inoculated under the skin or into a vein, is innocuous when injected into the lungs through the trachea. In all animals the protective mechanism of the lung is far more efficient than that of the blood stream.

In lobar pneumonia the extensive lesion is simply an expression of the efficiency of the individual's protective mechanism. This protective mechanism may be adequate or inadequate. That it is often adequate is shown by the early disappearance of the infectious agents in the exudate, not only in the experimental pneumonias of the dog and of the rabbit, but also in the lesions of fatal infections in man. Extensive lesions, however, are only incited by pneumococci of exalted virulence, and yet in the dog, and possibly also in man, the organisms must possess or acquire even greater virulence to induce the systemic manifestations of lobar pneumonia.

In man and in the dog extensive lung involvement may cause little discomfort, whereas serious symptoms often develop when the lesion of the lungs is comparatively slight. But apart from all this, the disease symptomatically is a systemic reaction to the poison, whether this poison be derived from the lung lesion or from the bacteriemia. For this reason, if pneumonia as it appears in man is to be investigated experimentally in animals, and if this study is to be complete, the bacteriemia of the rabbit and the pneumonic lesion of the dog must be studied.
Recovery of man or animals is due to the immunity incited. From the first, research has been directed in turn to one after another of the several phases of immunity, and in the course of this study attempts have been made to cure the disease by treatment with sera from immunized animals. But previous research was focussed on one phase of immunity and not on immunity as a whole; hence it failed to demonstrate satisfactorily the mechanism of recovery from pneumococcus infection, and the attempts to cure the disease by serum therapy failed to yield convincing results.

SUMMARY AND CONCLUSIONS.

From the results of this study of the action of immune sera on pneumococcus infection it is evident that immune sera vary greatly in their curative value. Immune sera possess protective action, but protective action is not necessarily indicative of curative action.

Treatment with the serum of normal rabbits may prolong the course of pneumococcus infection in the rabbit. This action, however, is slight and not always manifest. Sera from animals immunized with dead pneumococcus cells which had been washed free from their products, failed to exert materially greater curative action than normal sera. Sera from animals immunized with culture filtrates free from pneumococcus cells possessed, in some instances, a slight curative value, but often this curative action was not apparent.

In animals actively immunized, however, the presence of an immunity to culture filtrates was readily demonstrated. In the immunity produced by injections of dead culture material the strength was not sufficiently exalted for the sera to possess a practical curative value.

It was only after immunization with virulent living cultures that the blood serum acquired marked curative action. After pneumococcus infection in the rabbit had become established, treatment with this serum induced crisis and cured the animals.

From the results of the study of the mechanism of recovery it is evident that, despite the fact that virulent pneumococci are singularly insusceptible to the action of immune sera in the test-tube,
pneumococcus infection nevertheless conforms to the general law of infection.

Diphtheria and tetanus organisms give rise to powerful toxins, but the parasitism of these organisms is slight and their development is localized. Diseases produced by these organisms are toxemias and neutralization of their toxins by antitoxin puts an end to the disease.

The pneumococcus gives rise to toxic substances which are less active or are active only in the body tissues, but the parasitism of this organism is marked and its development is rarely localized. Nevertheless, the manifestations of the disease arise from the action of the bacterial poisons on the tissues. The neutralization of the pneumococcus poisons by immune serum puts an end to the symptoms of the disease, but the pneumococci survive as harmless parasites until destroyed by lysis or phagocytosis.

The neutralization of the pneumococcus poison may take place suddenly and completely as in crises; or, it may be incomplete with exacerbations of infection, as in lysis. Crisis, as it occurs in the lobar pneumonia of man and in the bacteremia of the rabbit, is simply one phase of recovery, and recovery does not differ fundamentally, whether it is sudden and complete as in crisis, or incomplete and prolonged as in lysis, or whether the pneumococci are destroyed by lysis extracellularly as in the rabbit, or intracellularly as in the phagocytosis of the dog and man.

Since the recovery of animals from pneumococcus infection differs in no essential from that of man, since the unaided protective mechanism of man as compared with that of susceptible animals is exceptionally efficient, and since it is possible by treatment with sera from animals highly immunized with living cultures of virulent pneumococci to cure pneumococcus infection in the most susceptible animals, it is difficult to conceive of the infection in man failing to yield similarly to the administration of such sera.

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