A STUDY OF THE MECHANISM OF RECOVERY FROM LOBAR PNEUMONIA.

By RICHARD H. P. SIA, M.D., OSWALD H. ROBERTSON, M.D., AND SHUTAI T. WOO, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research, New York, and the Department of Medicine, Peking Union Medical College, Peking, China.)

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Investigations into the mechanism of recovery from lobar pneumonia of recent years have brought forward an increasing amount of data to show that the principal evidence of acquired immunity consists in the development of humoral immune substances at or about the time of crisis. Yet the majority of authors writing on this subject express considerable caution in drawing conclusions as to the causal relation between the immune bodies and the recovery process even though certain of these substances or reactions have been shown to be directly concerned in the destruction of pneumococci and protection of the body from invasion. The reasons for this reservation of judgment are principally as follows: First, the occurrence of immune substances in the serum has not been observed as a constant phenomenon in all cases recovering from pneumonia; second, the appearance of these immune bodies, while generally coinciding with the onset of recovery, may occur 1 or 2 days before crisis or in some cases not until afterwards; third, serum immunity has been detected infrequently in cases terminating fatally; fourth, while the promotion of phagocytosis and intracellular destruction has been found to be one of the chief manifestations of acquired antipneumococcus serum action, the evidence for pneumococcidal power of the blood at the time of recovery is not generally considered as convincing. Furthermore, doubt has been cast on the importance of phagocytosis as a factor in the disposal of pneumococci in the body by the failure to demonstrate any degree of engulfment of these microorganisms by the cells of the lung exudate secured from patients during the period of recovery.

Finally, the fact that the introduction of large quantities of immune serum containing a high concentration of specific antibodies has been found by a number of investigators to be ineffective in cases of pneumonia after the 2nd or 3rd day of the disease, raises the question as to the ultimate rôle played by these serum substances.

The above considerations have led certain workers to seek other explanations for the sudden termination of infection in lobar pneumonia. Changes in the H ion concentration of pneumonic exudate in relation to the acid death-point of pneumococci have been studied (1). Investigation of the mobilization of enzymes in the blood at the time of crisis (2) have also brought to light interesting facts but they do not afford a satisfactory interpretation of the recovery process.

Our findings in a study of experimental pneumococcus infection in cats and rabbits (3) suggested that it would be profitable to pursue further by the same methods the inquiry as to the relation of acquired humoral immunity to the mechanism of recovery from lobar pneumonia in man. It was found that at the time of recovery from experimental disease there appeared constantly in the serum, immune properties characterized not only by opsonic, agglutinative, and mouse-protective activity, but also by the power to promote to a marked degree the killing of virulent pneumococci in rabbit serum-leucocyte mixtures. None of these changes occurred in animals with fatal infection. The degree of pneumococcus-killing power potential in the recovered animal's blood made it appear that this action played an important part in the disposal of pneumococci. While the development of humoral immune bodies was usually more marked and could be more closely related to the onset of recovery in the pneumococcus-resistant animal (the cat) than in the susceptible animal (the rabbit), yet the latter showed the same type of serum immune change and hence it seemed probable that in the human, whose resistance lies somewhere between that of these two animals, a similar immune reaction occurs.

Methods.

The methods employed in carrying out the pneumococcidal, opsonic, and agglutinative tests have been described in previous publications (4–6). The pneumococcidal-promoting power of the serum was tested by adding it in progressive dilution to rabbit serum-leucocyte mixtures containing small quantities of

TABLE I.

Determination of Pneumococcidal-Promoting Power of Serum during Course of Lobar Pneumonia, Pneumococcus Type II.

Human serum dilution 0.1 cc. + normal rabbit serum 0.2 cc. + rabbit leucocyte suspension 0.1 cc. + pneumococcus suspension 0.1 cc.

Serum sample in relation to lysis	Amount of standard pneumococcus suspension	Dilutions human serum	Growth as shown by color changes at hrs.*			Pneumococci in stained film at 72 hrs.
			16	48	72	
2 days before	10^{-6}**	1-10	++++			+
	"	1-20	++++			+
1 day before	"	1-10	++++			+
	"	1-20	++++			+
Day beginning	"	1-10	0	0	0	0
	"	1-20	0	++++		+
	"	1-40	++++			+
	"	1-80	++++			+
	"	1-160	++++			+
Day completed	"	1-40	0	0	0	0
	"	1-80	0	0	0	0
	"	1-160	0	0	0	0
	"	1-320	0	0	0	0
	"	1-640	++++			+
	"	1-1280	++++			+
3 days after	"	1-40	0	0	0	0
	"	1-80	0	0	0	0
	"	1-160	0	0	0	0
	"	1-320	0	0	0	0
	"	1-640	++++			+
	"	1-1280	++++			+
12 days after	"	1-20	0	0	0	0
	"	1-40	++++			+
	"	1-80	++++			+
	"	1-160	++++			+
	"	1-320	++++			+
	"	1-640	++++			+
Controls	10^{-7}	0	++++			+
	"	0	++++			+

* Degrees of methemoglobin formation.

** This amount represents 500 to 1000 pairs of pneumococci.

highly virulent pneumococci. Since these mixtures have no growth-inhibitory action on such organisms, any pneumococcidal effect which occurs may be attributed directly to the added test serum. The amounts of the various constituents used in the reaction are given in Table I. Opsonic and agglutinative tests were performed by mixing pneumococci which had completed their active growth phase[1] with serum, in a ratio of 1 part of pneumococcus suspension to 20 parts of serum. After sensitization for 1 hour the pneumococci were then separated from the serum by centrifugation, the sedimented mass broken up with a capillary pipette, macroscopic agglutination noted, and phagocytic tests performed in the usual way with especially prepared rabbit leucocytes. The degrees of opsonic action and agglutination were noted in terms of plus signs ranging from + slight but definite, to ++++ which was very marked. Since all the specimens of serum from one patient, inactivated after separation and kept on ice, were tested at one time it was possible to make comparative readings on them. The different tests, pneumococcidal, opsonic, agglutination, and mouse protection, were usually made on the same day. Occasionally this was not possible.

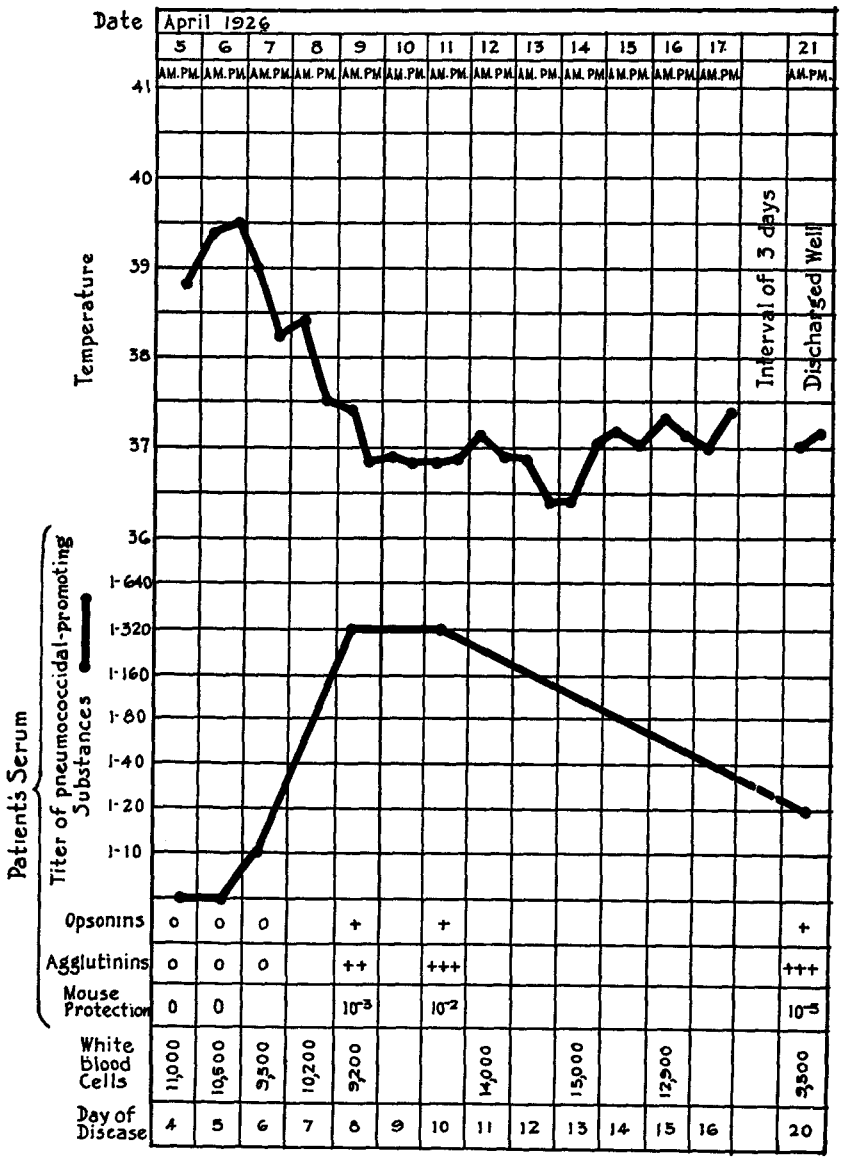
The pneumococci used in the tests with Type I sera were highly virulent strains. The homologous organisms isolated from both blood and sputum as well as a stock culture were used. In the tests with serum from pneumonia Type II and Group IV it was necessary to use the serum and leucocytes of young rabbits as none of the strains from these patients were virulent, nor could they be made virulent for full grown rabbits (7). Controls with young rabbit serum and leucocytes showed no opsonic or agglutinative action for these Type II and Group IV strains of pneumococci which were highly virulent for the young animal.

Clinical Cases.

The following study represents observations made on seventeen cases of lobar pneumonia comprising five cases of Pneumococcus Type I, six of Type II, two of Type II atypical, and four Group IV. Sixteen of the patients recovered. One died.

It may be stated briefly at the outset that the results of the study in human cases were essentially the same as those obtained in cats with experimental pneumococcus infection. Furthermore no constant differences in reaction were observed between the three types of pneumonia studied. The blood serum in the early stages of the disease was found to be without pneumococcus immune principles. Then, about the time of crisis, the serum acquired demonstrable

[1] Type II pneumococci, however, were used in the active growth phase. The significance of the growth phase in relation to opsonic and agglutination tests is discussed in an earlier publication (*J. Exp. Med.*, 1927, xlvi, 239).



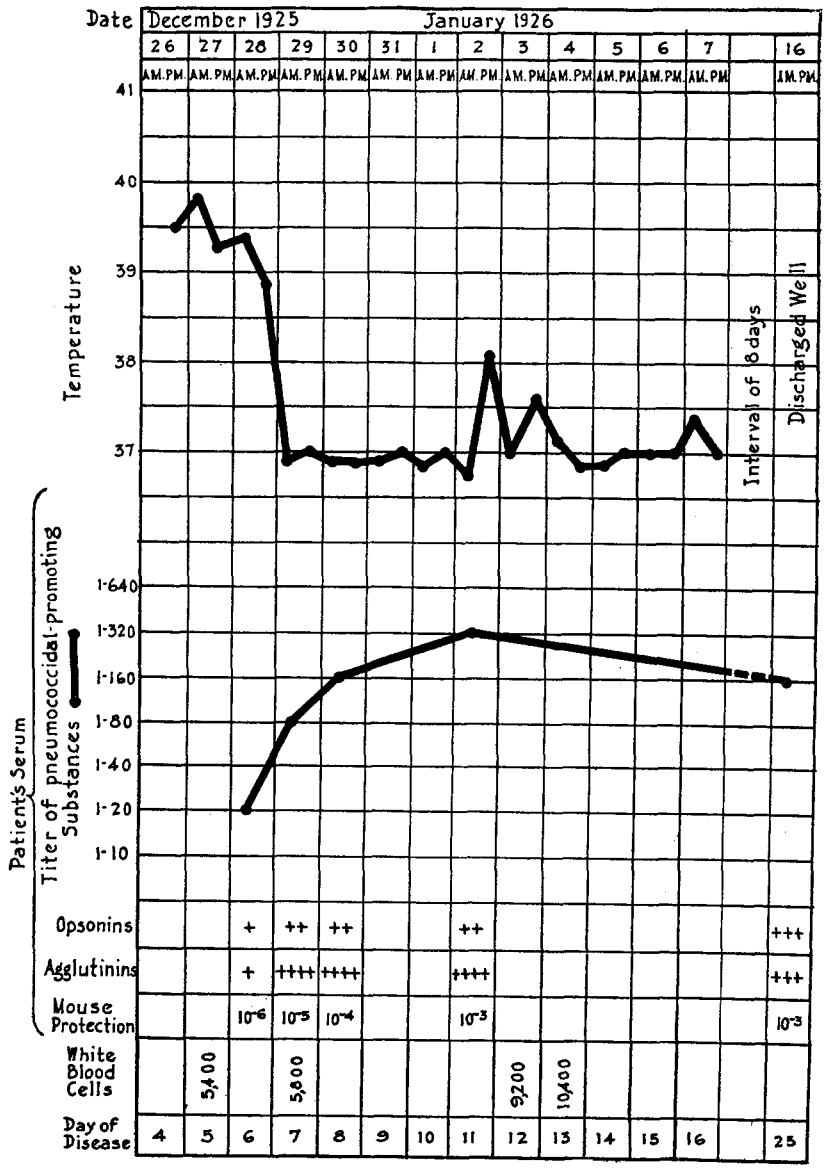
TEXT-FIG. 1. Case 13455. Lobar pneumonia, Pneumococcus Type II.

pneumococcidal-promoting power as well as opsonic, agglutinative, and mouse-protective properties. Text-fig. 1 illustrates well the sequence of changes found to occur in cases where the observations were begun early in the disease course. Tests on the blood specimens taken from this patient on the 4th and 5th days of disease were negative (Table I). On the 6th day, which marked the beginning of the fall in temperature, the serum showed the titer of pneumococcidal-promoting substances of 1:10. Opsonins and agglutinins were not demonstrable at this time. On the 8th day when the temperature had reached normal the titer of pneumococcidal-promoting substances had risen to 1:320 and opsonins, agglutinins, and mouse-protective bodies were evident.

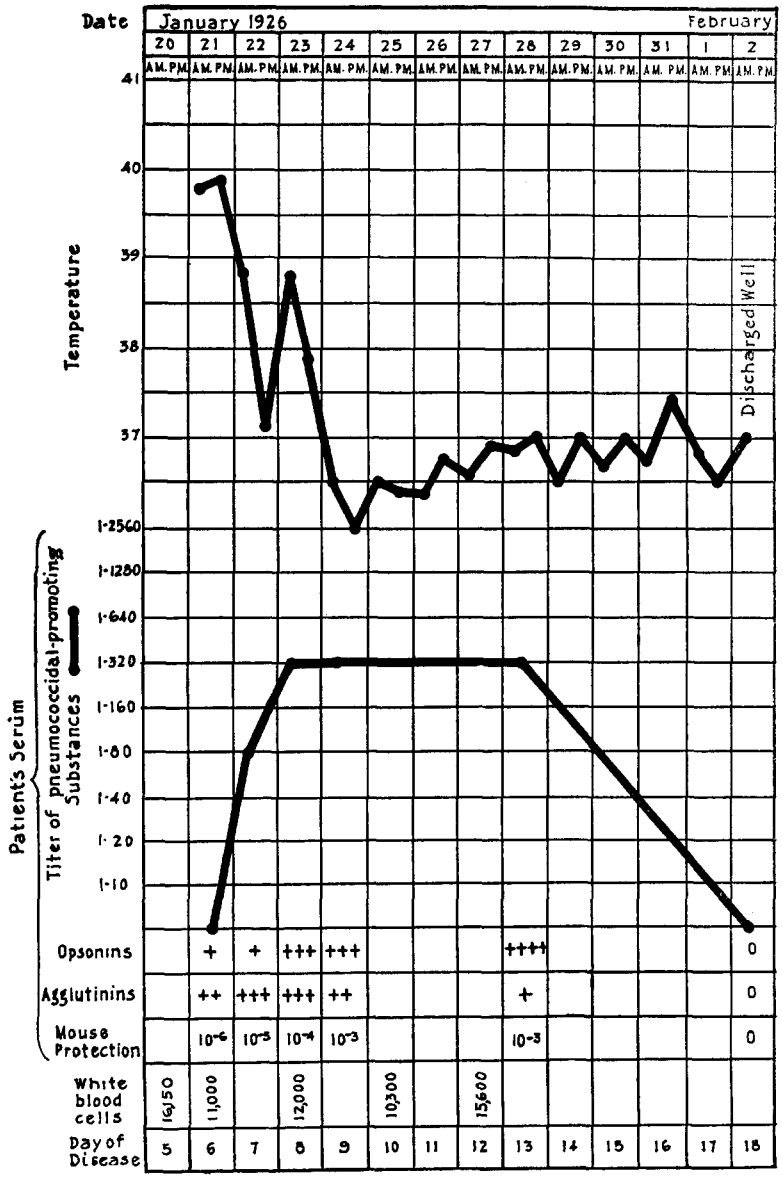
The occurrence of demonstrable immune substances in the blood as shown especially by the pneumococcidal-promoting power, appeared to coincide generally with the beginning of defervescence. In only one instance (Text-fig. 4) were immune properties found in the serum more than a very short time (hours) before the temperature began to fall. In one other case pneumococcidal-promoting substances failed to appear until after the temperature had reached normal. It is quite possible that a more complete series of observations made immediately preceding the initiation of recovery might show a constantly earlier appearance of serum immune properties, although the results of tests such as are exhibited in Text-figs. 1 and 3, in which the immune reactions were found to be present initially to only a slight degree, suggest that they had not been detectable much earlier. In support of this supposition is the fact that once this immune property appeared in the blood it showed in most instances a rapid quantitative increase.[2]

The titer of pneumococcidal-promoting substances usually reached its height within 48 to 72 hours. Then after some days at this high level it began to fall. Just how long the serum continued to show immune properties was not determined but that the persistence of passive immunity varies greatly from patient to patient was shown by the finding in some cases that all traces of humoral immunity had

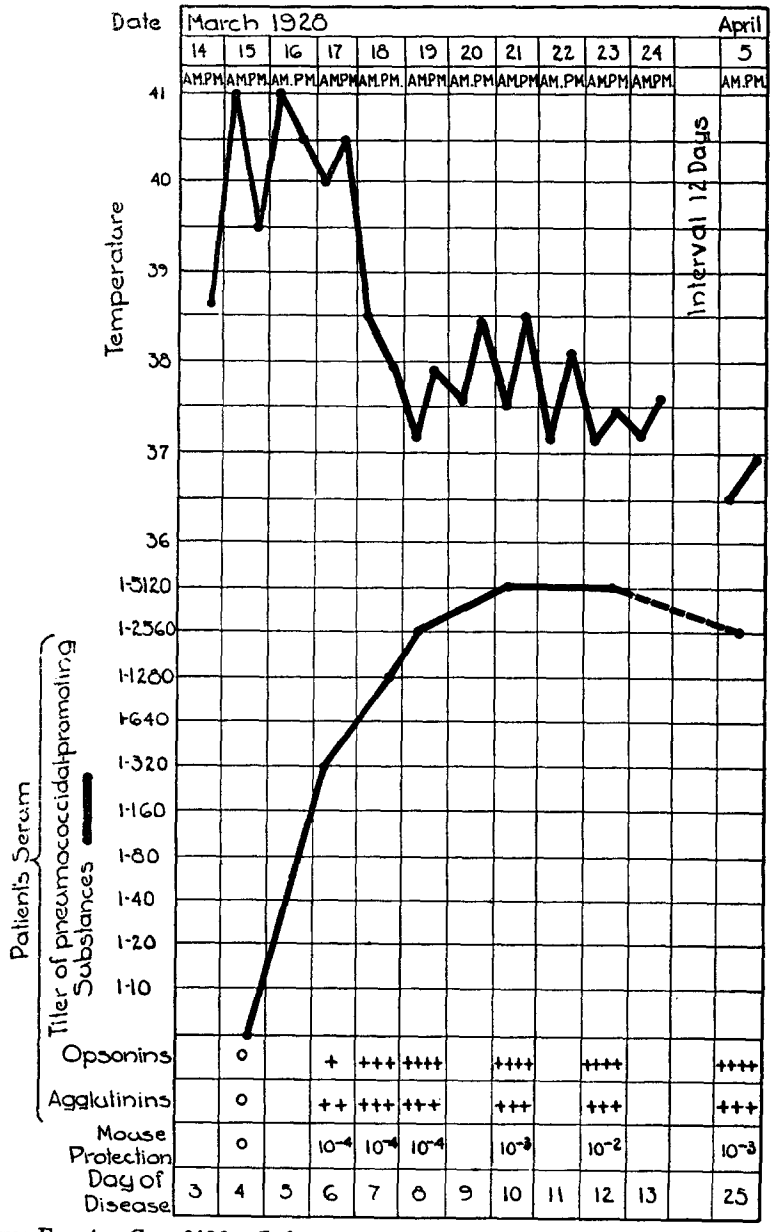
[2] Our observations in experimental animals (3) in which a rapidly diminishing blood invasion occurred 24 to 48 hours before the termination of the disease make it seem highly probable that immune bodies are elaborated and perhaps present in low concentration for some time before crisis.



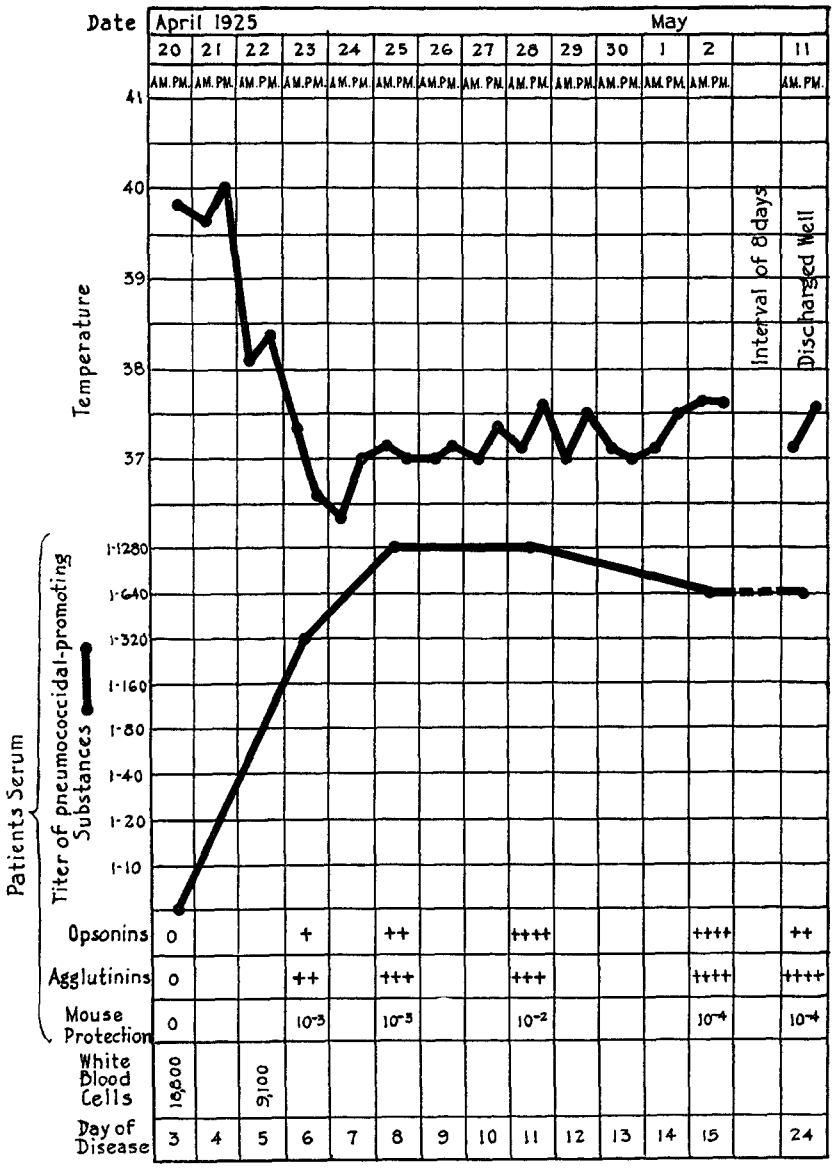
TEXT-FIG. 2. Case 12656. Lobar pneumonia, Pneumococcus Type II.



TEXT-FIG. 3. Case 12827. Lobar pneumonia, Pneumococcus Type I.



TEXT-FIG. 4. Case 3193. Lobar pneumonia, Pneumococcus Type II atypical.

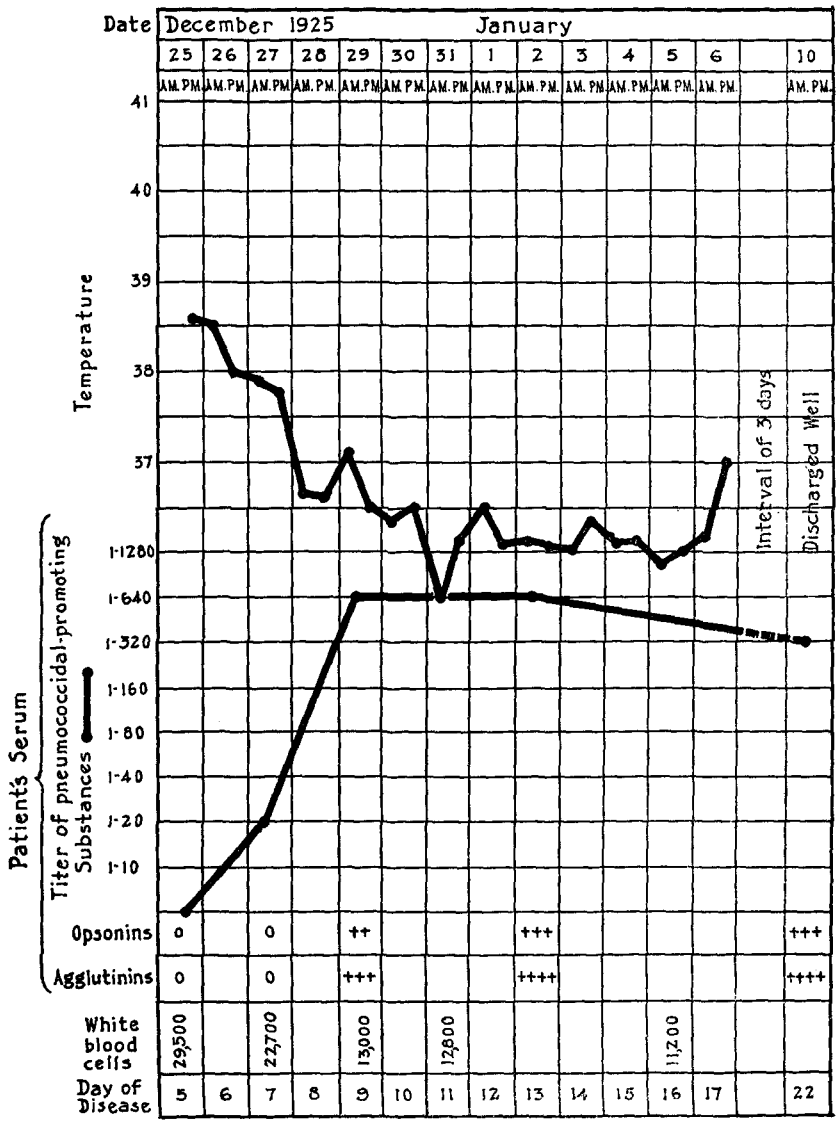


TEXT-FIG. 5. Case 10608. Lobar pneumonia, Pneumococcus Type II.

disappeared within 10 days after crisis (Case 12827, Text-fig. 3), while in others, and these constituted the majority, at the end of weeks or months serum immune properties were still evident though much diminished. Note Case 4960, Text-fig. 7, tested after 74 days. The persistence of immune substances in the serum seemed to be associated to a certain degree with the initial intensity of immune body production. Thus, those patients in whom serum immunity was demonstrable for the longest periods showed a relatively high pneumococcidal-promoting serum titer at outset, and those in whom the serum immunity disappeared earlier showed an initial low or at most moderately elevated titer. However, these observations are too few to permit any conclusions concerning this point.

Only one patient of the sixteen recovering showed a positive blood culture. This was taken on the day before the appearance of serum immune substances which occurred at the time of crisis, hence it was not possible in this series of cases to determine whether the occurrence of detectable pneumococcidal-promoting substances in the blood marked the termination of blood invasion as was always found to be the case in cats recovering from experimental pneumococcus infection.

In the majority of the cases all the different manifestations of serum immunity became demonstrable at the same time as in Cases 12656 and 3193, Text-figs. 2 and 4. In others, the appearance of pneumococcidal-promoting power in the serum seemed to precede or occasionally lag behind the occurrence of opsonic, agglutinative, and mouse-protective action (Text-figs. 1, 3, and 6). These variations may, however, be more apparent than real since the different means employed to bring out the several reactions may not be strictly comparable in their power to detect minute traces of the immune substance or substances. Furthermore, a comparison of the changing intensity of the four reactions through the period of observation revealed a general parallelism. This was closest between the pneumococcidal-promoting power and the mouse-protective action which are more susceptible to accurate quantitative estimation than are the opsonic and agglutinative reactions as carried out in this work. It was found frequently, especially at the beginning of recovery, that equal parts of serum and pneumococcus suspension failed to bring out opsonic activity while employing 20 parts of serum to 1 of suspension produced a well defined reaction.



TEXT-FIG. 6. Case 7797. Lobar pneumonia, Pneumococcus Type II.

In striking contrast to the findings in patients recovering, are those made on the case progressing to a fatal termination (Text-fig. 8). Repeated tests of the blood serum made during the course of the disease in this patient failed to reveal any evidence of the development of immune properties. Tests for opsonic, agglutinative, and mouse-protective action were not made in this case but judging from the findings in experimental pneumococcus infection (3), it is probable that these reactions were also lacking here. There was a persistent slight blood invasion but it did not increase during the course of the disease.

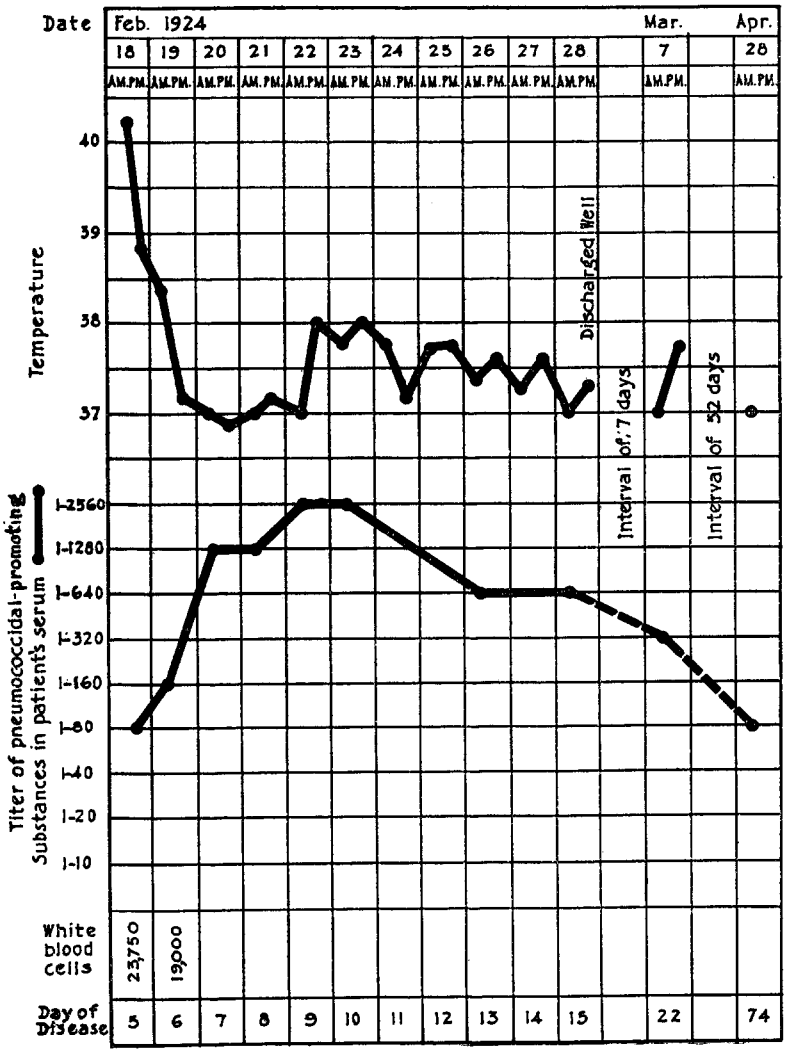
Specificity of Immune Substances.

The pneumococcidal-promoting substances occurring in the serum at recovery were found to be strictly type-specific. Pneumococcidal tests in which one serum was used with several types of pneumococci and also several types of serum with one type of pneumococcus, showed killing of the pneumococci only in those tubes containing the homologous serum and organisms. The opsonic and agglutinative reactions were also found to be specific to type.

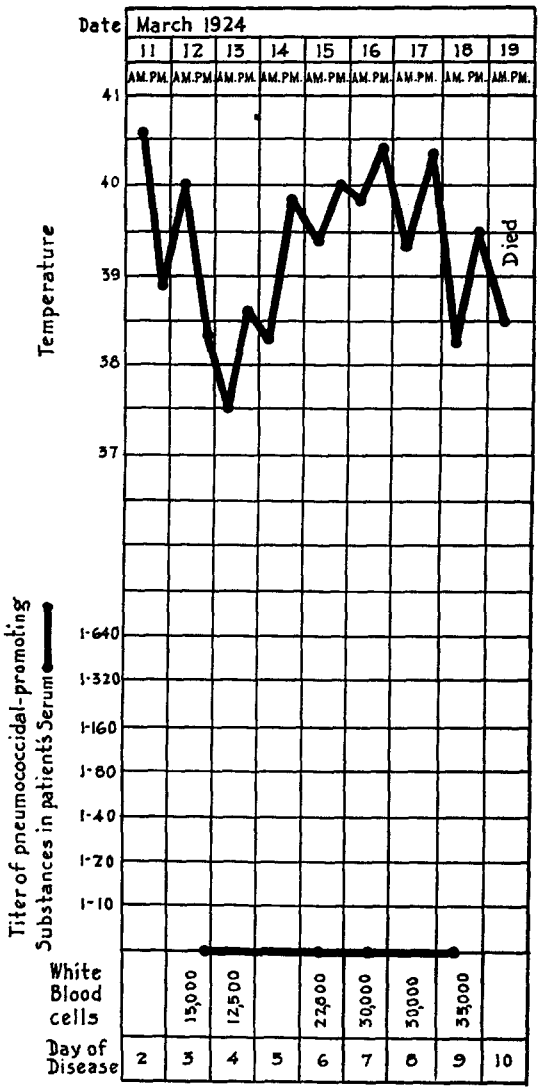
White Blood Cells.

The observations on the leucocytic response in this series of pneumonia patients are much less complete than those made in the experimental infected animals described in the preceding paper to which reference has been made (3). However, certain points are apparent and these are in general similar to the findings in the experimental disease. In the majority of the human cases there was a moderately increased or high white count just before the onset of recovery. In others, however, the number of leucocytes remained about normal and in one instance slightly below normal during the critical period (see Text-figs. 1 and 2). The fatal case showed an increasing white count towards the end of the disease reaching 35,000 the day before death.[3] These findings are in agreement with the inferences drawn from the animal studies, namely, that the leucocytes probably play a secondary rôle in the mechanism of recovery from pneumococcus infection, but

[3] There was no evidence of complications in this patient although this could not be definitely determined since an autopsy was not obtained.



TEXT-FIG. 7. Case 4960. Lobar pneumonia, Pneumococcus Type I.



TEXT-FIG. 8. Case 4983. Lobar pneumonia, Pneumococcus Type II.

the well substantiated clinical observation that a low white count is generally to be considered as of unfavorable prognostic import, indicates that a certain minimum number of white blood cells are necessary to provide suitable conditions for recovery.

Complications and the Presence of Immune Substances.

Two of the patients in this series, a Group IV and a Type II pneumococcus pneumonia, developed empyema at a time when there were demonstrable pneumococcidal-promoting properties in the serum. In one instance the titer was low, 1:40, in the other it was 1:640. Both patients recovered.

DISCUSSION.

While the above findings do not warrant the conclusion that the development of specific antipneumococcus properties occurs constantly in the serum of all patients recovering from lobar pneumonia, they do indicate that these serum changes are of very common occurrence and perhaps are present to varying degrees in every case. The fact that certain workers, Chickering (8), Clough (9), Lister (10), and others, have failed to demonstrate the presence of opsonins and agglutinins in a small per cent of pneumonia cases at the time of crisis may simply indicate the occurrence of considerable variation in the degree of antibody response. We have shown that the use of equal parts of serum and pneumococcus suspension employed in the earlier studies may fail to bring out a low concentration of opsonins and agglutinins. Chickering mentions that in some of his cases agglutinins were detected on 1 day only. However, the absence of mouse-protective action in the critical and postcritical serum observed by certain investigators in occasional cases recovering from lobar pneumonia is of greater significance on account of the marked sensitiveness of this test.[4] Dochez (11) found in a series of ten recovering cases that the serum of one failed to show protective power against the homologous organism. Clough (12) studying twelve cases found protective action in the serum of only nine. In the three patients in whom the serum showed no protection, only one serum specimen was obtained in each case and this, 1 to 3 days following recovery. Other authors, and most recently

[4] Mouse protection seems to be fully as delicate an indication of the presence of antipneumococcus immune substances as is the test for pneumococcidal action.

Baldwin and Rhoades (13), have found protective substances constantly in the serum of recovering patients.

Previous studies by other investigators have shown that in the majority of cases the appearance of circulating immune bodies coincides closely with the time of crisis or lysis. But in a small percentage of pneumonia patients immune substances have been detected 1 to 2 days beforehand. Baldwin and Rhoades (13) found protective bodies in twelve out of twenty cases at least 2 days prior to the day of crisis. However, a number of their reported cases had received specific therapy of one kind or another so that it is not clear how many of them developed the passive immune properties spontaneously. Does this occasional finding of immune properties in the serum sometime before crisis constitute a serious objection to the assumption that immune body development is related intimately to the mechanism of recovery? While the relationship between demonstrable humoral immunity and recovery would be much clearer if serum immune substances always appeared at or immediately before crisis, the fact that they may be present in the blood a day or two before the apparent beginning termination of the disease process does not seem to provide a sufficient reason for denying them an important rôle in the recovery mechanism. We know relatively little about conditions in the body which influence the functioning of opsonins, protective bodies, etc. Furthermore, structural peculiarities in the local lesion may well produce variation in the rate at which the immune substances penetrate or there may be other factors of a more general nature upon which the effective action of these antipneumococcus bodies largely depends. May not the early appearance of acquired immune bodies in a given case of lobar pneumonia represent a condition somewhat analogous to that which is frequently observed after the introduction of antipneumococcus serum Type I? Following the injection of an adequate amount of immune serum the blood acquires immune properties but the crisis may not occur for several days. However, the blood invasion ceases and the pneumonic process stops spreading. It has been shown by Baldwin and Cecil (14) that the appearance of protective bodies in the blood of spontaneously recovering cases marks the termination of blood invasion, a finding with which our observations in experimental pneumococcus infection agree. It is not possible that the spread of the pneumonic process

also ceases at this time? Our one patient in whom humoral immunity was detected 12 to 18 hours before crisis (Text-fig. 4) did show physical signs of beginning resolution 24 hours before the critical fall in temperature. We have found no observations on this point in the literature.

There exists also the possibility that the termination of the disease depends on the development of antitoxic as well as antibacterial immunity, as was suggested years ago by Cole (15), Wadsworth (16), and others.

Failure to detect immune substances until 24 hours or more after crisis has been reported less frequently than their early appearance. This condition might be due to an unusually marked neutralization of perhaps a rather low concentration of these bodies at the time of recovery.

The finding of antipneumococcus immune properties in the serum of patients going on to a fatal termination has been confined as far as we can determine to cases developing complications. In Baldwin and Cecil's (14) three fatal cases showing circulating humoral immune substances there was a complicating empyema. In Clough's (9) one reported case, an active endocarditis was probably present. The development of such secondary foci of infection in patients in whom the pneumonic process has been checked and who show evidence of considerable serum immunity is a further indication of the complexity of the recovery mechanism.

Of even more importance than the finding of antipneumococcus substances in the serum during recovery is information as to their mode of action. The demonstration in this study that a serum showing such properties possesses the power to promote the destruction of highly virulent pneumococci provides more direct evidence of the function of these immune bodies than has heretofore been obtained. That immune serum promotes phagocytosis and intracellular digestion in the body as it does in the test-tube seems highly probable although it should be pointed out that we have made no determinations of the activity of the pneumonic leucocytes. Investigations in this field by other workers (17–19) have shown that the leucocytes apparently function actively during the height of the disease.[5]

[5] A discussion of this phase of the subject is taken up in an earlier publication (3).

The fact that the above findings in human cases of lobar pneumonia are in practically entire accord with the observations made in cats undergoing experimental pneumococcus infection enhances considerably the significance of the development of serum immune bodies at the time of crisis. In the cat it was possible to show that the acquisition of humoral immunity was associated with greatly increased antipneumococcus resistance. Furthermore variations in the degree of acquired immunity were found to be associated in a general way with the corresponding fluctuations in concentration of demonstrable serum immune bodies. But while these observations suggest that the reaction of the body to pneumococcus invasion is the same in animals and in man despite the great dissimilarity in the character of the lesions produced, they do not indicate certainly that the mechanism of recovery is identical in both instances. The peculiar character of the local lesion in lobar pneumonia would seem to introduce certain special conditions to be reckoned with in the human body's defense against this organism and which may involve the operation of processes other than those essential for recovery in less intensely localized pneumococcus infection. In a discussion of this question, Cole (20) suggests that the destruction of pneumococci in the lung lesion may depend on local factors quite different from those responsible for the destruction of the bacteria in the circulating blood. He mentions the marked changes which occur in the resolving lung exudate, the solution of fibrin acting as a possible relief from tension and affording an outlet for the exudate and the production of chemical compounds, soaps, fatty acids, etc., shown to have a destructive action on pneumococci. However, he points out the difficulty in inferring that the crisis depends mainly on the resolution of the exudate, in that these two processes do not necessarily occur synchronously. Cole's conclusion (21) that recovery depends largely on an adequate concentration of immune bodies in the blood is supported by the observations presented in this paper.

SUMMARY.

Employing a method devised for the investigation of natural immunity and experimental pneumococcus infection, a study has been made of the serum immune changes occurring during the course of

lobar pneumonia due to Pneumococcus Types I and II and Group IV, in man. It was found that at the time of crisis or lysis the blood serum acquired constantly the property of promoting pneumococcus killing to a relatively marked degree. Other evidences of antipneumococcus reaction—mouse protection, opsonins, and agglutinins—were also demonstrable in the blood at this time. These immune changes appeared in the majority of cases at the beginning of recovery and failed to occur when the disease terminated fatally. The fact that these observations in human cases are practically identical with previous findings in the experimental disease in cats, enhances considerably the significance of the development of serum immune bodies at the time of crisis since in the experimental animal it was possible to show that the acquisition of passive immunity was associated with greatly increased antipneumococcus resistance.

BIBLIOGRAPHY.

1. Lord, F. T., and Nye, R. N., *J. Exp. Med.*, 1922, xxxv, 689, 699.
2. Jobling, J. W., Peterson, W., and Eggstein, A. A., *J. Exp. Med.*, 1915, xxii, 568.
3. Robertson, O. H., Woo, S. T., Cheer, S. N., and King, L. P., *J. Exp. Med.*, 1928, xlvii, 317.
4. Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, xxxix, 219.
5. Robertson, O. H., Woo, S. T., and Cheer, S. N., *J. Exp. Med.*, 1924, xl, 487.
6. Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1927, xlvi, 239.
7. Woo, S. T., *J. Exp. Med.*, 1926, xliii, 623.
8. Chickering, H. T., *J. Exp. Med.*, 1914, xx, 599.
9. Clough, P. W., *Bull. Johns Hopkins Hosp.*, 1919, xxx, 167.
10. Lister, F. S., *Pub. South African Inst. Med. Research, No. 3*, December 22, 1913.
11. Dochez, A. R., *J. Exp. Med.*, 1912, vi, 665.
12. Clough, P. W., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 295.
13. Baldwin, H. S., and Rhoades, D. R., *Bull. Hyg. Lab., U. S. P. H., No. 141*, 1925, vii.
14. Baldwin, H. S., and Cecil, R. L., *J. Am. Med. Assn.*, 1926, lxxxvii, 1709.
15. Cole, R., *J. Am. Med. Assn.*, 1912, lix, 693.
16. Wadsworth, A. B., *J. Exp. Med.*, 1912, xvi, 78.
17. Tunnicliff, R., *J. Infect. Dis.*, 1911, viii, 302.
18. Rosenau, E. C., *J. Infect. Dis.*, 1906, iii, 683.
19. Eggers, H. E., *J. Infect. Dis.*, 1912, x, 48.
20. Cole, R., *Arch. Int. Med.*, 1914, xiv, 56.
21. Cole, R., *J. Am. Med. Assn.*, 1917, lxix, 505.