CHEMO-IMMUNOLOGICAL STUDIES ON LOCALIZED INFECTIONS.

FOURTH PAPER: EXPERIMENTAL PNEUMOCOCCIC MENINGITIS AND ITS SPECIFIC TREATMENT.*

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The purposes of this paper are to record the general progress of the chemo-immunological studies, and in particular to show that a fatal experimental disease similar to a not uncommon and grave disease of man has often terminated in recovery after a treatment formulated from these studies, and to indicate a way in which the corresponding disease of man may be treated with a reasonable hope of sometimes preventing its almost invariably fatal termination.

In the first paper has been related why and how we were led by consideration and experiment to attempt to arrest the progress of experimental pneumococcic infections by the local use of sodium oleate combined with immune antipneumococcic serum and boric acid. Among others, certain experiments were reported illustrating the results obtained by treating rats and mice inoculated in the peritoneal cavity. It was shown that appropriate treatment early after inoculation with massive doses of virulent pneumococci not only regularly saved the animals but also prevented the appearance of more than the slightest transitory signs of any illness. As a measure for preventing the development of infection the treatment was effective. As a curative measure for arresting the developing, or already established infection it was less effective, particularly in respect to the lapse of time after inoculation. Yet if we consider the severity of the test, the animals being highly susceptible to

* Received for publication, August 1, 1912.
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rapidly fatal septicemia, the results were encouraging enough to lead us to pursue the principle in the hope of developing it, and because of the promise which it afforded of a possible successful application to the treatment of certain localized pneumococcic infections of man.

Since the publication of the first paper the work has been continued along the same fundamental lines in the search for an improved means of treatment. At the same time, pending developments in this direction, we undertook to ascertain whether what was already in hand might be successfully applied to the treatment of an experimental disease which bears a closer resemblance to pneumococcic diseases of man than is afforded by the septicemic infections of the small laboratory animals, and thus offers more reliable indications of the practical value of the therapeutic principle.

In the second and third papers\(^1\) we recorded the results of the search for a more efficient agent than sodium oleate. Several substances were found to be much more powerful in destroying the pneumococcus in the test-tube, but they were less efficient than sodium oleate in controlling infections of the animal body. Also the attempts to find an agent which is superior to boric acid in preventing the serum inhibition of the sodium oleate failed. Hence we turned to the determination of the optimum proportions of the components of the therapeutic mixture of sodium oleate, immune serum, and boric acid, which had already proved itself.

What is meant by the optimum composition of the mixture will be apparent upon a brief restatement of the essential points regarding the action of the components alone and combined. The destructive action upon the pneumococcus of sodium oleate solutions varies throughout a wide range of concentration in direct ratio to the degree of dissociation. The destructive action is prevented wholly or in part by blood serum according to quantitative conditions. Alone in the animal body sodium oleate has little or no apparent restraining influence upon pneumococcic infections. In the test-tube a potent immune serum kills many of the pneumococci present but allows a subsequent rich growth of those which escape destruction, unless indeed a very small number be present, when all may be killed. The bactericidal action of immune serum does not depend

wholly upon, but is enhanced by, the presence and cooperation of phagocytes; and the intensity of action varies in direct ratio to the degree of concentration of the serum. In the animal body immune serum exerts a distinct protective action, but one efficient in saving the animal only under quite sharply defined conditions pertaining to quantity of culture and of serum. In other words, there is a minimum quantity of serum which will protect against even the most minute, otherwise certainly fatal quantities of culture, and no quantity of serum protects against more than a definite quantity of culture. Dochez\textsuperscript{a} directed attention to this phase of the protective action of immune serum and has recently made it the subject of a special investigation. He has pointed out the relationship between quantity of serum and of culture in determining protection or lack of it, and suggested the importance of its bearing upon the practical treatment of established infections with immune serum. We attach an additional importance to the facts as affording a standard of comparison for appreciating the greater efficiency of the mixture of sodium oleate, immune serum, and boric acid. In contradistinction to test-tube experiments, a given quantity of serum in the animal body is just as efficient, if previously diluted even to 10 per cent. with salt solution, as if employed undiluted. The presence of boric acid in a certain quantity is necessary to prevent serum inhibition of the oleate, but an excess defeats the purpose of its employment, since of itself, in large quantities, it prevents the bacteriolytic action of the sodium oleate.

After testing several species of animals in diverse portions of the body in which we attempted to produce a suitable disease, with strains of the pneumococcus varying in virulence, we finally employed for our study pneumococcic meningitis in monkeys produced by a highly virulent pneumococcus. The conditions which this experimental disease presents are favorable for testing the method and important because of their close similarity to the highly fatal pneumococcic meningitis in man. The experimental disease could be produced with ease, and, as it appeared later, with certainty. Except necessarily in the mode of infection it was remarkably like pneumococcic meningitis of man.

\textsuperscript{a}Dochez, A. R., Personal communication.
The clinical, bacteriological (including bacteremia), and pathological manifestations were nearly identical. It differed in that its course was more severe, and when untreated it terminated invariably in death. This statement is true of the disease produced, as it always was except once, by virulent pneumococcus. In a single instance a less virulent pneumococcus was injected and a meningitis produced that terminated in recovery without any intervention other than lumbar puncture performed for diagnostic purposes. The experiment has no bearing upon the particular theme of the paper, but is recorded to show how much the clinical result depends upon the infecting bacterium. It is an experimental confirmation of what clinicians are coming to realize, namely, that pneumococcal meningitis though a highly mortal disease is not necessarily always fatal; and it serves to indicate still further the close similarity of the experimental to the spontaneous disease.

The disease lent itself well, moreover, to constant observation of its course, and to direct local treatment, as lumbar puncture afforded indications of the influence of treatment during the course of the disease.

Before recording the individual experiments certain general features should be considered relating to the animal infected, the kind of pneumococcus employed, the mode of infection, and the method of treatment.

Macacus rhesus was chiefly used, but a few experiments were made with Cercopithecus callitrichus. All the monkeys were vigorous and seemed in good health. Genus, species, and sex appeared not to influence the results. But the individual factor was manifest. Most animals reacted to the inoculation in such a manner as to permit ready recognition of the type of the disease. Occasionally an individual proved excessively susceptible, seemed to oppose no resistance, and died of septicemia within the day. The monkeys seemed to be less resistant in the cold than in the warmer months. When and in what way these two factors of the individual and the season appeared to influence the experiment will be indicated in the discussion of the protocols.

The first three experiments were made with different strains of pneumococcus. They showed that a meningitis could be readily
produced, and allowed a study of the characteristics of the disease
and the influence of degree of virulence of the bacterium upon its
course. From the third experiment on, the same strain of pneumo-
coccus was used throughout. This organism was obtained in pure
culture by Dr. Dochez directly from the circulating blood of a
patient with lobar pneumonia. Suffice it to say that it was a char-
acteristic pneumococcus soluble in bile. In the beginning the or-
ganism was of high but not extraordinary virulence. It was first
inoculated into monkeys after five passages in mice when 0.000,001
of a cubic centimeter was fatal. Repeated control tests showed the
degree of virulence to be maintained throughout the ten months of
study. In the nature of its pathogenic action the organism was
well adapted to the purposes: it caused inflammation rather than a
rapidly fatal sepsisemia.

In the first experiment the culture used was a virulent pneumo-
coccus grown from the spinal fluid of a patient with meningitis. In
the second one a so called avirulent pneumococcus isolated directly
from the saliva of a well person was employed. The characteristics
of this organism are stated in the protocol of monkey 2.

At the beginning a few experiments were made by intracranial
inoculation. The disease produced in this way was most severe and
quickly caused the death of the animal. Besides, it did not lend it-
self well to the treatment which is preeminently a local one admin-
istered by lumbar puncture. The opportunities for fluid injected
intraspinally to reach the distant seat of severe inflammation in the
brain itself were small. Normally in the monkey the space in the
membranes is small and this is quickly further reduced by swelling
and the fibrinous exudate. Indeed the focus of infection in the
brain and its membranes seemed sometimes to have been cut off
from the spinal subdural space. On account of the fibrinous nature
of the pneumococcic inflammation this meningitic disease offers
greater difficulties in treatment than, for instance, epidemic cere-
brosphinal meningitis. We shall refer later to those cases of pneu-
mococcic meningitis which arise from middle ear disease and where
the cerebral meninges are first and most inflamed.

Hence the intracranial method of infecting the animal was aban-
doned early, and throughout the remainder of the work the animals
were inoculated and treated by subdural spinal puncture and injection.

Two kinds of immune serum which were efficient in protecting rats and mice against infection by the same strain of pneumococcus used to produce the meningitis were tested alone. The one was from a goat, the other from a horse, immunized by the intravenous injection of large quantities of live, highly virulent, homologous pneumococci. Later other monkeys were treated with a mixture of sodium oleate, the same immune serum, and boric acid.

The experiments are presented in three groups. Those in the first group show the nature of the untreated disease and at the same time serve as control observations from which the results of treatment may be deduced. Those in the second group show the results of treatment with immune serum; and those in the third, treatment with the mixture of sodium oleate, immune serum, and boric acid.

THE EXPERIMENTAL DISEASE.

The first experiment was performed by Dr. Flexner in the spring of 1910. We abstract from his notes:

May 24, 1910. *Macacus rhesus*; medium size. 2:00 P.M. Lumbar puncture; clear fluid; injected 0.4 c.c. of a thin suspension of a 24 hour culture on sheep serum agar, and a few drops of spinal exudate, from a case of pneumococcal meningitis (Schirck). 7:00 P.M. On perch; hair erected; not very sick. May 25, 10:00 A.M. Not very sick. L. p.;* small quantity of clear fluid containing a few polymorphonuclear leucocytes; no cocci. Injected 0.5 c.c. of a bouillon suspension from a fresh culture of same organism. 2:00 P.M. Monkey sick, lying partly down on perch. 4:00 P.M. L. p.; no fluid runs from needle. A little pus mixed with water remaining in needle is drawn up by the syringe. Film shows polymorphonuclear leucocytes and some Gram positive diplococci intra- and extracellular. May 26, 9:00 A.M. Animal lying on side; very sick. 10:00 A.M. General convulsion. 11:00 A.M. L. p. Cloudy fluid containing flakes of cells. Many leucocytes and diplococci some of which are intracellular. 11:10 A.M. General convulsion; some retraction of head; nystagmus; body limp.

May 27, died at 9:30 A.M. Autopsy at 11:00 A.M. The vessels of the cerebral meninges are injected and there is cloudy fluid in the meshes of the pia arachnoid, especially over the medulla. The ventricles contain an excess of cloudy fluid, but no purulent exudation. No macroscopic changes in the other organs. Films from the cerebral meninges and choroid plexus show many

*Abbreviation for lumbar puncture.
The experiment requires little comment. Clinically there were many of those manifestations of meningitis which occur in man. Septicemia supervened. Death occurred early. At autopsy the familiar morphological and bacteriological conditions were encountered.

In the second experiment an organism of that variety which is commonly called avirulent was used. It was isolated directly from the saliva of a well person on January 25, 1911. The colony was of the characteristic ring form. In subcultures the organism exhibited the typical cultural and morphological characters of the pneumococcus; it fermented inulin feebly, and was soluble in bile. 0.5 of a cubic centimeter of a broth culture proved harmless to a mouse. From the time of its isolation until the date of this experiment, i.e., for ten weeks, the organism had been kept continuously on plain agar at 37° C. by third day transplantations.

Apr. 4, 1911. Monkey 2. Cercopithecus callitrichus; rather large. 3:30 P.M. Under ether anesthesia injected into cranium through fine trephine opening in left frontal region 1 c.c. of a 24 hour plain broth culture of pneumococcus L 9. Animal conscious in five minutes. 4:30 P.M. Bright and active just as before inoculation. Apr. 5, 9:30 A.M. Animal sits on perch with head bowed; hair over anterior part of body erected. No paralysis or other focal symptoms. 3:30 P.M. A little worse; entire coat erect; little resistance to being captured. L. p. 1 c.c. of distinctly turbid fluid drops from needle; no coagula. Many pus cells and typical diplococci mainly extracellular; very little phagocytosis. Transplantation of one loop of fluid affords many colonies of pure pneumococcus. Apr. 6, 9:00 A.M. Animal lively; does not appear ill; coat smooth. 10:00 A.M. L. p. 4 drops of turbid fluid slowly. Many p. n. l. but fewer than yesterday; moderate number of large, and a few small, mononuclear leucocytes. Fewer diplococci than in yesterday’s fluid; no phagocytosis. Slight pure growth of pneumococcus. Apr. 7. Animal seems well. Apr. 10. Well. May 8. Continued well.

The experiment has already been referred to in the introduction. It led to the trial of another strain of pneumococcus, and one known to be virulent. The value of lumbar puncture in affording important information is apparent.

* Abbreviation for polymorphonuclear leucocytes.
In all the experiments now to be reported the virulent culture described before was used.

Apr. 9, 1910. Monkey 3. Cercopithecus callitrichus; very large. 11:30 A.M. Under ether anesthesia injected into cranium 1 c.c. of a 24 hour plain broth culture of pneumococcus X.5. Animal is recovering while the wound is being closed. Recovery complete in a few minutes. Apr. 10. Animal found dead at 7:00 A.M. Death occurred about 19 hours after inoculation.

9:30 A.M. Autopsy.—Upper lip retracted; both eyelids swollen; pupils one half dilated, round, equal. Lumbar puncture; two drops of slightly turbid fluid; very few p. n. l.; myriad diplococci; many typical pairs, also short chains; many degenerative forms. The brain shows yellow through the dura mater. The pia is milky and covered everywhere with a viscid exudate, greatest at the base of the cerebrum. The lateral ventricles contain a small quantity of the same exudate. The pia of the cord is milky everywhere, and there is a scanty exudate. The ventral cavity of the body presents no gross changes. Films from the surface of the cerebrum, the lateral ventricle, and the spinal cord disclose many p. n. l. and a great many cocci in pairs and short chains, with only an occasional example of phagocytosis. In the heart's blood are many typical pairs and short chains. The spinal fluid, lateral ventricle, and the heart's blood all afford a pure profuse growth of pneumococcus.

The quantity of culture was large, and the disease fulminant, septicemia quickly supervening. The experiment indicates how quickly the spinal meninges may become involved and how rich the early exudate may be in fibrin. The bearing of the nature of the exudate upon the efficacy of treatment is most important, as will appear in the succeeding protocols.

The next experiment was performed in the same way with a smaller quantity of culture.

Apr. 10, 1911. Monkey 4. Cercopithecus callitrichus; medium size. 11:40 A.M. Ether anesthesia; injected intracranially 0.1 c.c. (1 c.c. of a 1 to 10 broth dilution) of a 24 hour broth culture of pneumococcus X.5. Complete recovery in a few minutes. 5:00 P.M. Animal seems well. Apr. 11, 9:15 A.M. Animal sits bent forward slightly; coat slightly raised; eyes watery; pupils half dilated, round, and equal; respiration moderately accelerated. L. p. 3 drops of slightly turbid fluid very slowly; very few p. n. l.; few typical pairs of extracellular pneumococci. Moderate pure growth from one loop of fluid. P.M. Animal perhaps slightly worse. Apr. 12, 9:30 A.M. Worse; reclines on floor of cage in the corner; coat erect. Several attempts to obtain spinal fluid are unsuccessful. In the afternoon the animal's condition grows worse. Apr. 13,

*The Roman numeral refers to the name of the culture; the Arabic decimal indicates the serial number of the last mouse "passage"; and the exponent the generation in broth used.
A.M. Much weaker; lies on floor with head retracted. Made two unsuccessful attempts to obtain spinal fluid. During the afternoon the weakness slowly progresses.

Apr. 14. Found dead at 7:50 A.M. The body is fresh. Death occurred about 3½ days after inoculation. Autopsy 12 M. The inflammation is intense over the vertex of the cerebrum, and gradually diminishes towards the base and the medulla to become slight throughout the meninges of the cord. The cerebral cortex is yellow and the sulci are distended with pus and fibrin. The ventricles contain a moderate quantity of turbid fluid. Films from the brain show many p. n. l. and very many cocci in pairs and short chains; fewer in the scant spinal exudate. Profuse cultures from cortex, lateral ventricle, spinal cord, and heart's blood.

With the smaller quantity of injected culture the disease was slower in developing and the local inflammatory reaction was more marked. Spinal fluid could not be obtained after the end of the second day probably because of the thickening of the meninges and the absorption of the fluid by the fibrin in the exudate. The usual richness of the exudate in fibrin, a characteristic of pneumococcic inflammations in general, has in this particular disease an indirect effect in materially lessening the quantity of fluid which may be injected and in hindering its diffusion throughout the spinal canal and into the cranium.

The remainder of the experiments grouped under this heading were performed at intervals during the work largely for the purpose of often controlling the virulence of the culture, and the effect of treatment in companion experiments. In each of them the culture was injected by the spinal route.

May 8, 1911. Monkey 17. Macacus rhesus; medium size.
9:45 A.M. Injected, intraspinaUy, after the free withdrawal of 1.5 c.c. of clear fluid, 0.1 c.c. (1 c.c. of a 1 to 10 broth dilution) of a 24 hour broth culture of pneumococcus X.511. No symptoms. May 9, A.M. No signs of illness. 2:45 P.M. Animal is restless and eats less than usual. L. p. 1.2 c.c. of slightly turbid fluid freely. Sediment obtained by centrifugalization; many p. n. l.; few large and small mononuclear leucocytes; few extracellular diplococci. Profuse growth in culture. May 10, 9:00 A.M. Animal sits bowed on perch; hair raised slightly; weakness in movements upon being disturbed. 10:45 A.M. L. p. 12 c.c. less turbid fluid freely; several small white flakes; fewer cells; many cocci in pairs and a few short chains; slight phagocytosis. Profuse growth in culture. P.M. Animal becomes worse with great rapidity and dies at 5:00 P.M., 2½ days after inoculation.

Autopsy.—May 11, 9:00 P.M. The spinal membranes are moderately congested, moist, and the pia is milky. No fibrin is visible. The cerebral meninges
are glued together, and the cortex tears in several places as the dura is being
reflected. There is a general moderate congestion most marked at the base.
The pia is cloudy everywhere and opaque in the sulci. The ventricles contain a
small quantity of rather thick yellowish fluid. In films from the cortex there
are a great many pus cells and many diplococci for the most part extracellular
and only fairly well preserved. There is moderate phagocytosis. In the spinal
exudate there are fewer pus cells and diplococci and less phagocytosis. On the
surfaces of the lower lobes of both lungs is much mucilaginous exudate in sheets
and clumps containing many poorly staining pus cells and a moderate number
of typical pneumococci, of which a few are intracellular. Otherwise the ventral
cavity is negative. No bacteria seen in the heart's blood; culture negative.
From the cerebral and spinal exudate the pneumococcus grows profusely and
pure.

At first the animal offered considerable resistance, shown by its
physical condition, the polymorphonuclear cell reaction in the spinal
fluid, and the slow multiplication of the diplococcus. Then it
showed signs of illness and became worse with great rapidity, while
coincidently the cellular reaction subsided and the cocci grew pro-
fusely. The absence of cocci from the blood at autopsy indicates
that the meningitis itself may cause death without the superven-
tion and help of septicemia. There was a metastatic pleurisy of
slight extent and degree.

The experiment reported next was done in the autumn after the
summer interruption of two months, during which time the culture
was preserved in the dried spleens of infected mice.

Sept. 19, 1911. Monkey 32. Macacus rhesus; medium size.
11:00 A.M. L. p.: three attempts to obtain fluid failed; on the third attempt,
injected 0.1 c.c. (1 c.c. of a 1 to 10 broth dilution) of a 24 hour broth culture of
X.9. With the needle and syringe still in place withdrew and reinjected 0.3 c.c.
for assurance. No symptoms. During remainder of the day the animal seems
well. Sept. 20, 9:00 A.M. Animal not so active as yesterday. 11:00 A.M.
Ill; rather quiet; deliberate in movements; eyes dull; coat beginning to rise.
2:00 P.M. All symptoms more marked; weakness evident as animal springs;
head retracted at times. 4:00 P.M. Worse; coat much raised. Sept. 21, A.M.
Animal is worse; quite weak, but still moves about; coat rough. P.M. Condition
about the same. Sept. 22, 9:22 A.M. Animal much worse; no longer
jumps but climbs slowly up grating of cage to perch. P.M. Very weak; sits
quietly huddled; does not move when touched. Sept. 23, 9:00 A.M. Prone;
apparently dying; head much retracted. Death at 10:30 A.M., 4 days after
inoculation.

Autopsy.—Performed at once. The brain and cord are moist. The pia
everywhere is thickened, opaque, and yellowish white, more over the cerebral
hemispheres than along the cord. The ventricles are slightly distended with turbid
fluid. Diplococci are present all along the cord and over the brain beneath the
dura. They are not numerous except in the fibrinous exudate covering and
infiltrating the pia of the convexity of the brain, where there are enormous
numbers. Phagocytosis is slight. At the bases of both lungs there are a few
deposits of a dry fibrinous exudate containing pus cells and diplococci. From
the cortex, lateral ventricle, spinal cord, and pleural exudate the pneumococcus
grows profusely; from the heart's blood, in a few isolated colonies.

The experiment indicated that the organism had preserved its
virulence. The disease progressed gradually and steadily. Again
the inflammation appeared to be greater in the cerebral than in the
spinal meninges although the inoculation was made into the spinal
canal. The dry pleurisy occurred again. And, as in the preceding
experiment, septicemia did not seem to be necessary to cause
death. The few cocci found in the blood at autopsy were probably
the expression of an agonal invasion rather than of a true septi-
cemia. The animal of this experiment was the companion to one
inoculated at the same time in the same way and treated later (com-
pare monkey 33 of the third group).

Dec. 12, 1911. Monkey 45. Macacus rhesus; medium size. 1:55 P.M. L. p.:
about 0.7 c.c. of clear fluid; injected 0.1 c.c. of a 22 hour broth culture of X.115.
Slight retraction of back upon completion of injection passing away in a few
seconds. For the remainder of the day until 6 o'clock, when it is observed for
the last time, the animal seems perfectly well. Dec. 13. Animal found dead at
8:00 A.M. Lived about 15 or 16 hours after inoculation.

Autopsy.—At 9:30 A.M. Body fresh. There is a moderate quantity of yel-
low, sticky exudate all along the spinal pia, and over the brain, being most
abundant at the base. The ventricles contain a modicum of opalescent fluid.
In the meningeal exudate everywhere there are many pus cells and enormous
numbers of diplococci, many of which are intracellular. Cultures are profuse
and pure. Films from the left lateral ventricle show a few mononuclear cells,
no p. n. l., and no cocci. No growth upon transplantation from this source.
Heart's blood: moderate number of typical capsulated pairs and a few short
chains; profuse growth in culture. Both lungs are slightly congested and edem-
atosous, but there is no consolidation. Otherwise the ventral cavity is negative.

This experiment shows the fulminant disease. It is remarkable
that so intense a purulent inflammation with fibrin formation could
occur in such a short time. At this season, during the cold of mid-
winter, the disease ran regularly a shorter course than it had done
in the spring and autumn. Possibly also this animal was unduly
susceptible, for no change was ever made in the manner of preserv-
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Two more experiments to illustrate the untreated disease very similar to the one just described may be summarized. The monkeys were inoculated in the usual way late in the afternoon of December 14, 1911, and on January 5, 1912, respectively. Seen twenty and forty-five minutes later the animals seemed well; but both died during the night, one about thirteen hours, and the other about nineteen hours after the inoculation. The autopsy in each instance showed a general purulent cerebrospinal meningitis and septicemia. The rapidity of the development is evident.

It was, as already stated, in mid-winter that the disease was so much more severe than it had been before. Therefore the quantity of culture inoculated was reduced to one fifth of what had been regularly used before. The protocols of the experiments appear in the second and third groups.

To summarize: the experiments show that virulent pneumococci when injected into the posterior cavity of monkeys produce regularly an inflammation of the meninges, and particularly of the pia mater, attended by bacteremia; that the inflammation becomes quickly purulent and fibrinous in character, extends readily from the cerebral to the spinal meninges and vice versa, and is so regularly attended by certain definite general symptoms that the whole constitutes a definite disease entity.

INFLUENCE OF TREATMENT WITH IMMUNE SERUM.

In all, eleven experiments were made with immune serum. In two not only the animal was treated but also the culture before it was inoculated. That is, in order to make the simplest test of the serum, a large quantity, many times that required to protect a rat against the same quantity of culture as the monkeys received, was added to the culture, the mixture incubated, and then inoculated into the monkey, which later received still more serum. In nine experiments of this group, as well as in all those of the third group, the animal was first inoculated with the culture alone and then later treated. The time allowed to elapse between inoculation and
the beginning of treatment was usually determined by the physical condition of the animal and the character of the spinal fluid. As a rule, in the treatment with immune serum alone, this interval was short. The results of early treatment were so little encouraging that there was no reason to increase the interval between inoculation and the beginning of serum treatment. Once begun the treatment was usually repeated once a day as long as the animal lived; or, as occurred in two instances, until the physical condition of the animal was much improved and the spinal fluid gave no growth of the coccus. The quantity of serum injected was the maximum of what was considered safe, and was determined by the quantity of fluid which had been withdrawn, and the condition of the animal while the slow injection was being made. In the beginning of the disease it was almost always possible to inject two cubic centimeters with no apparent embarrassment to the animal; later, as the disease progressed, less spinal fluid could be withdrawn and less serum injected, but even then it was usually possible to give at least one cubic centimeter without appreciable harm. In some experiments concentrated serum was used; in others a 40 per cent. dilution. Of itself the serum seemed to be harmless.

9:55 A.M. Under ether anesthesia injected intracranially 0.1 c.c. of a 24 hour broth culture of X.5'. Recovery complete in a few minutes. 1:55 P.M. No signs of illness noticed. L. p.: 0.5 c.c. of slightly blood-tinged fluid. In the sediment there are no more leucocytes than the presence of blood accounts for, and no cocci are seen. Twelve colonies of pneumococcus grow from the planted fluid.

Injected 2 c.c. of immune serum (goat 5, bleeding of February 16, 1911). No symptoms attend or follow injection.

Apr. 15, A.M. Animal is slightly ill; sits quietly but moves with agility when disturbed; coat somewhat fluffy. 10:15 A.M. L. p.: 2 drops of clear fluid; very few cells, about half of which are polymorphonuclear and half mononuclear; no cocci seen. Scant growth of pneumococcus in culture. Injected 2 c.c. of the same immune serum.

Apr. 16, 10:15 A.M. Animal slightly worse; not so quick in movements; head droops a little. L. p. no fluid obtained. Injected 2 c.c. of immune serum without resistance. Apr. 17, 10:30 A.M. Slightly worse. L. p.: no fluid obtained. Injected 2 c.c. of immune serum; slight struggle towards close of injection. Until noon the animal remains in about the same condition. P.M. Much worse; lies down sometimes; no longer eats.

Apr. 18, 10:00 A.M. Very weak; lies on side, eyes swollen. L. p.: no fluid
obtained; believing that the animal would die if left alone, we injected again 2 c.c. of immune serum. The last half of the injection is attended with slight struggle and embarrassment of respiration. During the remainder of the day the animal’s condition seems about the same as before the injection. Apr. 19. Animal found dead at 8:00 A.M. It died 48 days after inoculation.

Autopsy at 10:00 A.M. Intense inflammation of cranial meninges; the sulci are widely distended by a purulent exudate rich in fibrin. There is a small area of red softening on the superior surface of the right lobe of the cerebellum. The spinal cord is enveloped throughout by a yellowish sheath about 2 mm. thick representing the pia and the fibrinopurulent exudate. There are many p. n. l. and enormous numbers of cocci in pairs and short chains in the cerebral and spinal exudate. Phagocytosis is slight. In culture profuse pure growth of pneumococcus from exudate and heart’s blood.

In this experiment treatment was begun early and only four hours after the inoculation while the animal still seemed well; and although the bacteria had already reached the spinal fluid probably only slight multiplication had occurred, for they were too few in number to be seen in films. Even on the second day the animal was still very strong. At the best the serum only delayed the fatal issue a little while. It may not have reached the brain in more than small quantities much diluted, but even in the spinal canal where it was present and could not have been greatly diluted it did not prevent the growth of the cocci and the production of an inflammation almost as severe as that in the cerebral meninges.

Apr. 21, 1911. Monkey II. *Macacus rhesus*; large.

11:10 A.M. Under ether anesthesia injected intracranially 0.1 c.c. of a 24 hour broth culture of X.5⁴. Complete recovery at once. 3:10 P.M. Animal seems a little torpid; otherwise no signs of illness are detected. L. p.: 1.2 c.c. of turbid fluid freely; moderate number of p. n. l., more than a moderate number of extracellular diplococci. Profuse growth in culture. Injected easily 2 c.c. of immune goat serum. No symptoms. Also injected intravenously 5 c.c. of immune serum. This injection is followed by a mild shock of short duration. Seven minutes later the animal has recovered completely. 4:30 P.M. The animal huddles against another in the cage; moves with agility when disturbed; coat slightly raised. The animal was observed no more this day, and was found dead at 7:30 the next morning. It probably lived 8 or 9 hours after the inoculation.

*Autopsy.*—11:30 A.M. The membranes of the brain, chiefly over the convexity, are milky from the presence of a moderate quantity of yellowish white exudate containing some fibrin. Films show many p. n. l. and very many diplococci. In the spinal canal there is less exudate but yet p. n. l. and many diplococci. The heart’s blood contains many diplococci. Profuse growth of pneumococcus occurs in all cultures.
In this experiment the animal was unusually susceptible, for in four hours it had become ill and the bacteria had reached the spinal fluid and begun to multiply. It was on this account that the intravenous injection of immune serum was made. At least it was not effective in delaying death. The experiments were so little encouraging that an attempt was made to afford the serum better opportunities to exert any beneficial action of which it might be capable. To this end the culture and serum were mixed and incubated and then injected in order to see if infection would be prevented.

Apr. 21, 1911. Monkey 12. Macacus rhesus; small.
4:00 P.M. Under ether anesthesia injected intracranially a mixture consisting of 0.1 c.c. of a 24 hour broth culture of X.56 and 1 c.c. of immune goat serum. The mixture had been incubated for 1 hour and 10 minutes at 37° C. and then kept in the refrigerator for 30 minutes. A culture of the mixture just before it was injected furnished a moderate growth of pneumococcus. The animal quickly recovered and seemed well for the remainder of the day. Apr. 22. The animal may be a little agitated; otherwise it seems well. 11:00 A.M. L. p.: 1 c.c. of turbid fluid freely; a moderate number of p. n. l., a few mononuclear cells, and no cocci. No growth in culture. Apr. 23. Animal seems well throughout the day.

Apr. 24, A.M. Still seems well. At 3:30 P.M. the animal is first noticed to be ill. It is greatly agitated, shaking at the grating of the cage. In a few minutes it lies prone and trembling with roughened coat. On being taken up an almost complete flaccid paralysis of the left anterior extremity and a spastic paresis of the left posterior extremity is apparent. There is slight ptosis of the left upper eyelid. 4:00 P.M. Animal weaker. L. p.: turbid fluid flows quickly under pressure; a few p. n. l. and mononuclear cells; more than a few diplococci; no phagocytosis; profuse growth in culture. Injected immune goat serum, stopping at 1.6 c.c. upon the appearance of struggle and slight embarrassment of respiration; recovery in one minute. 6:00 P.M. Condition about the same as before the injection. Animal found dead at 7:30 P.M., 38 days after the inoculation.

Autopsy.—April 25, 10:30 A.M. There is an intense fibrinopurulent inflammation of the pia of the brain and cord, which is more pronounced in the brain where the sulci are widely distended by a thick yellow exudate. The cortex everywhere is much congested, even within its substance. There is a large area of red softening in the right temporal lobe. The ventricles contain a moderate quantity of turbid fluid. In films from the brain and cord there are many p. n. l. and enormous numbers of diplococci everywhere. Profuse growth in cultures from the brain and heart's blood.

In this experiment the immune serum had already effected a considerable reduction in the number of viable cocci in the culture be-
fore it was injected. The condition of the animal for the first two
days and the character of the spinal fluid seemed to indicate that the
infection if not prevented was retarded. Then symptoms of illness
appeared more or less suddenly and the animal very rapidly weak-
ened and died at about the time the untreated controls did. The
autopsy indicated that the disease was developing all the while the
animal showed few or no chemical signs and explained the focal
symptoms.

It was at this point that the intracranial method of inoculating
was abandoned and the intraspinal substituted. The next experi-
ment resembled the one just described except that the mixture of
culture and serum was injected into the spinal canal, and the animal
itself was later given a regular course of serum treatment regardless
of its apparent condition.

1:30 P.M. Injected intraspinally a mixture consisting of 0.1 c.c. of a 24
hour broth culture of X.5a and 1 c.c. of immune goat serum. The mixture had
been incubated for one hour at 37° C. A transplantation from it just before it
was injected furnished a moderate growth of pneumococcus. No symptoms
followed the injection. Remainder of the day animal seems well. Apr. 26.
Animal seems perfectly well. 10:00 A.M. L. p.: 1.5 c.c. of moderately turbid
fluid freely; a moderate number of p. n. l. and a few mononuclear cells are
seen in films. There are a few bodies, about half intra- and half extracellular,
which appear to be swollen cocci. Here and there an occasional diplococcus is
identified. Transplantation gives rise to no growth. Injected 1.8 c.c. of im-
mune serum; considerable struggling. During the afternoon the animal seems
well. Apr. 27, 2:50 A.M. No evidence of illness. L. p.: 1 c.c. of fluid, about
half as turbid as that obtained yesterday, flows slowly; p. n. l. predominate; no
bacteria seen; no growth occurs. Injected 2 c.c. of immune serum; only slight
struggling. Apr. 28. Animal seems perfectly well. 9:45 A.M. L. p.: 1 c.c.
of faintly turbid fluid; a few cells about half of which are mononuclear; no
bacteria seen; no growth occurs. Injected 1.8 c.c. of immune serum; much
struggling. Apr. 29. Well.

The animal remained well for one month when it developed diarrhea, be-
came emaciated rapidly, and died on June 30.

Autopsy at 4:00 P.M. Body much emaciated. Nothing abnormal is dis-
covered in the central nervous system. There is an extensive ulcerative enteri-
tis and hyperplasia of the mesenteric lymph nodes. Transplantations from the
cerebral cortex, lateral ventricle, spinal cord, and heart's blood give rise to no
growth.

In this experiment the immune serum was given the maximal
advantage. When incubated with the culture before inoculation
Richard V. Lamar.

and later administered three times to the animal it prevented the occurrence of infection. Such prevention of infection, while undoubtedly indicative of a beneficial action of the serum, is to be sharply distinguished from the arrest of an established infection. In the next experiment the test of the serum was made somewhat more difficult.


11:50 A.M. Injected intraspinally 0.1 c.c. of a 24 hour broth culture of *X.5*.

At 1:50 P.M. animal well. L. p.: 3 drops of opalescent fluid slowly; a few mononuclear cells and an occasional pair of cocci in films; slight growth in culture. Injected 2 c.c. of immune goat serum. Animal seems well remainder of the day. May 2, 9:40 A.M. Animal may be a little agitated; otherwise no signs of illness can be detected. L. p.: about 0.7 c.c. of a slightly turbid fluid containing one small white flake; a fair number of white cells, half p. n. l. and half mononuclear; no bacteria seen; no growth. Injected 2 c.c. of immune serum. May 3, A.M. The animal eats with normal greed and seems well. 11:10 A.M. L. p.: 1 c.c. of opalescent fluid slowly; more white cells than yesterday; ratio about the same; no bacteria seen; no growth. Injected 2 c.c. of immune serum. May 4, 10:05 A.M. Animal has continued well. L. p.: 2 drops of clear fluid; an occasional p. n. l. and a very few mononuclear cells; no bacteria seen; no growth. No serum injected.

The animal was kept under observation for two months during which time it remained well.

Immune goat serum given two hours after the inoculation and once on each of the two succeeding days seemed to prevent multiplication of the bacteria and the occurrence of infection, although the animal was apparently intoxicated for one day after the inoculation.

These two were the only instances in which an immune serum prevented the infection and death of the animal. In all other experiments where a longer period than two hours was allowed to elapse between inoculation and the beginning of treatment not only did all of the animals die, but the disease followed much the same course as in the untreated control animals. These experiments are summarized in table I (page 598).

The experiments call for little discussion. They show that immune serum had a distinct, though only slight, restraining influence upon infection. When administered within two hours it prevented the occurrence of infection in two instances. When given
### TABLE I.

<table>
<thead>
<tr>
<th>Date</th>
<th>Designation of animal</th>
<th>Time elapsing between inoculation and beginning of treatment</th>
<th>At beginning of treatment</th>
<th>Subsequent treatments</th>
<th>Result</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 10, 1911</td>
<td>No. 19(^7)</td>
<td>8 hrs.</td>
<td>Slightly ill, excited</td>
<td>Very few p.n.l., many extracellular diplococci; profuse growth</td>
<td>None.</td>
<td>Died 20 hrs. after inoculation.</td>
</tr>
<tr>
<td>May 13, 1911</td>
<td>No. 21(^7)</td>
<td>4 hrs.</td>
<td>Slightly ill</td>
<td>Slight multiplication of cocci; slight growth</td>
<td>1 and days later.</td>
<td>Died 2(\frac{1}{2}) days after inoculation.</td>
</tr>
<tr>
<td>Oct. 10, 1911</td>
<td>No. 36(^7)</td>
<td>18 hrs.</td>
<td>Apparently well</td>
<td>Few p.n.l.; myriad diplococci; profuse growth</td>
<td>1 and days later.</td>
<td>Died 3(\frac{1}{2}) days after inoculation.</td>
</tr>
<tr>
<td>Jan. 11, 1912</td>
<td>No. 50(^8)</td>
<td>10 hrs.</td>
<td>Perhaps a little excited; otherwise well</td>
<td>Very few diplococci, some intracellular; scant growth.</td>
<td>1 day later.</td>
<td>Died 5(\frac{1}{2}) days after inoculation.</td>
</tr>
<tr>
<td>Jan. 24, 1912</td>
<td>No. 52(^8)</td>
<td>8(\frac{1}{2}) hrs.</td>
<td>No signs of illness</td>
<td>Many p.n.l. and extracellular diplococci; profuse growth</td>
<td>1 and days later.</td>
<td>Died 2(\frac{1}{2}) days after inoculation.</td>
</tr>
<tr>
<td>Jan. 30, 1912</td>
<td>No. 53(^8)</td>
<td>8(\frac{1}{2}) hrs.</td>
<td>Slightly ill, restless</td>
<td>Many p.n.l. and extracellular diplococci; profuse growth</td>
<td>None.</td>
<td>Died 21 hrs. after inoculation.</td>
</tr>
</tbody>
</table>

\(^7\) Immune goat serum was employed.

\(^8\) Immune horse serum was employed.
later the first injection seemed usually to restrain infection, but the restraining action was of short duration, the disease quickly developing into its usual course and producing death. An even apparently beneficial action of subsequent injections was rare. Thus the serum was utterly powerless to stop an infection once well begun and to prevent the death of the animal.

INFLUENCE OF TREATMENT WITH A MIXTURE OF SODIUM OLEATE, IMMUNE SERUM, AND BORIC ACID.

In the experiments of this group the animal was first inoculated in the spinal canal. Afterwards, according particularly to the animal's physical appearance and the state of the spinal fluid, treatment was begun. As a rule, the treatment was repeated once a day during the life of the animal, or until the spinal fluid gave no, or very little, growth of pneumococcus. Each cubic centimeter of the mixture contained 0.1 of a cubic centimeter of a 1 per cent. aqueous solution of Merck's or Kahlbaum's sodium oleate, 0.2 of a cubic centimeter of the immune antipneumococcic serum, and 0.7 of a cubic centimeter of a 5 per cent. aqueous solution of boric acid. The mixture was always freshly prepared for each injection. In the protocols it is designated by the letters T. M. As in the experiments with immune serum, usually two cubic centimeters were injected each time during the early stages of the experiment, later it was often not possible to inject more than one cubic centimeter without causing symptoms of pressure. Of itself the mixture was harmless, both in infected animals and in normal ones employed to determine this point.

The nineteen experiments will be first recorded in the form of a table (table II) showing the gross features and result of each, after which a few that possess features of special importance or interest will be described at greater length.

From the table it is apparent that in those instances in which recovery took place it did so only after repeated injections of the mixture of sodium oleate, immune serum, and boric acid. In only one instance, that of monkey 28, were as few as three treatments given. The average number was five or six. This means that an actual disease was treated, as was clear from the animal's general
physical condition, its behavior, and from the continuous presence of pus and live pneumococci in the cerebrospinal fluid. After each treatment it was usual to notice an improvement in the physical condition, a clearing of the spinal fluid, and a reduction in the number of diplococci. This was true particularly of the first injection, which was usually followed by a disappearance of the bacteria from the circulating blood, and not only in those instances where recovery occurred but also often even in those which terminated fatally. The subsiding of the bacteremia is probably due, as Dr. Flexner\textsuperscript{11} has said in his lecture upon local specific treatment, not only to the control of the local infection but also to the action of immune principles which have diffused from the cerebrospinal fluid into the general circulation.

An example of the disease terminating in recovery is afforded by the protocol of monkey 35.

\textbf{Sept. 26, 1911.} Monkey 35. \textit{Macacus rhesus}; fairly large.
4:05 P.M. L. p.: clear fluid; injected 0.1 c.c. of a 20 hour broth culture of \textit{X.ii} (made directly from the heart's blood of a mouse). At 6:00 P.M. no change is apparent. Sept. 27, 9:00 A.M. Animal slightly ill; eyes watery and not so bright as yesterday; coat raised. 10:04 A.M., 18 hours after inoculation. L. p.: 2.5 c.c of opalescent fluid quickly, under pressure. Films from the sediment show a few p. n. 1. and about as many mononuclear cells; an occasional pair of extracellular diplococci. Culture gives moderate growth of pneumococcus.

Injected 2 c.c. of the mixture of sodium oleate, immune serum, and boric acid (T. M.) No symptoms. Throughout the remainder of the day the animal may be a little better.

Sept. 28, 9:00 A.M. Condition about the same; animal still moderately ill. 9:15 A.M. L. p.: 2 c.c. of turbid fluid freely; many p. n. 1. and a few mononuclear cells; no cocci seen; no growth.

Injected 2 c.c. of T. M. Condition remains the same remainder of the day.

Sept. 29, 10:50 A.M. Animal better; coat still fluffy. L. p.: 1.3 c.c. of turbid fluid freely; a great many p. n. 1. and many capsulated extracellular diplococci; an occasional pair intracellular; profuse growth. Injected 2 c.c. T. M.

Sept. 30, 8:45 A.M. Condition same as yesterday. L. p.: 1 drop of turbid fluid; many p. n. 1.; fewer diplococci, some involuted and Gram negative, and an occasional pair intracellular. Profuse growth but less than yesterday. Injected 1 c.c. of T. M.

Oct. 1, A.M. Condition the same. 4:05 P.M. L. p.: 4 drops of slightly blood-stained fluid; few p. n. 1., no cocci seen. Two colonies in the culture. Injected 2 c.c. of T. M.


<table>
<thead>
<tr>
<th>Date</th>
<th>No.</th>
<th>Time after inoculation</th>
<th>Condition at beginning of treatment</th>
<th>Nature of spinal fluid</th>
<th>Subsequent course</th>
<th>Result</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 14, 1947</td>
<td>36</td>
<td>4 hrs.</td>
<td>Slightly ill; fever; chills; moderate growth.</td>
<td>None obtained; one at the day three days</td>
<td>46 hrs.</td>
<td>Recovered</td>
<td>No illness.</td>
</tr>
<tr>
<td>May 21, 1947</td>
<td>42</td>
<td>3 hrs.</td>
<td>Slightly ill; fever; chills; moderate growth.</td>
<td>None obtained; one at the day three days</td>
<td>46 hrs.</td>
<td>Recovered</td>
<td>No illness.</td>
</tr>
<tr>
<td>May 24, 1947</td>
<td>43</td>
<td>3 hrs.</td>
<td>Slightly ill; fever; chills; moderate growth.</td>
<td>None obtained; one at the day three days</td>
<td>46 hrs.</td>
<td>Recovered</td>
<td>No illness.</td>
</tr>
<tr>
<td>June 2, 1947</td>
<td>44</td>
<td>2 hrs.</td>
<td>Slightly ill; fever; chills; moderate growth.</td>
<td>None obtained; one at the day three days</td>
<td>46 hrs.</td>
<td>Recovered</td>
<td>No illness.</td>
</tr>
<tr>
<td>June 5, 1947</td>
<td>45</td>
<td>2 hrs.</td>
<td>Slightly ill; fever; chills; moderate growth.</td>
<td>None obtained; one at the day three days</td>
<td>46 hrs.</td>
<td>Recovered</td>
<td>No illness.</td>
</tr>
<tr>
<td>June 15, 1947</td>
<td>48</td>
<td>2 hrs.</td>
<td>Slightly ill; fever; chills; moderate growth.</td>
<td>None obtained; one at the day three days</td>
<td>46 hrs.</td>
<td>Recovered</td>
<td>No illness.</td>
</tr>
</tbody>
</table>

**TABLE II.** The therapeutic mixture contained immune goat serum. The therapeutic mixture contained immune horse serum.
Oct. 4. Still improving. Oct. 6. Animal well. Yet fearing a possible relapse (compare No. 34) l. p. was done at 4:00 P.M. 3 drops of blood-tinged fluid; no excess of p. n. l. over normal ratio to red blood cells; no cocci seen; two colonies in culture. Injected 1 c.c. of T. M.

Nov. 5. Observed daily, animal has shown no signs of illness until today when there is diarrhea. Nov. 7. Diarrhea worse; emaciation. Nov. 10. Animal found dead this morning. Autopsy at once: moderate emaciation. Nothing abnormal found in the posterior cavity. There is an acute colitis of severe degree; hyperplasia of the mesenteric lymph nodes.

Upon microscopic examination nothing abnormal is found in the spinal cord or its membranes.

In the experiments which terminated fatally the disease followed one of two well characterized and readily recognizable courses. Either the animal was gravely ill when the treatment was begun and died during the first or second day, nearly always from septicemia; or the animal was not so ill at the beginning of treatment, the customary improvement followed the first injections, recovery seemed to have taken place, the treatment was discontinued, and then later what may be designated a relapse occurred and was quickly followed by death. In one instance (protocol 39), after apparent recovery a massive fibrinous pneumonia, instead of a relapse, developed and led to death. The meningitis was healed.

The first type of fatal disease is illustrated by the protocol of monkey 43.

Nov. 27, 1911. Monkey 43. Macacus rhesus; medium size.

1:40 P.M. L. p.: clear fluid; injected 0.1 c.c. of a 24 hour broth culture of X.114. No evidences of illness during remainder of day. Nov. 28, 8:00 A.M. Animal quite ill. 9:15 A.M. Very ill. 9:35 A.M. Seems worse than twenty minutes ago; very weak; can no longer sit up. L. p.: 1.3 c.c. of very turbid fluid containing flocculi; very many p. n. l. mostly poorly staining and an enormous number of diplococci nearly all extracellular. Very profuse growth. Likewise profuse growth from four drops of blood taken from the ear. Leucocytosis about 25,000. Injected 1.5 c.c. of T. M. No immediate change in the animal's condition, but it steadily weakens. Death at 12:15 P.M., 22½ hours after inoculation.

Autopsy.—1:30 P.M. Slight congestion of spinal meninges. There is a scant, sticky, yellowish white exudate all along the surface of the pia. It contains many p. n. l. and still a great many diplococci but not nearly so many as were in the fluid drawn off prior to the treatment. Extreme phagocytosis has occurred. Most cocci are within pus cells and many pus cells are crowded with the organisms. Cultures furnish moderate growth. The cerebral pia is more congested than the spinal, and there is more exudate. In the heart's blood are a few diplococci. Profuse growth occurs from it.
The second type of fatal disease, that of apparent recovery followed, upon discontinuation of treatment, by late relapse and death, is illustrated by the protocol of monkey 29.


10:50 A.M. L. p. : 1 c.c. of clear fluid; injected 0.1 c.c. of a 24 hour broth culture of X.5°. 2:15 P.M. Animal uneasy. 5:30 P.M., 7 hours after inoculation. Animal slightly ill; hypersensitive along spine; coat ruffled. L. p. : 2 c.c. of turbid fluid; many p. n. l., an occasional pair of cocci extracellular; more than moderate growth. Injected 2 c.c. of T. M. May 26, 10:30 A. M. Somewhat better. L. p. : 2 c.c. of turbid fluid; more p. n. l. than yesterday; only one pair of diplococci seen; a single colony grows from the transplant. Injected 2 c.c. of T. M. Condition remains the same during remainder of day. May 27, A.M. Much better; animal springs up and down as normally. May 28. Well. May 29. Still seems well.

May 30, 9:20 A.M. The animal is found with coat slightly raised, sitting on its perch. It cannot be made to come down; cries when disturbed. Made many attempts, in several spaces, to obtain spinal fluid; all failed; even three made under light ether anesthesia. Finally, injected with little resistance 1.5 c.c. of T. M.; no pressure symptoms observed. The animal quickly recovers from the ether and upon being put into its cage climbs upon the perch and remains sitting as before. 2:00 P.M. Still sits upon perch; seems about the same.

4:30 P.M. Animal dead. Death occurred 5½ days after inoculation.

Autopsy.—May 31, 9:30 A.M. The spinal cord beneath the dura is enveloped throughout its length by a thick, yellowish white, translucent sheath comprising the pia infiltrated and covered by a fibrinous exudate, the whole averaging from 1 to 2 mm. in thickness. Films show many p. n. l. in all stages of disintegration, and an occasional coccus, but so few that only after a search of several minutes are a few swollen pairs found within a single pus cell.

The pia of both cerebral hemispheres and to a less extent that of the cerebellum is finely congested and studded with abundant punctate hemorrhages. Films from the cerebral cortex contain many well preserved p. n. l. and many typical extracellular diplococci. There is less similar exudate at the base of the brain. The ventral cavity of the body is negative. Cultures from the exudate in the lumbar and thoracic regions of the spinal cord and from the heart's blood remain sterile. From the right cerebral cortex the pneumococcus grows abundantly and pure.

In this experiment the effect of the injections in almost sterilizing the spinal canal is apparent. The clinical "relapse" and death of the animal were evidently due to the progress of the inflammation in the cranial cavity which had been cut off from communication with the spinal subdural space by the fibrinous exudate.
DISCUSSION.

The experimental data presented have more than a theoretical interest. They should indeed be considered with respect to their application to the treatment of a highly fatal disease of human beings. Pneumococcic meningitis is far from being a rare affection, and the number of reported recoveries from the disease is very small. There exists at present no effective treatment that has come to be at all generally employed. The few instances in which antipneumococcic serum has been applied have not yielded results that inspire confidence in its sole employment. Even when administered by direct intraspinal injection the effects have been doubtful. Matthes\(^2\) so treated three patients and all died; Grober,\(^3\) two, one of which recovered and one died; and Schlesinger\(^4\) and Kleinschmidt,\(^5\) one each, both recovering. The total number is small, and although three of seven treated with serum recovered there have been reported ten more instances of recovery following no more radical treatment than mere lumbar puncture and withdrawal of fluid.\(^6\) The reasons for the inefficiency of serum treatment are now rendered fairly clear. An antipneumococcus serum is at best active only against the homologous organism or organisms the types of which have been employed in its preparation. Under optimal experimental conditions the extent of its efficacy is confined within a brief space of time; and in general this will probably be found equally true of the so called spontaneous human pneumococcus meningitis. The outlook for its successful employment alone in the human disease is not encouraging.

The therapeutic mixture of sodium oleate, boric acid, and antiserum holds out greater promise. The scope of its efficacy is far wider than that of the antiserum alone. It remains still to be determined whether its action is as closely restricted within the limits of homologous strain of pneumococcus as is that of the anti-

\(\text{\textsuperscript{2}}\) Matthes, M., Med. Klin., 1908, iv, 733.
\(\text{\textsuperscript{3}}\) Grober, J., München. med. Wochenschr., 1910, lvii, 1332.
\(\text{\textsuperscript{4}}\) Schlesinger, H., Wiem. med. Wochenschr., 1911, lxii, 46.
\(\text{\textsuperscript{5}}\) Kleinschmidt, Med. Klin., 1911, vii, 1195.
\(\text{\textsuperscript{6}}\) Rolly, Fr., Deutsch. med. Wochenschr., 1911, xxxvii, 774; Kleinschmidt, loc. cit.
serum. This point, which is of high importance, is under examination at present so that a decision should soon be reached. 17

Fortunately the number of strains or types of pneumococci appears not to be large. Neufeld and Händel 18 have noted that most strains of pneumococcus are subject to the action of an immune serum prepared with a single culture of their type I pneumococcus, which is the culture employed for immunizing the goat and horse used in these experiments. Neufeld and Händel recognize four serum-specific types of pneumococcus, three of which are infrequent. It may happen that the specificity is not absolute with respect to the oleate serum mixture, and it is also, of course, possible that the obstacle to the usefulness of a single immune serum may be obviated by preparing a suitable polyvalent serum.

Other considerations which may affect the results of the employment of the oleate serum mixture are clinical in nature. From the experiments it appears that pneumococcal infections, started in the region of the brain, follow a severer course, and are less amenable to treatment than those started in the region of the spinal cord. In both, the experimental procedures produce traumatic injury and this injury gives to the infection a graver character when inflicted on the tissues of the brain than when inflicted on those of the cord. A class of pneumococcal inflammation of the meninges in man arises from infections of the membranes about the brain; namely, in middle ear disease, fracture of the base of the skull, infection of the accessory air sinuses, operations on the nasal tissues, etc. It remains to be ascertained to what extent this is a determining factor in the control of natural pneumococcus meningitis.

A great number of cases of pneumococcus meningitis in man follow blood infection with the pneumococci and are secondary to in-

17 Tentative experiments performed on rats and mice with two cultures of atypical strains of pneumococcus, obtained from the circulating blood of pneumonic patients, indicate that the atypical strains stand midway between the highly soluble typical pneumococcus and the insoluble Streptococcus pyogenes in respect to solubility in sodium oleate. The experiments indicate further that the atypical strains are resistant to the action of the mixture of typical antiserum, sodium oleate, and boric acid. Experiments are in progress to determine the effect of polyvalent immune sera which carry the specific antibodies for the atypical strains.

flammations of the lung, pleura and heart, and arise generally throughout the meninges. In these cases the therapeutic control of the meningitis need not be equivalent to the suppression of the pathological conditions as a whole, to which the patient may still succumb. On the other hand, it is often just the concurrent meningitic infection that makes the general clinical conditions grave, so that with its abatement the outlook for the cessation of the other inflammation becomes much improved. Fortunately, the direct attack upon the meningitic infection is capable of affecting the blood infection since the eruption of pneumococci from the meninges into the blood is arrested while the ready escape of the therapeutic mixture from the meninges into the blood provides a favorable condition for the action of the immune serum upon the pneumococci already present in the blood, as well, possibly, as those situated in the interior of some organs.

The experiments show that the time interval between onset of infection and beginning of the specific treatment is a very important factor. The reasons for this are several: the degree of the infection and attending intoxication; the numbers of multiplying diplococci; the nature of the inflammatory exudate,—whether serous, purulent, or fibrinous,—that effects the penetration of the mixture to the diplococci and the remote parts of the cerebrospinal meninges. A purulent and fibrinous exudate is not only permeated less readily by the mixture, but its high protein content tends to reduce the action of the oleate upon the pneumococci upon which the efficiency of the mixture so much depends. Hence serous or seropurulent inflammations will probably be more subject to control than purulent and fibrinous ones. And yet in practice this distinction may make less difference than in the experiments in which the course of the infection is abnormally rapid. Should it be found that infection of the spinal meninges is sometimes controlled, while that of the cerebral meninges and ventricles is not, it will be well to consider the injection of the mixture directly into the cerebral ventricles, as has been successfully done in the serum treatment of epidemic meningitis.  

Footnotes:

9 Flexner, Simon, loc. cit.
Finally, the specific treatment described is not necessarily confined to meningitis but may be applicable to other local pneumococcal infections, such as those of the pleura, joints, and possibly the peritoneum. The determining conditions will be similar: the type of pneumococcus, the accessibility of the focus of infection (whether isolated by adhesions or communicating with the cavity into which the mixture is injected), the nature of the inflammatory exudate, and the coexistence of other slight or severe infections in inaccessible organs or parts. The oleate soap obviously possesses higher affinity or greater avidity for certain bacteria (e.g., pneumococcus) than for protein in general, since the boric acid suffices to hold it apart from serum protein under conditions in which it still attacks the bacteria.

CONCLUSIONS.

Virulent pneumococci injected into the cranial or spinal cavities of monkeys produce constantly a meningitis closely resembling pneumococcus meningitis in man, except that the experimental disease pursues a more rapid course to the invariable death of the untreated animal.

An homologous immune pneumococcus serum injected into the spinal canal exerts a restraining influence upon the disease to the extent that when employed early it prevented, exceptionally, the occurrence of infection and thus saved the life of the animal, and when given later it at first retarded the disease but subsequently exerted no beneficial action and was powerless to save life.

A mixture of sodium oleate, immune serum, and boric acid exerted regularly a more powerful action than immune serum alone, and not only prevented the occurrence of infection but also, when administered repeatedly, arrested the progress of an actually established infection and led, often, to the enduring and perfect recovery of the inoculated animal.

It is proposed to employ a similar mixture in the direct treatment of pneumococcal meningitis and possibly of still other accessible local pneumococcic infections in man.