THE NEUTRALIZATION OF ANTIPNEUMOCOCCUS IMMUNE BODIES BY INFECTED EXUDATES AND SERA.

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That sterile filtered inflammatory exudates have the power to modify the course of infection was noted long ago. The chief discussion concerning the nature of this phenomenon has been carried on by Bail and his associates and by those who have contraverted their views. Bail[1] gave to the hypothetical substances existing in pathological exudates, which alter the course of infection, the name "aggressins." He thought that they were secreted by the bacteria during their growth in the animal body and acted by inhibiting or by neutralizing the defensive mechanisms of the body. According to this investigator it is to these substances that the bacteria owe their power to invade the body tissues and therefore it is upon their ability to form them that the property of virulence depends.

Wassermann and Citron[2] have opposed the view of Bail that these so called aggressins represent substances set free by the bacteria during their struggle against the protective agencies of the body, and believe that they represent merely bacterial substances which may go into solution either within the body or during growth or autolysis *in vitro*, and that these substances act by fixing the humoral immune bodies and so rendering them ineffective; that the mode of action of these substances therefore does not differ from that of dead bacteria. It is not german to the present discussion to consider the large amount of evidence which has been brought forward to support the contending views, the chief purpose of the present paper being to record the demonstration of large amounts of substances which neutralize immunity principles in the blood and exudates of animals infected with pneumococci, and to indicate the importance of these substances in the specific therapy of acute lobar pneumonia.

The part which these substances play in experimental pneumococcus infections, especially their effect on phagocytosis, has been studied by Hoke,[3] Rose-

[1] Bail, O., *Arch. Hyg.*, 1905, lii, 272.

[2] Wassermann, A., and Citron, J., *Deutsch. med. Woch.*, 1905, xxxi, 1101.

[3] Hoke, E., *Wien. klin. Woch.*, 1905, xviii, 348.

now,[4] Tschistowich and Jurewich,[5] Zade,[6] and Nunokawa.[7] Tschistowich and Jurewich have made the observation that thoroughly washing virulent, non-phagocytable pneumococci in salt solution is sufficient to render them phagocytable. Rosenow, as well as Tschistowich and Jurewich, has also observed that treating non-virulent, phagocytable pneumococci with the washings from, or extracts of virulent pneumococci is sufficient to render the former non-phagocytable and therefore virulent, and he has found that this property is retained even after washing in salt solution. This writer has given to the hypothetical substances which may be extracted from virulent pneumococci the name "virulin," and Tschistowich and Jurewich have given to the substances which they have obtained by somewhat similar methods the name "anti-phagin."

It is evident that these observations, especially those of Rosenow, would render necessary an entirely different conception of the phenomenon from that held by Wassermann and Citron.

In a considerable number of experiments, however, I have been unable to confirm the observations of Rosenow that non-virulent pneumococci may absorb and fix something derived from virulent pneumococci which renders the former virulent, and for the present, therefore, I am inclined to accept the explanation offered by Wassermann and Citron, especially since this conception is sufficient to explain all the following observations.

The first observations on which this communication is based were made on the fluid removed from the chests of persons suffering from empyema. The fluid from these cases was examined for its content in pneumococcus immune bodies as tested by agglutination and protection. Similar tests of the patient's blood showed that it possessed well marked protective and agglutinative properties, and we were therefore surprised when we found that the empyema fluid possessed no such powers. A probable explanation seemed to be that, although the immune bodies were originally present in the exudate, they had been absorbed by the bacteria present, just as they may be from immune blood serum when bacteria are added *in vitro*. It occurred to us, however, to test this empyema fluid after removal of bacteria, to determine whether or not there might be present soluble substances,

[4] Rosenow, E. C., *J. Infect. Dis.*, 1907, iv, 285.

[5] Tschistowich, N., and Jurewich, Y., *Ann. Inst. Pasteur*, 1908, xxii, 611.

[6] Zade, M., *Z. Immunitätsforsch., Orig.*, 1909, ii, 81.

[7] Nunokawa, K., *Z. Immunitätsforsch., Orig.*, 1909, iii, 172.

which would fix or divert the immune substances contained in immune serum. The following is a protocol of one experiment.

Case 1.—E. R.; age 19 years. Acute lobar pneumonia followed by empyema due to Pneumococcus Type I. 200 cc. of thick pus were removed at operation. A portion of the fluid was centrifugalized at high speed for 30 minutes, the super-

Agglutination Tests					
0.4cc Serum I	+	0.4cc empyema fluid		0.1cc Cult. I	2 hrs. 37° - 18 hrs. ice
" " (1:10)		" " "		"	++
" " (1:20)		" " "		"	++
" " (1:40)		" " "		"	+
" " (1:50)		" " "		"	−
" " (1:100)		" " "		"	−
" " (1:200)		" " "	30 min at 37° C.	"	−
" " (1:10)		NaCl		"	++
" " (1:20)		"		"	++
" " (1:40)		"		"	++
" " (1:50)		"		"	++
" " (1:100)		"		"	++
" " (1:200)		"		"	++
———		Empyema fluid undiluted		"	−
———		" " 1:20		"	−
———		" " 1:40		"	−
———		" " 1:100		"	−

TEXT-FIG. 1. Protocol of an experiment showing the inhibiting action of empyema fluid on the agglutination of pneumococci by immune serum.

natant fluid was removed to a fresh centrifuge tube and again centrifugalized for 1 hour, and finally diluted with an equal quantity of isotonic saline solution and again centrifugalized for 30 minutes. The perfectly clear fluid as examined mi roscopically contained no organisms.

This fluid was then tested for its power to cause agglutination of Type I pneumococci and also for its power to inhibit the agglutination of pneumococci by Type I immune serum. The results are given in Text-fig. 1.

The fluid was then tested for its power to inhibit the protective action of immune serum against infection with Type I pneumococci as tested in mice. To guard against the possibility that the fluid, centrifugalized as noted above, might contain an occasional pneumococcus which might interfere with the result, it was heated for 30 minutes at 56°C. Cultures made from this fluid were sterile.[8] Text-fig. 2 gives the results of this experiment. They show in a striking way that empyema fluids may contain large amounts of soluble substances which inhibit the action of immune serum.

Protection Tests					
Culture dilution cc.	0.2cc. i.h.s. + 0.2cc. empyema fluid	0.2cc. i.h.s. 0.2cc. n.h.s.	0.2cc. NaCl + 0.2cc. empyema fluid	Culture control	Empyema fluid alone cc.
0.1	D. 20 hrs.	S.	D. 30 hrs.	—	0.5 S
0.01	" 21 "	"	" 16 "	—	0.3 "
0.001	" 30 "	"	" 38 "	—	0.2 "
0.0001	—	—	" 30 "	—	0.1 "
0.00001	—	—	" 30 "	D. 30 hrs.	
0.0000001	—	—	" 30 "	" 30 "	

TEXT-FIG. 2. Protocol of an experiment showing the inhibiting action of empyema fluid on the protection of mice by immune serum.

Similar tests have been carried out with a series of these empyema exudates removed by aspiration or at operation. The results have not been so striking in all the cases examined as those shown in the above protocol, though some degree of inhibition has been present in all infected cases. Several sterile serous fluids aspirated from the chest of pneumonia patients, however, have not exhibited this phenomenon. The degree of inhibiting action is apparently dependent upon the degree of infection and the time the infection has lasted before aspiration is performed.

These observations indicate why it is that infections in the partially immunized animal tend to be focal and why, when an animal is infected with organisms of slight virulence, the infection tends to remain localized. It is probable that as soon as bacteria begin to grow in tissue spaces these inhibiting substances appear in the in-

[8] In making the tests with mixtures of serum and fluid, the mixtures were allowed to incubate for 30 minutes at 37°C. before injection.

flammatory exudate, and when the fluid is not readily absorbed the substances accumulate in large amounts, so that finally, as in empyema, it is practically impossible to produce a focal immunity reaction until the focus is opened and the fluid, with its content of neutralizing substance, is removed by drainage, when the bacteria remaining are no longer protected from the natural or artificial defensive mechanisms of the body and so may be overcome. This conception agrees in the main with the view held by Bail and others, though the application of the theory has previously been made rather to the problems of virulence and infection than to those of recovery. The observations previously mentioned also indicate that favorable results can hardly be expected from the treatment of these focal infections with immune serum, either administered intravenously or injected directly into the focus itself unless the pathological exudate has previously been removed. We have made one attempt to treat a patient suffering from empyema by the direct injection of immune serum into the cavity, but without apparent effect. These observations offer the explanation for the failure. In the treatment of focal infections with immune serum, without drainage, it would be necessary to inject sufficient serum to neutralize all the inhibiting substances present, as well as the amount necessary to prevent the harmful activities of the bacteria themselves.

Our next problem was to discover whether or not the inhibiting substances appear in the blood as a result of septicemia. This was first investigated by inoculating rabbits with very large injections of pneumococcus and testing the blood removed during the height of infection for the presence of these substances. To show that this action is due to soluble substances, and not to the bacteria present, the bacteria have been removed from the serum by filtration before testing. The results of one of these experiments are given in the following protocol.

Rabbit 1.—Weight 1,300 gm. July 11, 1916, 12 noon. Inoculated intraperitoneally with 1 cc. of peritoneal exudate of a rabbit previously infected with Type II pneumococci. 5 p.m. Blood culture shows innumerable numbers of pneumococci.

July 12, 10 a.m. Animal very sick. Blood removed by heart puncture. Serum removed from clot and passed through a Berkefeld filter. Culture of filtered blood sterile. The filtered blood was tested for its power to inhibit the

agglutinating action of immune horse serum, Type II. Table I shows the results obtained.

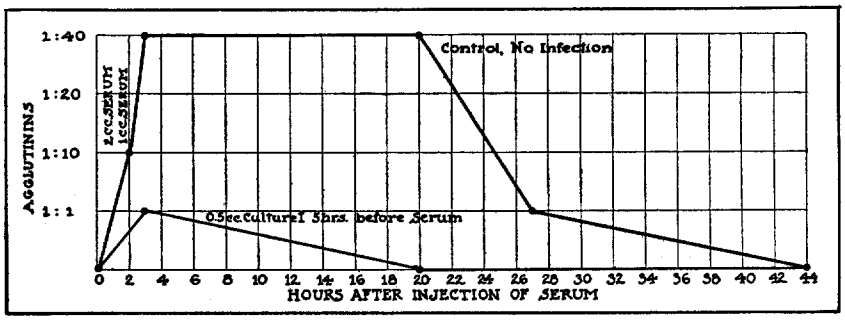
TABLE I.

Agglutination.		Results after 2 hrs. at 37°C. and 18 hrs. on ice.
0.4 cc. of Immune Serum II (1:40) + 0.4 cc. of filtered serum	After 30 minutes at 37°C. 0.1 cc. of Culture II was added to each tube.	−
0.4 cc. of Immune Serum II (1:50) + 0.4 cc. of filtered serum		−
0.4 cc. of Immune Serum II (1:100) + 0.4 cc. of filtered serum		−
0.4 cc. of Immune Serum II (1:200) + 0.4 cc. of filtered serum		−
0.4 cc. of Immune Serum II (1:40) + 0.4 cc. of salt solution		++
0.4 cc. of Immune Serum II (1:50) + 0.4 cc. of salt solution		++
0.4 cc. of Immune Serum II (1:100) + 0.4 cc. of salt solution		++
0.4 cc. of Immune Serum II (1:200) + 0.4 cc. of salt solution		++
0.4 cc. of Immune Serum II (1:400) + 0.4 cc. of salt solution		+
0.8 cc. of salt solution		0

The results of this and other similar experiments show that specific inhibiting substances such as those which are present in pathological exudates may also be present in the blood when an animal is suffering from a severe septicemia.

Another method which has been used for testing the presence of inhibiting substances in the blood is the following. A rabbit is infected with pneumococci and after the infection has reached its height immune serum is injected intravenously. At the same time, and as a control, a normal rabbit receives the same amount of immune serum intravenously. Within a few minutes and at varying periods following the injection of the serum, samples of blood are removed from both rabbits and tested for their content in antibodies. For this purpose agglutination is employed. If no neutralization of antibodies occurs, it is evident that the content of the blood in agglu-

tinins immediately following the injection should be the same as though the immune serum had been diluted *in vitro* with a quantity of fluid equal to the blood contained in the rabbit, and that by making repeated tests a curve showing the disappearance of the immune bodies by destruction or excretion may be constructed. As a matter of fact, numerous observations in normal rabbits have shown that when the rabbit's blood is tested within a few minutes following the injection of immune serum, its content in agglutinins is about that to be expected when the probable volume of blood in the rabbit and the consequent dilution of the immune serum is calculated. On the other hand, when a similar injection is made into an infected animal,



TEXT-FIG. 3. Curves showing the agglutinating power of the serum of normal and infected rabbits following the injection of immune horse serum.

the agglutinating power of the serum obtained from it is much less than that calculated from the probable dilution; indeed, agglutinating power may be entirely absent. Moreover, when the agglutinating power is present, though lower than that of the serum of the normal rabbit, and curves are made to show the disappearance of the agglutinating power, it is found that the agglutinins disappear much more rapidly from the serum of the infected rabbit than they do from the serum of the uninfected rabbit. Text-fig. 3 shows in a graphic manner the results obtained in one of these experiments.

In these experiments the possibility cannot be excluded that the fixation or neutralization of antibodies is due to the presence of bacteria circulating in the rabbit's blood, but previous observations make it

improbable that the entire phenomenon can be due to this. It seems probable that the neutralization is due to a considerable extent to the presence of soluble inhibiting substances.

This last method of study is directly applicable to patients, and a study of this kind in patients is of importance since it is difficult to produce in animals pneumococcus infections which last over a period of a week or longer, such as those which occur in man. Moreover, it was hoped that this study would offer indications for proper dosage of serum and might even be applicable in the treatment of the individual case. In a series of cases, therefore, the serum has been tested for its content in agglutinating antibodies both before and following the administration of immune serum.

The method of procedure was as follows: Samples of the patient's serum were obtained before any immune serum was administered and also 5 minutes following the first dose. Where more than one dose was administered (and successive doses have usually been given with 6 to 8 hour intervals) other samples were obtained immediately before and 5 minutes following each subsequent dose. Finally, following the last dose, in certain cases, samples were obtained at varying periods to observe the persistence of agglutinins in the blood. The samples from each patient were kept on ice until all had been obtained and they were then tested on the same day and with the same technique for the presence of agglutinins. The agglutination tests were made by the macroscopic method. In each of a series of small test-tubes was placed 0.9 cc. of the serum, or of the diluted serum. To each of the tubes was then added 0.1 cc. of an 18 hour broth culture of pneumococcus of the type to which the infection was due and corresponding to the serum which had been injected. The results were read by transmitted light after 2 hours at 37°C. and again after the tubes had remained on ice over night.

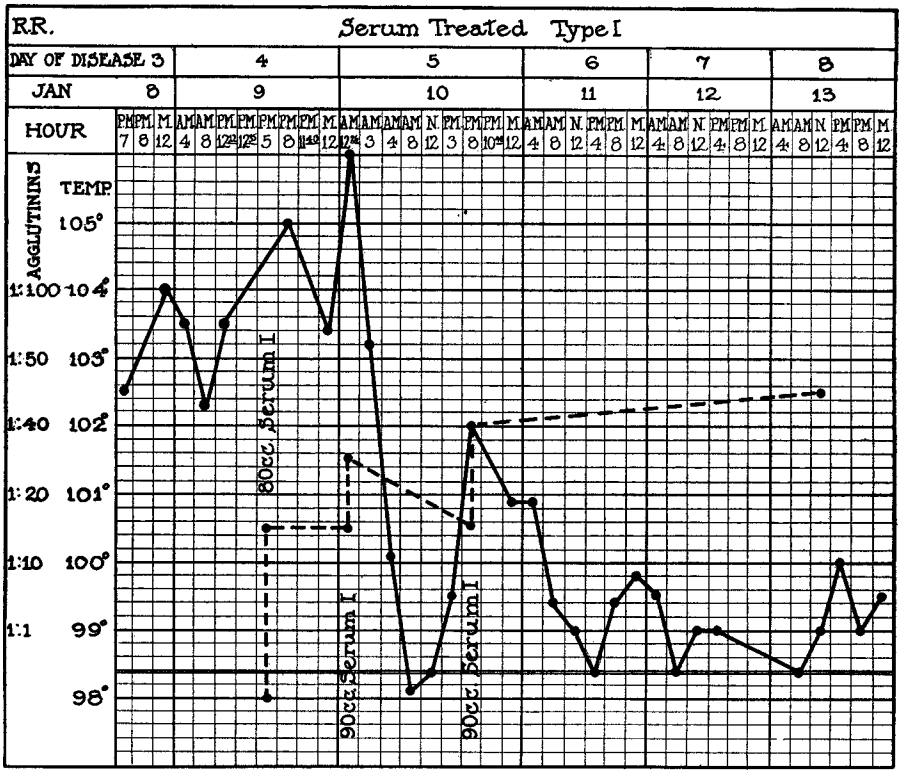
Employing these methods, agglutinin curves have been made from 30 cases suffering from Type I infection and receiving serum, 9 cases suffering from Type II infection, and also serum treated, 7 cases with Type II infection, who received no serum, 4 of Type III, not serum treated, 2 of Type IV not serum treated, and 1 case due to Type IV infection who, through a mistake in diagnosis, received several doses of Type I serum, making a total of 53 cases.

Cases Due to Type I Infection Treated with Serum.

Of these cases, 3 were treated on the 2nd day, 5 on the 3rd day, 5 on the 4th day, 4 on the 5th day, 9 on the 6th day, 3 on the 7th day, and 1 on the 9th day. All but two of the cases recovered. The charts and protocols of several cases, which illustrate the relation of agglutinin titer to the clinical course and to the temperature curve, are given below.

Case 1.—R. R., student; age 22 years. This patient was admitted January 8, 1917 at 7 p.m. suffering from pneumonia involying the left lower lobe. The onset had been quite typical with chill, 48 hours before admission. He was moderately sick; temperature 102.5°F., pulse 115, respirations 30. The leukocytes numbered 31,000 and the blood culture was positive, the plates showing one colony per cc. of blood. The sputum was bloody; a small amount was at once inoculated into a mouse. The following morning tests made of the growth in the peritoneal cavity of the mouse showed that the patient was suffering from an infection with Type I pneumococci. 12.17 p.m. The intravenous injection of antipneumococcus serum was commenced. Although the serum was given slowly, after he had received about 35 cc. he had some signs of serum intoxication, suffusion of the face, respiratory difficulty, and he vomited several times. The administration of serum was therefore at once discontinued. No tests were made of the agglutinating power of his serum before or after this treatment. The patient's condition did not materially change during the afternoon and at 5 p.m. serum was again administered; this time 80 cc. were given without any untoward symptoms. A sample of blood was taken just before and another one 5 minutes following the administration of the serum. When tested later, it was found that the blood before administering the serum contained no agglutinins for pneumococcus; the sample of blood taken following the administration of serum agglutinated Type I pneumococcus in a dilution of 1:15. This represents a concentration of antibodies fully equal to that which might be expected, taking into consideration the titer of the serum injected and the probable volume of the patient's blood. The patient's condition did not materially improve after this injection, so that another dose of 90 cc. of serum was administered at 12 midnight. A specimen of blood which was obtained just before this injection showed that the agglutinating power had not diminished during the time intervening since the preceding dose, and the specimen of blood taken 5 minutes after the serum was injected showed an increased concentration of agglutinins, so that now agglutination occurred with a 1:30 dilution of serum. Immediately following this injection the temperature rose to 106°F. and he had a shaking chill. The temperature then began to fall, being only 98.1°F. at 8 a.m. With this fall in temperature, the patient's condition markedly improved. During the day, the temperature again rose slowly, without, however, any other unfavorable features. As the temperature at 8 p.m. was

102°F., it was decided to administer another dose of serum, and 90 cc. were given, without any reaction. The test of the patient's serum obtained before this treatment showed that the agglutinins had fallen slightly, agglutination occurring in a dilution of 1:15, but after the treatment the titer again rose to 1:40. Following this treatment the patient made a good recovery.

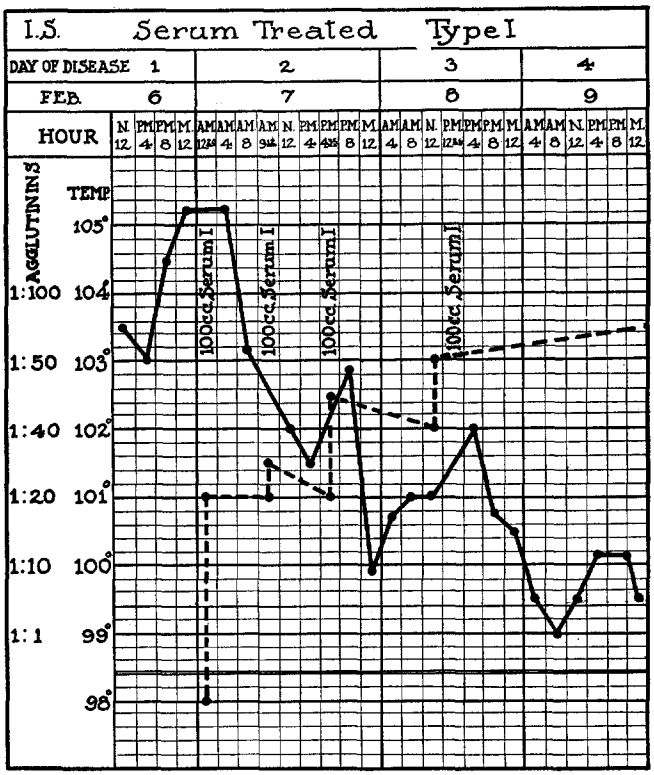


——— Temperature curve.
- - - - - - Curve of agglutinin titer.

TEXT-FIG. 4. Chart showing the curve of the agglutinin titer and the temperature curve of R. R.

In this case, therefore, which was mild or of only moderate severity, treated early in the disease, the administration of immune serum was followed by a satisfactory concentration of antibodies in the blood and there was no evidence of fixation or neutralization of the injected immune substances. This is graphically shown in Text-fig. 4.

Case 2.—I. S., tailor; age 32 years. This patient was admitted about 8 hours following the initial chill. There were definite signs of involvement of both lower lobes and he presented all the characteristic features of acute lobar pneumonia. His temperature on admission was 103.5°F., pulse 120, respirations 48. He appeared seriously sick; the blood culture taken on admission was positive. The diagnosis of the type of infecting organism was made by inoculation of sputum



TEXT-FIG. 5. Chart showing the curve of the agglutinin titer and the temperature curve of I. S.

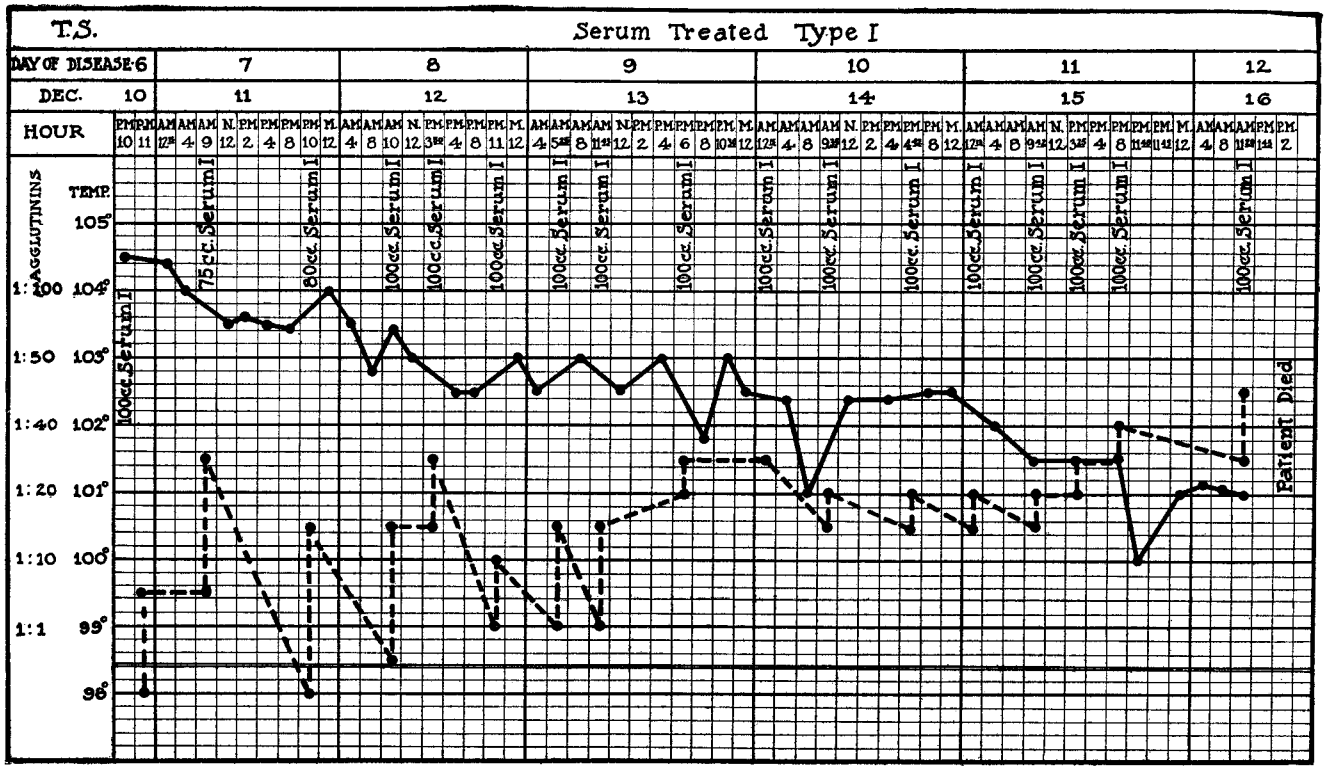
into a mouse, and at 12 midnight, 12 hours after admission, he was given his first treatment. As the curves presented in Text-fig. 5 show, the first dose of serum imparted to the blood a well marked power of agglutination, and each subsequent dose was followed by an increase of this property. With the increase of agglutinins in the blood there occurred an improvement in the patient's condition, the temperature fell, and he finally recovered completely.

The two examples given above illustrate the effects of serum treatment in the cases due to Type I infection, when the serum is given early and in large amounts, and when the infection has not reached too high a grade before the treatments are commenced. Since in twenty-eight out of the thirty cases recovery followed the administration of the serum, we did not have great opportunity to study cases of this type in which the serum was not effective. In all the cases treated with this type of serum agglutinins could be demonstrated in the patient's blood 2 to 3 minutes after the administration of 75 to 100 cc. of serum. The agglutinating power varied somewhat, though in most cases it occurred with a dilution of 1:10 or more. The refinements of the method are not sufficient to justify our calculating in each case the probable dilution and the consequent probable loss in agglutinins in the short interval elapsing before the first tests were made. In general, where the administration of subsequent doses of the serum has not led to a prompt increase of the agglutinins in the blood above the previous level the fall of temperature has been longer delayed and more serum has been required than in the cases in which a regular step-like rise took place. In comparing the temperature and agglutination curves in these cases it has been necessary to keep in mind the fact that the temperature alone does not offer a safe and sure criterion for judging the patient's condition and therefore for the effectiveness of the serum. In view of this fact it has been surprising to see the considerable uniformity with which the temperature and agglutination curves run in opposite directions. With rise of agglutinating power the temperature curve falls.

Of more importance than the immediate rise in agglutinins following the first dose is probably the persistence of the agglutinins in the blood during the subsequent 8 to 10 hours elapsing before the following dose of serum is given. In only five of the cases did a decrease during this period occur. In three of the cases the loss occurred only following the first dose. Following the subsequent doses the concentration reached a high level and persisted. The data in these three cases is not sufficient to enable us to state categorically that this loss indicated a greater severity of infection, though taken in connection with our other observations this seems probable. Two of these three cases were treated on the 4th day and one on the 6th. They

required two, three, and four doses of serum respecti ely and all made good recoveries following the serum treatment. In one of the other cases in which the agglutinating power disappeared before the following dose was given, the concentration of immune bodies following the first three doses was such that agglutination did not occur with dilutions greater than 1:5, and following the first two doses this power disappeared completely before the subsequent dose was given. It was only after numerous doses had been given that the concentration of agglutinins reached any considerable level and persisted. Altogether this patient required eleven doses of serum given over 7 days. Treatment was commenced in this patient on the 2nd day, but it was not pushed with great vigor at the start, the first dose being 80 cc., with 18 hours elapsing before the administration of the second dose of 70 cc., and 12 hours again elapsing before the administration of the third dose of 80 cc. This case suggested very strongly the inadvisability of inactive treatment at the start. This patient ultimately recovered and there occurred no extension of the lesion to other lobes, but the temperature remained high for 10 days and he was very ill. Finally, the last case in which the agglutinins disappeared between subsequent doses and in which there occurred difficulty in causing a persistent concentration of immune bodies in the patient's blood by the administration of immune serum was one of the two cases which ended fatally. The curves taken from the record of this case are shown in Text-fig. 6. It is apparent from the curves that it was not until treatment had been continued for 3 days, and nine doses had been given, that a persistent concentration of immune bodies at a high level was attained. Even following this there was a constant tendency for the concentration of immune bodies in the serum to fall, rather than to rise.

It should be noted that the treatment in this patient was commenced only on the 6th day, and 11 and 13 hours elapsed between the first and second, and the second and third doses, respectively. He was desperately ill on admission; temperature 104.5°F., pulse 136, and the blood culture showed an extremely high grade of infection, over 300 colonies per cc. In spite of this he lived until the 12th day. It seems that in this case the serum prolonged life. The infection and intoxication, however, at the start were so great that, although the



TEXT-FIG. 6. Chart showing the curve of the agglutinin titer and the temperature curve of T. S.

infection could be kept down, the intoxication could not be recovered from. In this instance it is likely, judging from the experimental observations, that the presence of large amounts of soluble inhibiting substances in the blood prevented the action of the immune serum. It is probable that in such cases very late in the disease these substances may be so large in amount that no practical amount of immune substances can neutralize them. If these conceptions are correct, the importance of giving very large doses of immune serum at the beginning of treatment is apparent.

In the other fatal case, persistent high concentration of immune bodies in the patient's blood was obtained without difficulty Nevertheless, the patient's condition did not improve and repeated doses of serum were administered. Type I pneumococci had been obtained from the sputum, and the blood culture showed 47 colonies per cc. of the same organisms. Treatment was commenced on the 6th day and the patient died on the 10th day. The pathological changes were extensive in both lungs. The autopsy showed a very widespread tuberculous involvement of both upper lobes and the upper portion of the lower lobe on each side. At the base of one lung, however, was a small area of complete consolidation, differing in appearance from the remainder of the tuberculous lung. This proved on study to be a typical acute diffuse pneumonic process and from it Type I pneumococci were cultivated. We have here an instance in which the serum was apparently effective against the specific infection, but death occurred on account of factors associated with the primary and extensive tuberculosis.

A further interesting case in this connection was one due to Type IV infection. Owing to a mistake in the early determination of the type of infection the patient received several doses of Type I serum before the mistake was discovered. In this patient, although he was quite ill, the administration of the serum caused a prompt appearance of agglutinins in the blood and this increased with the subsequent doses, without any material fall.

These studies of agglutination curves in the cases of Type I infection, however, while instructive and suggestive, do not after all give definite proof that the effect of immune serum is limited by the presence of soluble substances in the blood. When they are con-

sidered, however, in the light of the observations on the Type II cases which follow, the evidence becomes much more suggestive.

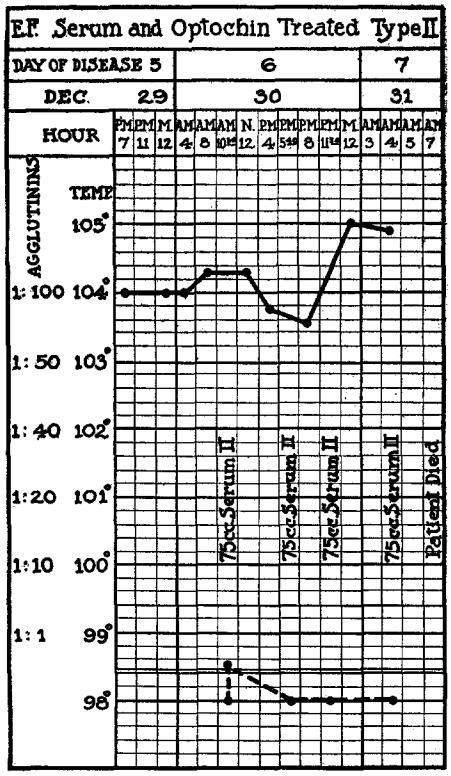
Cases Due to Type II Infection.

The reader should be reminded at the outset that it has been impossible to produce a serum against Type II pneumococci which is as active either *in vitro* or *in vivo* as is the serum against Type I pneumococci. Whereas the horse serum against Type I infection is of such a strength that 0.2 cc. will regularly protect a mouse against 0.1 cc. of virulent culture, it has been impossible to produce an immune Type II serum of any greater activity than that 0.2 cc. will protect a mouse against 0.01 cc. of culture. Moreover, the active Type I sera usually cause agglutination of homologous organisms in dilutions of 1:400 or over; the Type II sera usually cause agglutination in dilutions no greater than 1:200. It should also be noted that the capsule formation of Type II pneumococci is more highly developed than is that of Type I pneumococci. Dochez and Avery[9] have pointed out that production of precipitable substances in the blood and urine of infected animals apparently bears some relationship to this property of capsule development, the Type III organisms, which possess large capsules, forming most of this substance, the Type II organisms, which have smaller capsules, producing less, and the Type I organisms, which have small capsules, producing still less. While it is not certain that the substances in the infected animals which give rise to fixation of antibodies are identical with those concerned in the precipitation phenomenon, it seems likely that this is the case.

Studies of the agglutinin content of the blood were made in nine cases of Type II infection which received Type II serum. Of these patients, four recovered and five died. Of the patients who recovered, in two treatment was commenced on the 3rd day, in one on the 4th day, and in one on the 5th day. Of the fatal cases, treatment was commenced in one on the 3rd day, in one on the 5th day, and in the others on the 6th day. In all the four cases which recovered, a satisfactory and persistent concentration of agglutinins in the blood appeared. In one of the fatal cases, practically no agglutinins ap-

[9] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, xxvi, 477.

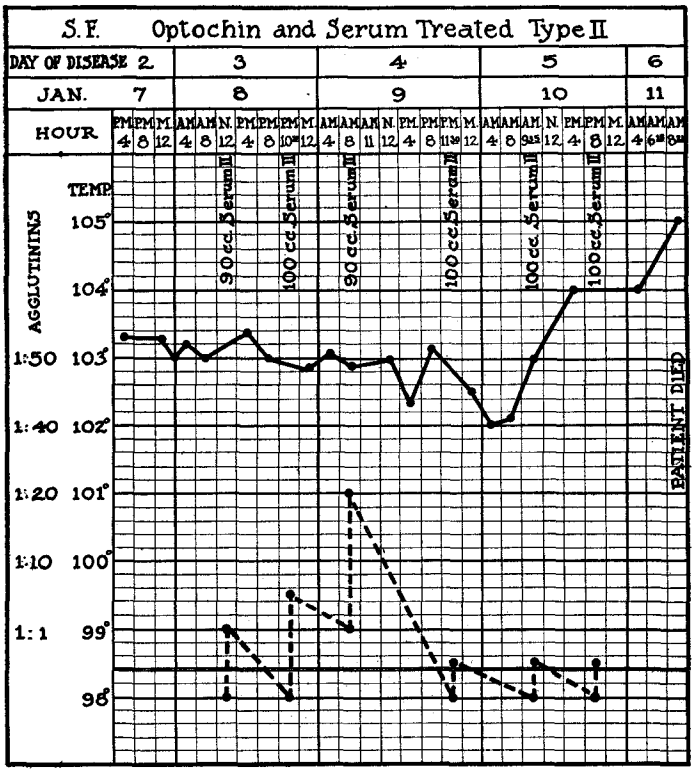
peared in the blood in spite of four doses of serum; in one a satisfactory concentration appeared only after three doses, and then disappeared; in another a satisfactory concentration was obtained only on the 9th day, after five doses of serum; in another, while a satisfactory concentration of agglutinins was obtained on the day



TEXT-FIG. 7. Chart showing the curve of the agglutinin titer and the temperature curve of E. F.

treatment was commenced, the 6th day, the patient was at that time suffering from meningitis, from which he died 2 days later. In the remaining case only one dose of serum was given, 2 hours before death; no agglutinating power appeared in the blood. Text-figs. 7 and 8 show graphically the results of observations made in two of the fatal cases.

Case 1.—E. F., porter; age 46 years. This patient was admitted on the 5th day of the disease with an extensive lung involvement and very severe septicemia, the blood cultures showing 1,000 colonies per cc. His condition was very serious; temperature 104°F., pulse 104, respirations 64, and, in spite of four doses of serum within 24 hours, the patient died. It will be noted that immediately following the administration of 75 cc. of serum, the undiluted blood showed power



TEXT-FIG. 8. Chart showing the curve of the agglutinin titer and the temperature curve of S. F.

of agglutination, but this property had disappeared within 7 hours, and following the subsequent doses the blood showed no agglutinating power whatever. It is evident, therefore, that in this severely infected case the immune bodies in the serum disappeared from the blood as fast as they were administered.

Case 2.—S. F., clerk; age 31 years. This patient, though admitted early in the disease, was extremely ill; temperature 103.3°F., pulse 160, respirations 56. The blood culture showed 400 colonies per cc. and the following morning, before the

first dose of serum was given, the culture showed 1,600 colonies per cc. Following the first two doses of serum there occurred an immediate appearance of agglutinins in the blood in low dilutions, which, however, disappeared or became minimum in amount before the succeeding doses. In spite of the extreme grade of blood infection, the number of organisms present in the blood diminished following these two doses, the cultures on the morning of the 4th day showing only 20 colonies per cc. There was a satisfactory increase in agglutinins following the third dose, but it will be noted that 15 hours were allowed to elapse between this dose and the succeeding one, and during this time the agglutinins had entirely disappeared and the subsequent doses produced little or no effect on the agglutinin content (Text-fig. 8). The patient died on the 6th day.

Five patients suffering from Type II infection who received no serum were also studied to observe the appearance of agglutinins in the blood. All these recovered. In four of these instances at the end of the disease there developed well marked power of agglutination; in one of them agglutination in the serum obtained on the 10th day occurred in a dilution of 1: 100. In the fifth no agglutinins appeared in the blood, though this was not studied later than the 12th day. This patient received optochin, as did, however, several of the cases in which agglutinins developed.

DISCUSSION.

Neufeld and Haendel,[10] Dochez,[11] and others have shown that specific immune substances usually appear in the blood during recovery from lobar pneumonia. This is shown by an increase in protective power of the blood for mice against homologous infection. Clough[12] has made similar observations and he and others have also noted that in certain instances the protective power is accompanied by the power of inducing *in vitro* phagocytosis of virulent homologous pneumococci which are not phagocytable in normal serum. It would seem, therefore, that bacteriotropins represent one form of immune body playing a part in this protective phenomenon. Bull[13] has brought forward experimental evidence which indicates strongly that the phenomenon of agglutination is of great importance in the

[10] Neufeld, F., and Haendel, *Arb. k. Gsndhtsamte.*, 1910, xxxiv, 166.
[11] Dochez, A. R., *J. Exp. Med.*, 1912, xvi, 665.
[12] Clough, P. W., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 295.
[13] Bull, C. G., *J. Exp. Med.*, 1915, xxii, 457.

action of immune serum in pneumococcus infection. It is possible that several different antibodies or phenomena take part in the mechanism of pneumococcus humoral immunity. The observations I have mentioned, as well as unpublished observations made in this laboratory, indicate strongly that natural recovery in pneumonia is associated with the development of humoral immunity and probably occurs because of this development. In the individual case, however, the factors which determine recovery or death cannot be stated so simply. In mild cases probably a very slight grade of humoral immunity may be sufficient to prevent progress of the disease, a grade of immunity which can be detected with difficulty by our present means. In other instances the reaction required on the part of the body may be very great and the immunity phenomena exhibited by the serum when tested outside the body may be very vigorous and marked.

The phenomenon of agglutination offers one ready means for testing the degree of humoral immunity. It is, however, not the only one and it is unsafe to judge of the immunological effectiveness of a serum solely by its agglutinating strength. The protective power and agglutinating power of immune horse serum, however, tend to run parallel. Consequently, the study of agglutinating power of the blood of patients, such as has been made in the present instance, must be of considerable value in indicating the presence or absence of humoral immunity. If recovery in pneumonia is due to the development of humoral immunity, the study of its appearance during recovery and especially of its appearance following treatment with immune serum, should be of significance. In commencing the study it was thought that the method might be employed to graduate the dosage of immune serum in the treatment of the individual case. If recovery is due to the appearance of immune bodies in the blood, the ideal serum treatment would be such that sufficient serum be administered to produce the required concentration of immune bodies and no more. It soon became apparent, however, that such a method, testing the blood before and after the administration of each dose, involved so much time and labor that it would not be of practical value. It has seemed, however, that the repeated tests of the serum in a series of cases, as has been done here, give us considerable

knowledge of the mode of action of the serum and offer valuable suggestions for the routine dosage and mode of application of the serum. The studies have further indicated strongly that during infection not only must sufficient immune substances be added to bring about a concentration sufficient to sensitize all the bacteria, produce their agglutination, opsonification, etc., but in addition there must be a sufficient amount administered to neutralize any soluble substances present in the serum which have the property of neutralizing and fixing the immune substances. It is realized that the occurrence of these soluble, fixing substances in the blood of infected patients has not been directly demonstrated. The experimental observations in animals previously described, however, make it altogether probable that these substances are present in severe infections. It must be admitted that in most instances where there was failure of immune substances to appear in the blood, or where the immune bodies disappeared very rapidly following their administration, bacteriemia was shown to be present before the first dose of serum was administered. In several cases, however, the blood infection could not be demonstrated after the first dose, and nevertheless, rapid disappearance of the immune bodies occurred following the subsequent doses. In one instance in which the rapid disappearance of immune bodies occurred, the blood cultures taken both before and after the administration of serum were sterile. However, it seems likely that in all cases when fixation of immune bodies occurs, blood infection has at some time been present, though the possibility that the fixing substances may, in certain instances, arise entirely in local foci cannot be excluded.

The nature of the substances bringing about the fixation can at present only be conjectured. The demonstration, however, by Dochez and Avery[9] of substances giving rise to precipitates in the blood and urine of infected patients makes it probable that the same substances are responsible for the phenomenon of fixation that we have studied. They have apparently shown that these substances may be excreted or formed by the bacteria during their growth, and it is also probable that substances contained in the bacteria and set free during their dissolution may give rise to the same phenomenon.

The observations made in this study have a practical bearing on the

question of the therapeutic administration of immune serum. The amount of serum necessary to be given does not depend merely on the weight of the patient and therefore on the consequent dilution of the serum in the body. It is also not entirely dependent on the degree of infection present. If the patient is treated early before large amounts of the soluble substance are present, a moderate amount of serum may be sufficient, even though the grade of blood infection may be considerable. On the other hand, if the infection has continued for a considerable time, and large amounts of soluble, fixing substance are present in the blood, the amount of serum required may be very large. It is therefore evident that it is important that the patient be treated as early as possible and before large amounts of these fixing substances are formed. Moreover, the importance of treating very actively at the start in order that all these fixing substances may be at once neutralized and the progress of the infection immediately and entirely overcome is apparent. It is therefore our plan at present to treat all patients with Type I infection with large initial doses, and to repeat the treatment every 6 to 8 hours as long as may be necessary. It is possible that the Type II serum is less effective than Type I serum not only because its concentration of immune bodies is less than that of Type I serum, but also because the power of pneumococci of this type to produce fixing substances is more highly developed than is that of pneumococci of Type I.

CONCLUSIONS.

1. In empyema fluids resulting from infection with pneumococci there are present large amounts of soluble substances which have the property of neutralizing pneumococcus antibodies.

2. Similar substances are found in the blood of infected rabbits.

3. When immune serum is injected into infected rabbits the immune substances disappear very quickly, and therefore are prevented from activity in overcoming the infection.

4. When immune serum is administered to patients severely infected with pneumococci, the immune bodies may also disappear very rapidly, and this disappearance is probably associated with the presence of such soluble substances in the blood.

5. The serum only becomes effective when these substances are neutralized.

6. The study of agglutination curves is of value in showing why in certain instances favorable results have not followed the use of immune serum.

7. It is important that in severely infected patients the serum be administered early in the disease and that the initial dosage be large.