THE PNEUMOCOCCIDAL POWER OF WHOLE BLOOD

II. Estimations in Lobar Pneumonia[1]

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Our primary purpose in using the whole blood technique described in a previous communication (1) for measuring pneumococcidal power was its application to the study of lobar pneumonia in patients with the disease. Seventy-six separate observations have been made on 35 different patients. These observations have been grouped according to their relation to the stage of the disease, as follows: (*a*) before the crisis, (*b*) at the crisis or later, (*c*) with complications and (*d*) about the time of death. In two cases the changes were compared with the findings of other serological reactions at various stages of the disease. The whole blood immunity, as affected by the administration of serum, was also studied in a few patients. All patients observed were suffering from lobar pneumonia caused by the Type I pneumococcus, unless otherwise stated.

The results of our tests with blood, the serum of which was highly mouse protective, differ considerably from the results obtained when blood from individuals with no history of pneumonia and with no serum protection for mice was tested.

The protocols of two typical tests are given in table 1. In the first protocol is shown the pneumococcidal power of the blood of a person who had no history of pneumonia and who had no serum immunity detectable by the usual mouse protection test. The blood of this subject killed 1000 pneumococci. The blood agar plate planted with

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0.1 cc. of the contents of the first tube in which growth occurred, (number 5), became brown, that is, it contained an uncountable number of pneumococcus colonies. In the second protocol the pneumococcidal power of an immune blood is shown. The immune blood killed 100,000 organisms. In two tubes in which actual sterilization did not take place there was a reduction in the number of organism originally added to the blood. This was shown by the subsequent growth in the blood-agar plates from tubes 2 and 3 of less than 5000

TABLE 1

Specimen protocols

1. Blood from patient with no history of pneumococcus infection. 2. Blood from patient convalescing from lobar pneumonia due to Type I pneumococcus.

Tube number	Number of organisms	Protocol 1. Non-immune blood			Protocol 2. Immune blood		
		Color change	Culture		Color change	Culture	
			Amount	Growth		Amount	Growth
			cc.			*cc.*	
1	10^8				+++	0.1	∞
2	10^7	+++	0.1	∞	0	0.6	∞ −
3	10^6	++	0.1	∞	0	0.6	1,250 colonies
4	10^5	0	0.6	∞	0	0.6	0
5	10^4	0	0.6	∞	0	0.6	0
6	1,000	0	0.6	0	0	0.6	0
7	100	0	0.6	0	0	0.6	0
8	100 (plasma)	Diffuse growth	0.1	∞	Diffuse growth	0.1	∞
9	0	0	0.6	0	0	0.6	0

pneumococcus colonies when 100 million and 10 million organisms had originally been introduced into the tubes. Such an inhibition of the growth of pneumococci was produced only by the blood of individuals who had a degree of serum immunity easily demonstrable by the mouse protection test.

WHOLE BLOOD IMMUNITY DURING THE ACUTE PHASE OF LOBAR PNEUMONIA

During the febrile stages, before the crisis of lobar pneumonia in patients who are destined to recover, it is likely that the immune

mechanism is actively at work. Ten blood samples were examined from 7 different subjects in this active stage of Type I lobar pneumonia. None of these patients received any antipneumococcus serum or any other specific treatment. Although the first patient to be considered below finally died with erysipelas, he is included in this series because he passed through a distinct crisis and appeared to recover from the acute phase of lobar pneumonia. The 6 other patients made complete and permanent recoveries.

The blood specimens examined both for pneumococcidal power and serum protection will be taken up in the order of their relationship to the day of crisis. The description of "the day of crisis" that has been adhered to is as follows: a day on which the rectal temperature reached 101°F., and following which a marked improvement in the condition of the patient was manifest.

Our earliest observation during the course of lobar pneumonia was made on the second day of the disease, five days before the crisis. The findings throughout this case are illustrated in figure 2. The blood cultures taken at the bedside yielded 12 Type I pneumococcus colonies per cubic centimeter. Growth occurred in the heparinized blood, even when no pneumococci were added.

The next observations in order of their approach to the crisis are those on three cases made three days before the drop of temperature to 101°F. In these cases the pneumococcidal power showed wide variations. In one case, in which the blood culture was weakly positive, no pneumococcidal power was present, though no spontaneous growth occurred in 0.5 cc. of blood to which no pneumococci were added. Of the two other patients, both of whom had negative blood cultures, blood from one killed 10,000 pneumococci, and blood from the other killed 1,000,000. Neither of these patients showed any serum immunity when tested by the routine protection test in mice.

Two observations were made on the second day before the crisis. Both of these patients had positive blood cultures, and both showed whole blood immunity for the Type I pneumococcus. In the first case, examined two days before the termination of the acute phase of the disease, the blood culture was weakly positive, 5 cc. of blood being required to produce growth. In this case the blood sample sterilized itself and killed 10,000 pneumococci, while the serum sample showed a

small amount of protection. This protection test was done after the serum had been kept in the ice box for a period of 20 days. In the second of these observations which was made on the 5th day of the disease (fig. 2) the blood culture showed 4 pneumococci per cubic centimeter of blood. The blood sample with heparin added sterilized itself, and in addition killed all pneumococci in the tube seeded with 1,000,000. No serum protection was found in this specimen when the test was done one month later. This test, as well as others, appears to indicate some independence of the whole blood immunity against the pneumococcus from serum protection for the mouse.

In the three cases examined for pneumococcidal power on the day before crisis, the blood itself was sterile, and the pneumococcidal power was of a high degree. Blood from one case killed 10,000 pneumococci, from another 1,000,000 pneumococci, and from the third 10,000,000 pneumococci. Tests with serum in the mouse likewise showed a considerable amount of protective power in each case.

These ten tests, made during the acute stage of Type I pneumonia in patients that ultimately recovered, may not include all possible combinations of immunological factors. They bring out, however, several points that may be summarized as follows: 1. The multiplication of organisms, already present in the patient's blood stream, after the blood has been withdrawn and subjected to the conditions of the test, is compatible with the patient's recovery from the acute stage of the disease. 2. The power of the blood to kill pneumococci introduced into it may develop (*a*) in the presence of bacteremia, and (*b*) independently of the serum antibodies as revealed by the routine mouse protection test.

WHOLE BLOOD IMMUNITY AT THE CRISIS AND AFTER THE CRISIS

The highest level in the development of whole blood immunity was reached the day before the actual fall in temperature to 101° and was maintained at that level for one week after the crisis. A total of 26 observations was made on 13 patients both on the day of the crisis and at subsequent intervals up to the 28th day after crisis. The thirteen tests made in the first week after crisis all showed a high degree of pneumococcidal power. In eight of these tests 100,000 organisms were killed by 0.5 cc. of blood and in five 1,000,000 organisms were

killed. All these bloods showed serum protection for mice and marked inhibitory power in the tubes in which complete sterilization did not take place.

WHOLE BLOOD IMMUNITY IN THE PRESENCE OF PNEUMOCOCCUS COMPLICATIONS

Blood samples from three patients suffering from complications due to the pneumococcus were measured for their pneumococcidal power. In each case the whole blood immunity was as high as that found after the crisis in uncomplicated cases of lobar pneumonia. In the first case, illustrated in figure 1, the development of whole blood immunity and serum protection for mice had been observed previous to the detection of empyema on the 12th day after the crisis. The blood culture was negative when empyema was recognized, and the change in whole blood immunity from the level previously established was slight. Ten thousand organisms were killed and considerable inhibition of growth occurred in tubes planted with as many as 100,000,000 organisms. Serum protection for mice was lower the day following the detection of empyema than it had been 4 days previously. The fall in protective power was from 100,000 M.L.D. to 1000 M.L.D. This is not an unusual change in uncomplicated convalescence from lobar pneumonia and may have little significance. In the second case the complication was arthritis, due to a Type II pneumococcus. Eight days after the detection of the purulent accumulation in the right shoulder joint and 29 days after the onset of pneumonia the pneumococcidal power was unusually high, 1,000,000 Type II pneumococci being killed. Inhibition occurred in the tube inoculated with 10,000,000 organisms. The blood culture was positive only in the flasks seeded with 5 cc. of blood. The blood serum contained no Type II protection for mice. The third case was a pneumococcus Type I endocarditis. This patient had received, during the 7th, 8th, 9th and 10th day of his disease, 195 cc. of concentrated antipneumococcus serum containing 390,000 units of protection. Pneumococcus endocarditis was diagnosed by means of blood cultures, the development of an aortic diastolic murmur, and petechiae which appeared on the 30th day of his illness. The degree of whole blood immunity was high. One hundred thousand Type I pneumococci were killed, and in the

tube inoculated with 10,000,000 organisms less than 5000 survived. One cubic centimeter of the patient's blood cultured in agar yielded 110 Type I pneumococcus colonies. The serum of a sample of blood obtained simultaneously with the samples for blood culture and whole blood immunity test protected a mouse against 100,000 M.L.D. of Type I pneumococci. This protection test was done 41 days after the blood was drawn.

WHOLE BLOOD IMMUNITY ABOUT THE TIME OF DEATH

A certain relation has been observed between the presence of pneumococci in the blood stream and a fatal termination in lobar pneumonia. It seemed desirable, therefore, to determine the degree of bacteremia and the pneumococcidal power of the blood in moribund patients. The blood of three patients each suffering from Type I pneumococcus pneumonia was examined, one 3 hours, one 12 hours, and one 15 hours before death took place. All three patients appeared moribund at the time the blood was taken for examination, and all three had positive blood cultures. There was no evidence of any whole blood immunity in two of the patients whose blood in vivo contained 84 and 304 colonies of pneumococci per cubic centimeter respectively; indeed, the blood became overgrown by the Type I pneumococci already present without further inoculation of organisms. In the case of the third patient, whose blood contained 42 Type I pneumococci per cubic centimeter three hours before death, the whole uncoagulated blood killed not only those present but also killed 10,000 additional Type I pneumococci.

RELATION OF IMMUNE REACTIONS TO CLINICAL COURSE

In two cases of Type I pneumonia observations were made several times during the course of the disease. The accompanying charts show the temperature, the white blood count, the number of organisms in the blood, the whole blood immunity, the agglutination qualitatively observed, and the mouse protection test.

The patient represented in figure 1 was a man 36 years of age, who entered the hospital 31 hours after the onset of lobar pneumonia. His first blood specimen was obtained on the third day of his disease, the first day charted in the figure. His temperature fell sharply to

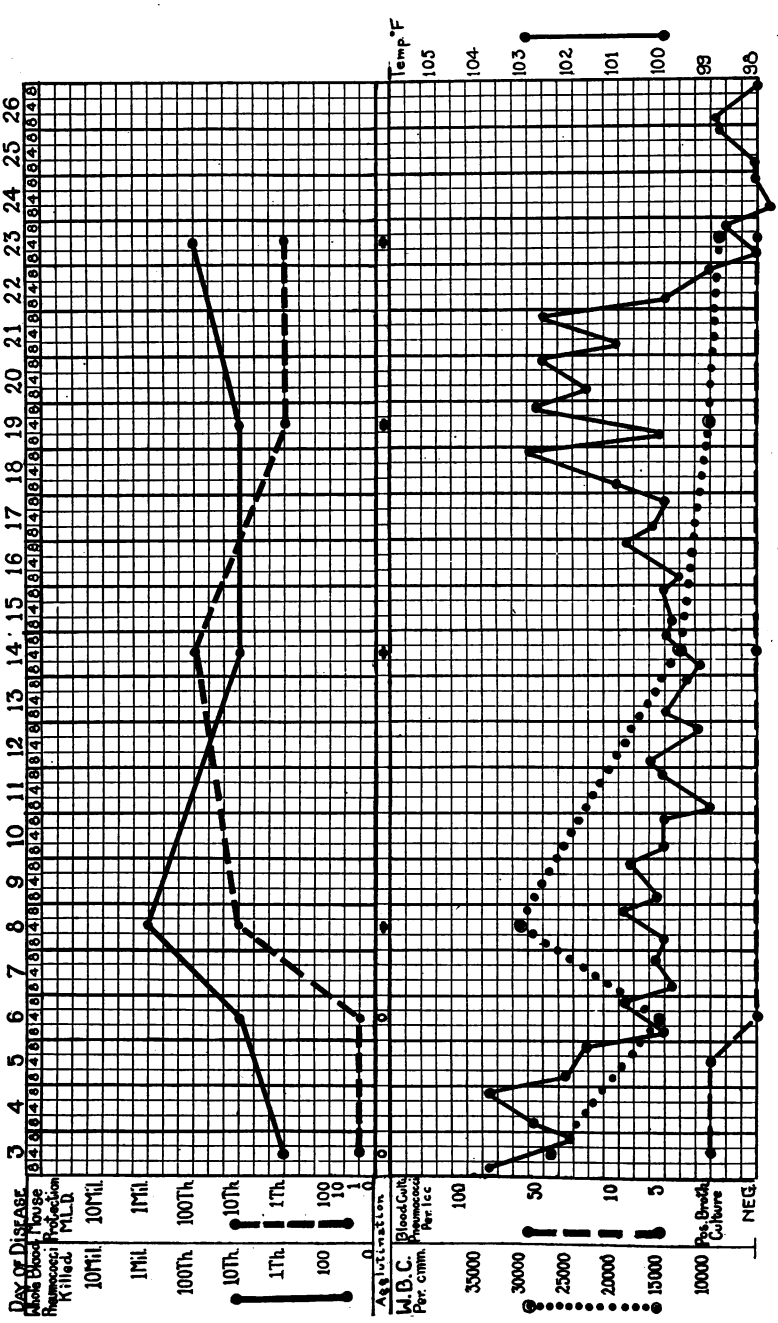

FIG. 1. OBSERVATIONS DURING THE COURSE OF A CASE OF TYPE I LOBAR PNEUMONIA FOLLOWED BY EMPYEMA. RECOVERY

100° on the 6th and 7th day, but rose again from the 14th to the 18th days. This was due to the development of empyema for which he underwent operation the 21st day of his illness. Recovery followed soon after thoracotomy was performed. This patient had a small amount of protection in his serum on the third day of his disease,

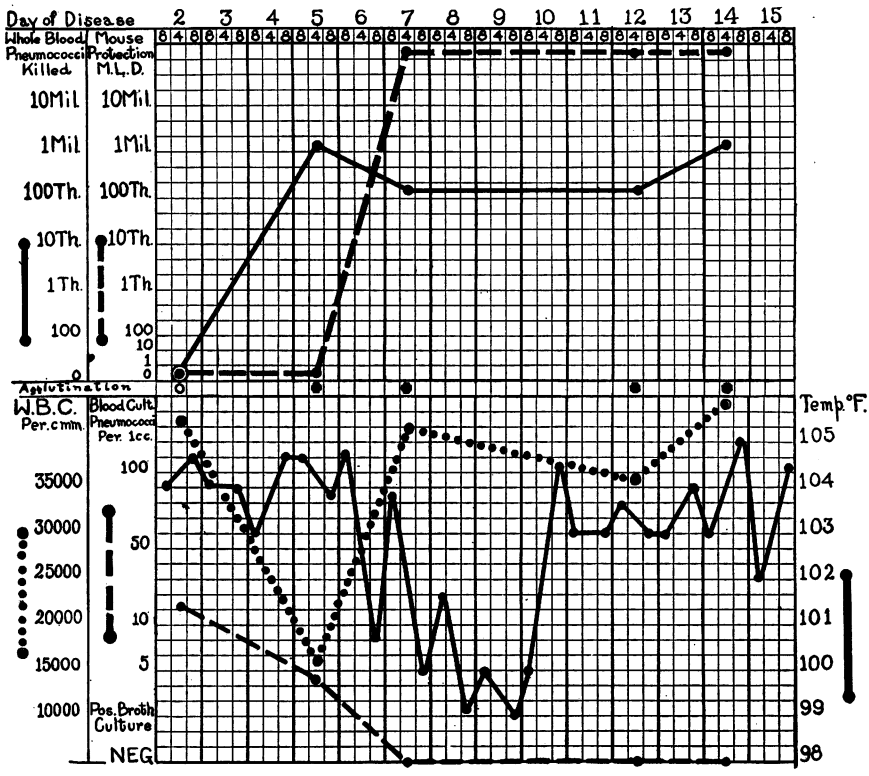

FIG. 2. OBSERVATIONS DURING THE COURSE OF A CASE OF TYPE I LOBAR PNEUMONIA FOLLOWED BY EMPYEMA AND ERYSIPELAS. FATAL TERMINATION

together with some pneumococcidal power, but showed simultaneously a positive blood culture in broth inoculated with 5 cc. of blood. An increase of pneumococcidal power was evident in the first specimen in which the blood culture was negative. Both pneumococcidal power and protective power reached a high point on the day of the sharp drop

in temperature. Agglutination appeared in the undiluted plasma on the day of the crisis.

Figure 2 represents the chart of a young man 28 years of age who entered the hospital on the first day of his disease. The first blood sample was obtained on the second day of his illness which is the first day shown on the chart. His temperature fell sharply to normal on the 7th day, rose again to 102°F., and then fell to 99°F. on the 8th day. On the tenth day it rose again and remained high until death on the 16th day. On the 13th day erysipelas appeared on the face. The patient died and at autopsy empyema was found in the left thorax. The pneumococcidal power of the blood appeared before the serum protection for mice. Agglutination appeared simultaneously with the appearance of protective power for mice. This patient, when first seen, differed from the preceding one in that he showed a more heavily infected blood culture. On the second day of his disease agar plates showed 12 Type I pneumococcus colonies per cubic centimeter of blood. In other cases, even shortly before death, a still greater number of organisms had been disposed of by the blood itself, but in this instance the organisms already present multiplied vigorously. At the second test made three days later, on the 5th day of the disease, there were 4 organisms per cubic centimeter in the patient's blood stream but these were killed and sterilization took place in tubes inoculated with as many as 1,000,000 Type I pneumococci. Serum protection for mice did not develop at this time, but was present on the 7th day, which was the day before crisis.

EFFECT OF SERUM ADMINISTRATION UPON WHOLE BLOOD IMMUNITY

It is of interest to note the effect of specific treatment on the ability of the blood to kill pneumococci. A patient with Type I pneumonia, on the fourth day of the disease was selected for the test (fig. 3). His blood contained enough organisms to give a positive culture when 5 cc. were inoculated into 50 cc. of broth. Before the administration of serum there was a slight amount of pneumococcidal power, sufficient to kill 100 pneumococci, in addition to those already present in the patient's blood stream. After the administration of 80 cc. of concentrated antipneumococcus serum containing 160,000 units of mouse

protection, the blood culture was sterile and whole blood pneumococcidal power had increased to such a degree that 1,000,000 pneumococci were killed. Protective power in the patient's serum and agglutination appeared simultaneously. Agglutination was still present the fourteenth day after the onset of the disease. The protective

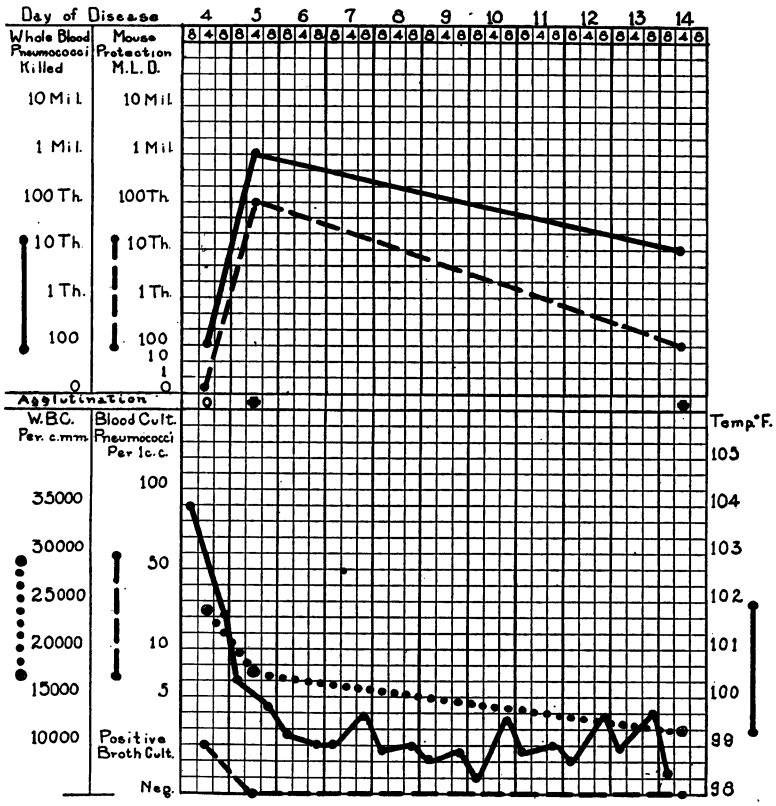

FIG. 3. OBSERVATIONS DURING THE COURSE OF A CASE OF TREATED TYPE I LOBAR PNEUMONIA WITH FAVORABLE OUTCOME

power for mice appeared to fall off more quickly than did the whole blood immunity.

DISCUSSION

This series of tests was designed to study the gross features of the whole blood immunity of man against the pneumococcus. The data

obtained does not bear directly on the partition of the pneumococcidal power of the whole blood between the humoral and cellular portions of the blood. A survey of the data discloses nothing inconsistent with the position taken by Robertson and Sia with respect to the immunity of animals (2) and man (3). Throughout our study in man serum immunity and whole blood immunity have been roughly parallel in the individual case.

The leucocyte is necessary for the reaction but, if the normal number is present, further increase has little or no effect upon the number of pneumococci which either the serum-leucocyte mixture or whole uncoagulated blood can kill. This point was brought out by Sia, Robertson and Woo (4) and may also be illustrated by our experiments on man. When an infection is at its height, such as before the crisis in lobar pneumonia, or in the presence of general septicemia, the white cell count is usually well above normal, with a high percentage of polymorphonuclear leucocytes. In the cases in this study that was frequently the time at which pneumococcidal power was low. With the drop in the count to normal the pneumococcidal power reached and maintained its highest level. In one test the maximum killing power, that effective against 1,000,000 pneumococci, appeared in the presence of 2,080,000 polynuclear leucocytes (4,160 per cubic millimeter), and, in another, in the presence of 11,800,000 polynuclear leucocytes (23,600 per cubic millimeter), or about five times as many. On the other hand, no pneumococcidal power was found in samples of blood which contained, at one extreme, 17,200,000 polynuclear leucocytes (34,400 per cubic millimeter) and at the other extreme 4,210,000 polynuclear leucocytes (8,420 per cubic millimeter).

Since certain degrees of whole blood immunity occur in normal subjects independently of serum protection, as detected by the usual mouse protection test, we should not expect the whole blood immunity in disease to follow exactly the serum protection for mice. In general, an increased power of the whole blood to kill pneumococci develops earlier in the acute stage of the disease than does protection for mice. Aside from this finding, however, serum immunity, as measured by the mouse protection test, and whole blood immunity run fairly parallel. The difference between serum protection and whole blood immunity may be due to a greater sensitiveness of the whole blood test.

Many writers have emphasized the importance of bacteremia in lobar pneumonia from the standpoint of prognosis and treatment. The presence of more than a certain number of organisms, especially later than the 5th day of the disease, is a fairly trustworthy indication of a fatal outcome. On the other hand, the presence of a few pneumococci in the blood in the early stages of the disease has little prognostic significance. A comparison between the findings of the whole blood test and the clinical course of the disease may give some idea of the significance of pneumococci in the blood and their relationship to the outcome in lobar pneumonia. On the whole, patients whose blood, when drawn, can kill whatever pneumococci are present therein, are doing well and those patients whose blood becomes overgrown by the organisms already present in the blood stream are doing badly. There are some conspicuous exceptions, however, to this general rule. On the one hand, the blood of one patient failed early in the disease to become sterile under the conditions of the test. Just before the crisis the blood of this patient developed the ability to sterilize itself and to kill a large additional number of pneumococci. On the other hand, we have observed a patient's blood that contained a number of organisms only three hours before death, which did sterilize itself. A special instance of the relationship of the pneumococcidal power of the blood to the outcome of the disease is manifested in pneumococcus complications. In the cases examined in this study (empyema, purulent arthritis, and endocarditis), the blood retained its ability to kill pneumococci present in the blood stream, and also the ability to kill a large number of pneumococci inoculated after the withdrawal of the blood. Although infection of the blood is an important symptom in the course of lobar pneumonia, it is apparently not the only factor associated with death, nor is it necessarily followed by death.

The actual mechanism of recovery or death does not seem to us adequately explained by the results of tests of blood or serum against pneumococci in vitro. In the 35 cases above, the whole blood immunity serum immunity, and the outcome of the disease are roughly parallel, except in the presence of purulent complications. Yet the correlation is not strict. Bacteria penetrate into the blood in the presence of bactericidal power, death occurs in the presence of few or many bacteria,

and occasionally in the presence of a certain amount of bactericidal power. The mechanism of recovery or crisis is difficult to understand in view of the difference in time between the appearance of the blood immune phenomena and the improvement in the condition of the patient. The missing correlation may lie, however, not in the lack of cause and effect, but in the lack of an exact knowledge of the mode by which the bacteria or their products produce death, or the immune products, on the other hand, produce crisis or recovery.

SUMMARY

1. The blood of 7 patients acutely ill from Type I lobar pneumonia killed from no pneumococci to 10,000 pneumococci in 6 observations made on the 1st, 2nd, 3rd, 4th and 5th days before the crisis. Four blood samples taken on the 1st, 2nd, and 3rd days before the crisis killed from 100,000 to 1,000,000 Type I pneumococci.

2. Thirteen blood samples examined during the first week after the crisis killed from 100,000 to 1,000,000 pneumococci.

3. Thirteen blood samples examined during the 2nd, 3rd and 4th week after the crisis killed from 1000 to 100,000 pneumococci.

4. Before the crisis, bacteremia was found to be associated with great variations in the pneumococcidal power of the blood; from no pneumococci to 1,000,000 pneumococci were killed. Bacteremia or sepsis about the time of death was usually associated with no pneumococcidal power, but in one case out of three, a moderate degree of pneumococcidal power and self-sterilizing power were present.

5. In the presence of pneumococcus complications such as empyema, purulent arthritis, and endocarditis, the patients' blood often contained pneumococci and was at the same time markedly pneumococcidal.

6. The administration of concentrated antipneumococcus serum to patients with lobar pneumonia produced a sudden rise in the pneumococcidal power of the blood to that level which is found in patients who have successfully passed the crisis.

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