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PNEUMOCOCCUS INFECTION AND LOBAR PNEUMONIA *

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PNEUMONIA, in many respects, certainly as a cause of death, is the most important infectious disease with which we have to deal. The symptomatic treatment is difficult and of doubtful utility; there is no well-established form of specific therapy. Nevertheless, up to within a very recent time, the investigation of the real nature of the process has been slight and fragmentary.

While the association of certain kinds of bacteria with this disease has been well established, much obscurity exists with regard to the mode of infection, the relation of the bacteria to the lesions and symptoms, the nature of recovery and, above all, with regard to the possibility of prevention or of cure. It has been in the hope of helping to shed some light on these problems that my associates and I have been making some clinical and experimental studies. It will be impossible to review in this paper all the work that has been done by others, and I shall have to content myself with presenting certain points of view which have been suggested mainly by our own work at the Rockefeller Institute.

Acute lobar pneumonia seems the best characterized of the acute lung affections. It has such a clear-cut clinical course that it is now generally considered a distinct clinical entity, and no more to be regarded simply as an infection of the lung than typhoid fever is to be considered an infection of the intestine. This, however, is a clinical point of view and it is possible that the same kinds of reaction occur in other localized pneumococ-

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cus infections as are present when the main seat of infection is the lung.

In a very large percentage of patients suffering from inflammation of the lung of the lobar type, pneumococci are present in the lesion. In isolated instances, other organisms, as *Bacillus influenzæ* or *B. pneumoniæ,* are found in pure culture. It has not been our purpose to consider these latter cases, but our attention has been given entirely to the group of cases in which *Diplococcus pneumoniæ* is present and is apparently the etiological agent. As is well known, diplococci, which at present cannot be differentiated from the pneumococcus, may be present in pneumonia of the lobular type (in which the clinical course is quite distinct from that in lobar pneumonia); they may also be present in other purely localized lesions in the body, entirely unassociated with any affection of the lung, and they may even be the organisms concerned in certain cases of septicæmia in man without any local lesions whatever. Moreover, organisms with identical characteristics, so far as yet determined, are found with so great frequency living on the mucous membranes of the mouth and throat of perfectly healthy individuals that they may be considered normal inhabitants of the mouth and throat cavities. In the face of such facts as these, how can it be maintained that *Diplococcus pneumoniæ* is the primary cause of such a well-characterized acute infectious disease as acute lobar pneumonia? In view of the present general consensus of opinion that this theory is true, one is indeed rash even to suggest the possibility that there may be another agent concerned. On the other hand, it is important that such a possibility should not be overlooked. Even though it should be shown, however, that pneumococci do not play the primary etiologic rôle in the natural infection, their association with the lesion and their frequent invasion of the blood render it evident that they play an important part in the process and probably the most important part in the outcome, just as do streptococci in certain diseases, such as smallpox and scarlet fever, of which it is generally believed that the natural infection is due to specific etiologic agents.

Up to within a relatively short time, the most important link in the chain of evidence that pneumococci cause pneumonia, namely the reproduction of the disease in animals, was lacking. Most important studies dealing with the experimental production of acute lobar pneumonia were published in 1904 by Wadsworth.[1] By carefully balancing the general resistance of the animal with the virulence of the race of pneumococci employed and by injecting the organisms intratracheally, he was able, in a series of rabbits, to induce a diffuse exudative pneumonia like the acute lobar pneumonia seen in man.

More recently Lamar and Meltzer,[2] and Wollstein and Meltzer[3] have succeeded in regularly producing a diffuse pneumonia of the lobar type in dogs, by injecting from 10 to 15 c.c. of the fluid culture directly into one bronchus through a rubber tube passed through the trachea, following the injection of the fluid by air blown through the tube, so as to force the infectious material into the finer ramifications of the bronchi. The pneumonia produced in dogs runs a more rapid course, resolution occurs earlier—in three or four days—and the mortality is much less than in pneumonia in man.

Using a similar technic, these investigators have produced diffuse lesions in the lungs of dogs with other micro-organisms. When streptococci are injected, the lesions tend to resemble more closely those seen in bronchopneumonia in man.[4] The observers lay stress on the greater tendency in this case to a leucocytic infiltration of the lung framework, and to a much-lessened formation of fibrin. These differences between the pneumonia produced by the injection of streptococci and that following the injection of pneumococci they ascribe to inherent differences in the nature of the micro-organisms concerned, and not to relative differences in virulence. By a similar method of intratracheal injection, Winternitz and Hirschfelder[5] have succeeded in producing pneumonia of a lobar type in rabbits. In these experiments also, large amounts of the culture material (4 or 5 c.c.) were injected.

From experiments which I have carried on, using the same method, it is evident that successful results in rabbits depend

somewhat on the race of organisms employed. If the organisms have very slight virulence, the animals may recover without lung lesions; if they are too virulent, a septicæmia quickly results and at necropsy only congestion and œdema of the lungs are present.

As Wadsworth showed, the lung consolidation is probably a manifestation of the resistance of the animal to the spread of the infection. The occurrence of the diffuse lung lesion is undoubtedly dependent on the same factors which are concerned in the differences in local reaction to the injection of virulent pneumococci in different races of animals. As is well known, mice and rabbits are very susceptible to pneumococcus infection, however induced, guinea pigs and dogs less and man possibly still less. The result of a subcutaneous injection of virulent pneumococci into a rabbit differs markedly from that seen when a similar injection is made into a guinea pig. In the former there is very little local reaction; a rapid general invasion of the organisms takes place, and the animal dies quickly from a marked septicæmia. In the latter a marked infiltration with much fibrin formation and a slowly progressive invasion of the subcutaneous tissues occur, while there is little or no general infection. That the time element plays a rôle in the formation of fibrin is well seen in the peritonitis induced in such susceptible animals as the mouse and rabbit. If an intraperitoneal injection is made of virulent pneumococci, death occurs within twenty-four hours, and in the peritoneum there is seen only a marked congestion, possibly hemorrhages, a serous exudation and usually no fibrin. If, however, the culture is less virulent and the animal lives forty-eight hours or more, there is usually considerable fibrin over the liver, and flakes of fibrin are seen throughout the cavity. The amount of fibrin increases in ratio with the length of time during which the animal is able to resist the infection.

The experiments of Wollstein and Meltzer,[4] however, tending to show that the peculiar property of stimulating the production of fibrin is possessed to a greater degree by pneumococci, whatever their virulence, than by streptococci, is of

very great significance. It is difficult to understand, however, just why this property should be a factor in the production of pneumonia of a lobar rather than of a lobular type. That this peculiar property should not be the only one concerned is made evident from the fact that the pneumococcus is the organism most frequently concerned in lobular pneumonia in children. Dochez[6] has shown that, during the acute stages of lobar pneumonia, there is an increase of fibrinogen in the blood. Nevertheless, the coagulation time of the blood is delayed, owing probably to an increase in antithrombin as well. We know of no observations which show whether or not so great an amount of fibrinogen exists in the blood in prolonged streptococcus infections in man as is present in pneumonia.

As the virulence of the race employed is of importance as regards the production of the local reaction, so also is the number of micro-organisms injected, as Kline and Winternitz[7] have shown. If the number of organisms is too small, no pneumonia results. It is well known that even in very susceptible animals a considerable number of virulent organisms is usually necessary to produce infection. Little attention has been given to the question why, when a considerable number of organisms are injected together, multiplication occurs and infection results, whereas if only a few organisms be injected, they are unable to multiply. Is it because in a culture of organisms certain ones are more resistant than others to the harmful influences, or is it simply accidental that, when a large number of organisms are injected, a few have a possible chance to escape?

Gillespie[8] has carried on some experiments with pneumococci which have a bearing on this problem. We, as well as others, have long recognized that in starting a culture of pneumococci in a large amount of bouillon, a litre for instance, a much larger inoculation is necessary in order to obtain growth than if the inoculation be made into 10 c.c., or if the culture be made on a solid medium. In the latter instance, one organism will usually produce a colony. By making the inoculations on filter-paper kept constantly wet by bouillon, it was shown that growth would occur with the inoculation of as small numbers

of organisms as are required on agar, and with much smaller numbers than are required to inoculate the bouillon. The differences in growth, therefore, are not dependent on differences in composition of the medium, and further experiments have shown that they are not due to differences in oxygen supply. It seems probable that for growth to occur, the bacterium must produce changes in the medium immediately surrounding it, and that when the opportunities for diffusion are great, such local changes cannot be kept sufficiently constant unless there be a considerable number of organisms in proximity. If this be the true explanation, it may have an important bearing on infection, not only with pneumococcus but with other micro-organisms as well. The presence of mucus in the smaller bronchi, for instance, might in this way favor the multiplication of micro-organisms and so favor infection. That very large numbers of bacteria are inoculated in the experiments of Meltzer and Winternitz probably explains to some degree why their experiments have been successful, where others have failed. A second factor in the success of this technic probably lies in the fact that considerable amounts of fluid are injected and this is blown into the terminal bronchioles. Meltzer has made the interesting suggestion that by this process the bronchioles are occluded and that in this way closed cavities are formed. It is generally recognized that as long as bacteria grow on exposed surfaces, they do no harm. It is only when the growth occurs in confined spaces that harmful results supervene.

While these experiments on the production of pneumonia in animals are of great value in showing that lesions resembling acute lobar pneumonia in man may be caused by pneumococci, they do not directly offer an explanation of the natural infection in man. It is hardly likely that in man an overwhelming infection ever occurs with numbers of bacteria so large as those used in the experiments in dogs. The usual recourse in this dilemma is to assume that the organisms concerned in natural infection have an increased virulence, or that the resistance of the host is lowered. By virulence in micro-organisms is usually meant adaptation to growth in the tissues of the host.

Since in pneumonia the organisms are cultivated from the lungs and at times from the blood, we know that they have virulence for man. We have no way of determining, however, whether or not all pneumococci growing in the mouths of healthy individuals are also virulent for man. The attempts to demonstrate conclusively that pneumococci isolated from cases of pneumonia in man are regularly of increased virulence for animals have not been successful. We have found that most of the pneumococci isolated from the blood of pneumonia patients have relatively high virulence for susceptible animals, yet while certain of these cultures, when freshly isolated, are of such virulence that 0.000001 c.c. of a bouillon culture will kill a mouse, we have also obtained cultures that required 0.5 c.c. to kill. The virulence of the race, therefore, does not seem to be the only deciding factor in the question why infection occurs, even though, as will be shown, it may be of considerable importance as regards the final outcome. It must be stated, however, that it is not certain that virulence for animals is identical with virulence for man. Usually high virulence of a given race of pneumococci for one susceptible animal, as the rabbit, indicates high virulence for another, as the mouse. Ungermann, however, has described a typical pneumococcus having high virulence for rabbits, but its virulence for mice, which was originally present, was lost. We have studied a race of pneumococci originally very virulent for mice, which, after passage through guinea pigs, increased its virulence for these animals, but became almost avirulent for mice and rabbits.

Against the view that pneumonia arises when organisms of increased virulence reach the lung is the fact that pneumonia rarely occurs in epidemics and is very slightly contagious. There are now a number of epidemic outbreaks reported, but it must be admitted that these are of rare occurrence, and all hospital experience is against contagion as a great factor in the spread of the disease. On the other hand, the fact that in certain times and places pneumonia occurs with greatly increased severity and frequency suggests that at these times the pneumococci concerned may have acquired increased virulence.

When the French attempted to build the Panama Canal, the incidence and mortality of pneumonia interfered as seriously with the work as did the occurrence of malaria and yellow fever. Even during the first years of the American occupation of Panama, the mortality from pneumonia was enormous. For its decrease there seems no adequate explanation. In South Africa, deaths from lobar pneumonia among the coolies constitute a serious menace to the continued working of the mines.

Little is known concerning diminished general resistance on the part of man to pneumococcus infection and its importance in natural infection of this disease. Clinical studies have quite conclusively demonstrated that the habitual use of alcohol increases susceptibility to infection or, at any rate, renders the subject less resistant when infection has once occurred. That exposure to cold, or especially sudden changes in temperature and chilling, play a part in infection can hardly be doubted, and there is some experimental evidence to show that animals suddenly chilled are more susceptible to infection with pneumococci than others.

The view that local changes in the lung are of importance for the occurrence of infection is interesting and suggestive. In most cases of lobar pneumonia the primary seat of infection is probably in the lung. Various writers have attempted to show that infection occurs through the blood-stream, but the evidence is not conclusive. Other localized pneumococcus infections in internal organs or cavities usually occur by extension, though this is not always demonstrable. The possibility that infection through the blood may occur in certain instances cannot be excluded. Pneumonia, as a part of a general infection, however, is generally of a lobular type.

Much has been said lately about the adaptation of certain organisms for certain tissues. In many cases, however, this adaptation is more apparent than real, and the mode and degree of infection play the larger rôle in localization.

In about 50 per cent. of the cases of pneumonia, a history of preceding coryza and cough may be obtained. In these cases it is possible that there occurs a downward infection along the

mucous membrane of the bronchi. The extension of the lesion through the lung from one lobe to another apparently takes place through the bronchi, as the study of large sections through lobes with beginning involvement shows. In the remaining half of the cases, however, the onset is sharp and sudden without history of bronchial involvement. Even in these cases some local change probably precedes the real infection. It is well known that thoracic trauma is frequently followed by pneumonia. The idea of Meltzer that infection may be facilitated by closure of the smaller bronchioles is most suggestive. It is possible that cold or chilling may stimulate the mucous glands so that the increase of mucus may produce favorable conditions for the growth of pneumococci, which are so frequently present in the upper respiratory tract.

That the lung is the chief seat of the disease, then, is probably due to the fact that infection occurs here, and that a local lesion results and not a general infection (at least not until late in the disease) is probably due to the fact that man is highly resistant to infection with pneumococci and that the anatomical conditions here permit of an extensive inflammatory reaction which opposes the spread of the infection.

But why does infection occur at all? Why does a person contract pneumonia? There is still considerable obscurity in regard to this phase of infection, not only in pneumonia, but also in many other infectious diseases of which the etiology is well known. The obscurity and difference of opinion in regard to tuberculosis are well known. Even our views with regard to infection in diphtheria have been disturbed by new observations. We all thought the transference of the infectious agent from the infected to the healthy throat was all that was necessary for infection in this disease. The observations of Moss, Guthrie and Gelien,[9] however, that in Baltimore there are four times as many carriers of virulent diphtheria bacilli as there are cases of the disease, are most disturbing. So far as can be determined, the bacilli from carriers differ in no way from the bacilli from patients with the disease. Moreover, the incidence of the disease seems to bear no relation to these carriers. The problem of

mode of infection is thus in a minor degree analogous to that in pneumonia, in which practically the entire population represent carriers of infection.

To explain the nature of infection we may say: First, there is a possibility that in pneumonia, as in diphtheria, the organisms causing infection differ inherently from those in normal throats, especially as regards adaptation or virulence for men. Definite evidence in favor of this would be most important, but at present there is none. Second, it is possible that the general resistance of patients to pneumococci is lowered, so that organisms, formerly living as harmless parasites, now invade the tissues and induce reaction. For this also we have no definite evidence. Third, the study of artificial infection in animals, as well as the course of the disease in man, suggests most strongly that local changes in the respiratory tract may precede the infection with pneumococci. Whether these are due to a primary infectious agent or to changes in the tissues due to other factors cannot be decided definitely at present. Finally, it is possible that infection depends on a combination of factors, virulence of organisms and general and local resistance each playing a part. Further knowledge along these lines is absolutely essential for prevention of this disease. To formulate rules or regulations for prevention at present seems useless, except as an experiment.

THE NATURE OF THE INTOXICATION

Whatever be the mode of onset in pneumonia, the production of the local changes in the lungs, as well as the general systemic manifestations of the disease, seems to be in some way related to the growth of pneumococci in the body. When micro-organisms grow within other multicellular organisms acting as host, the effects on the host are of two kinds: First, there is a local reaction, in which the bacteria are present in considerable numbers, as at the point of infection. Here are induced the changes spoken of as inflammation. In addition to this, however, there is practically always a reaction throughout the entire body, even when the local reaction is very mild and

evanescent. These general reactions are evidenced, not only by fever and nervous disturbances on the part of the host, but even in their absence by such effects as changes in the blood, especially the leucocytes, which indicate certain effects on the blood-forming tissues.

In most cases, the exact manner in which micro-organisms stimulate the tissues in which they are growing to a reaction which is called inflammatory, is still obscure. Since, however, identical reactions may be produced with non-living chemical substances, it is generally assumed that in the case of bacteria as well, non-living chemical substances are formed as a result of the bacterial growth, which substances are in themselves harmful. Whatever may be the exact relationship of the organisms to the local lesion, it is necessary to assume that the general manifestations of infection, and especially the effects on tissues far distant from the local lesion, are the result of soluble toxic substances which circulate in the blood or lymph. Since general manifestations similar to those in pneumonia are seen in other pneumococcus infections and even in general infections in animals without local lesions, it does not seem probable that these effects in pneumonia are due to disturbances in respiration associated directly with the lung lesions. It is possible, of course, that in pneumonia the general manifestations and effects on distant tissues and organs are due to the action *in situ* of bacteria which have gained access to the circulation and have been carried to these distant parts.

Very numerous observations have been made on the occurrence of bacteria in the general circulation in acute lobar pneumonia. During the past years blood-cultures have been made on most of the cases of pneumonia coming under my observation, and the results have not led me to change the conclusions arrived at ten years ago from the study of a series of cases, namely, that pneumococci are usually found in the blood only in the more severe cases, and the presence of the pneumococcus in the blood is of ill omen. It is possible that in all cases of pneumonia an occasional bacterium may be carried into the circulation, but the demonstration of this is difficult. That this may

occur, however, is not of prime importance, for the occurrence of an occasional organism could hardly explain the great degree of effect in distant tissues, as manifested by the general symptoms which we call intoxication.

The attempts to discover something of the nature of this circulating poison have been attended with much difficulty. It would seem that a more accurate knowledge of the metabolic disturbances in pneumonia might give a clue as to the nature of the intoxication. A series of studies with this object in view was undertaken.

Of late years attention has been drawn to the occurrence of functional disturbances, especially in infants, due to derangements in salt metabolism. It appeared of interest to learn whether or not specific changes in inorganic metabolism may be induced by pneumococci, which could account, in part at least, for the symptoms induced. The most striking disturbance in pneumonia is known to be the retention of chlorides, which is frequently almost complete during the acute course of the disease. Retention of chlorides to a lesser degree is known to occur in other infections, but Rowntree[10] has shown that this retention does not occur in influenzal pneumonia to nearly so marked a degree as it does in pneumococcus pneumonia. Medigreceanu[11] has carried on a series of studies of pneumonia in dogs and Peabody[12] has studied the question in cases in man. Peabody has shown that there is a retention not only of chlorine, but also of sodium and calcium while there is no retention of potassium and magnesium, but may be a loss. Further studies indicate that the retained substances are not stored in any one place, but are spread diffusely throughout the tissues.

It is not believed that these changes are specific for pneumonia, for they probably occur in other infections. They are most striking in pneumonia, since the changes between the febrile and afebrile state occur with such suddenness. It is not likely that these changes in themselves are responsible for any of the symptoms of the disease, but in view of the striking effects which have been induced by Meltzer by changing the balance in the inorganic salts in the body, this possibility must

be borne in mind. We have no knowledge of the reason for these changes in pneumococcus infection.

Pneumococci are known to produce acid readily, even, as shown by Hiss in albuminous mediums, containing no demonstrable sugar. It has therefore been suggested that the symptoms in pneumonia are the manifestations of an acidosis. Hamburger[13] has attempted to explain the chlorine retention on the basis of a febrile acidosis. The studies of Peabody,[14] however, have shown that the curves of chlorine retention and of ammonia excretion, which is generally considered the best indicator of acidosis, do not necessarily run parallel. The studies of inorganic metabolism have therefore given no conclusive insight into the nature of the intoxication.

In order, if possible, to obtain some knowledge regarding this problem by another method, Peabody has made studies of the gas exchange in the blood in pneumonia. He has found that in this disease the carbon dioxide in the venous blood is quite regularly low, in spite of the disturbances in gas exchange in the lung. At the same time there occurs an increase in the ammonia nitrogen in the urine, and the curves run somewhat parallel. These changes, which are indicative of increased acid formation, nevertheless correspond to changes that have been known to occur during fever and infection due to other causes, and are no indication of specific changes occurring in this disease. The carbon dioxide content of the blood does not bear a definite relationship to the severity of the disease, except that it is lowest in the most severe cases and in the terminal stages.

On the other hand, the study of the oxygen-content of the blood has revealed some interesting changes. Studies of the peripheral venous blood showed in certain cases a diminution in the oxygen-content of the venous blood. In studying the blood in one such case, it was found that the blood would not take up a normal amount of oxygen, and this in spite of the fact that the hæmoglobin content was normal. In a careful study of such blood by Butterfield and Peabody[15] it was found that this phenomenon was due to the formation of methæmoglobin. This

change also occurs regularly in the blood of rabbits[16] infected with the pneumococcus and has no relation to the lung lesion. It also occurs when the bacteria are grown in blood-containing mediums.

Usually the change into methæmoglobin in the animal body does not go so far that the methæmoglobin can be distinguished spectroscopically. In the test-tube, however, especially when hæmoglobin in solution is added to the culture, practically all the hæmoglobin may be changed into methæmoglobin. That this reaction is not simply due to the action of acids formed by the pneumococcus is shown by the fact that for the production of methæmoglobin far more acid is required than could be present in the body, and, moreover, that it may occur in cultures or filtrates that are alkaline in reaction. It is therefore evident that this change is due to the action of a poison formed by the pneumococci.

Peabody[17] further made a study of the blood in twenty-five cases of pneumonia to determine the frequency of the occurrence of this phenomenon and the time of its appearance. Of the cases which ended in recovery, in only one was there any indication of a diminution of the oxygen-absorbing power of the hæmoglobin. In all of the ten cases ending fatally, there occurred a progressive loss in the oxygen-content of the blood and in the oxygen-combining power of the hæmoglobin, and from the previous studies it is certain that these changes are due to the formation of methæmoglobin. In nine of the ten cases the blood-cultures were positive.

That these changes play a part in the fatal termination can hardly be doubted. The terminal symptoms of the disease may be accounted for by deficient oxidation. It is not likely, however, that these changes in the blood are in themselves the only factor in accounting for the fatal result; but they represent one of the factors, and are an indication of the intoxication which is the result of the growth of pneumococci in the body.

A second effect of the pneumococcus intoxication has been demonstrated by Medigreceanu[18] by estimating the amount of oxidase in the organs of animals dying from pneumococcus sep-

ticæmia, as compared with the organs of normal rabbits. In these studies Medigreceanu employed the method of Röhman and Spitzer, which is based on the property of tissues of oxidizing a mixture of naphthol and paraphenylendiamine into phenol. By comparing the tissues of normal animals with those previously infected with pneumococci, it has been found that this oxidase is generally diminished in the latter animals. By proper controls it has been possible to show that this change is due, not to the presence of pneumococci in the tests, but to some change which results in the tissue from the infection. Another effect of the action of the toxin on tissue function is thus made evident. It is therefore probable that, in addition to the lessened supply of oxygen by the blood due to the formation of methæmoglobin, there is also a lessened power of the tissues to carry on the proper oxidation function.

Finally, in order to obtain evidence of the presence of a poison, studies were made by Medigreceanu[19] to determine whether or not there was an increased output of substances known to have the property of neutralizing poisons arising in the body. Such a substance is glycuronic acid, and it was found that during the acute stages of pneumonia in man, in almost all cases, there is a definite increase in the output of this substance.

All these studies clearly indicate the activity in pneumonia of a circulating poison; but the direct demonstration of the presence of this toxic substance in the animal is more difficult. To this end the following experiments were performed. Each one of a series of rabbits was inoculated with an overwhelming dose of pneumococci. Then, just as death was imminent in from five to eight hours, the animal was bled to death, and as quickly as possible the blood was defibrinated, the serum passed through a Berkefeld filter to remove the bacteria and the filtrate injected intravenously into a normal rabbit. To our surprise and disappointment, the animals did not die, nor in a second series of rabbits treated in this way were we able to detect any minor harmful effects of such injections.

When one considers the conditions in pneumococcus infection

it is not surprising that there is great difficulty in demonstrating the presence of toxin in the animal, or even of demonstrating the production of toxin by the pneumococcus *in vitro*. The infectious diseases in which active specific toxins have been well demonstrated *in vitro* are diphtheria and tetanus. In these diseases, however, the conditions are unusual. Here a moderate number of organisms growing in the local lesion produce sufficient poison to bring about the most profound intoxication, and it is not surprising that the poison may readily be demonstrated *in vivo* and *in vitro*. In pneumococcus infection, the conditions are different. Even in general infection in the highly susceptible mouse or rabbit, the number of organisms growing in the body is enormous before the animal finally succumbs.

In the severe and fatal cases in man the blood may contain as many as 65,000 organisms per cubic centimetre; and when it is conceived that these are throughout all the body-fluids and the tissues, it is evident what immense numbers of bacteria are responsible for the intoxication and fatal outcome. In man, even when there is no marked invasion of the blood, the number of micro-organisms in the lung must be very large. It is probable that during the course of the disease bacteria are all the time undergoing degeneration, so that from the beginning to the end, large numbers of bacteria have been present. Also the amount of toxin present at any one time may be very small, yet when acting on tissue-cells for six or seven days may produce marked effects.

The suggestion has been made that in pneumonia the symptoms are due to the absorption of products of digestion of the pathological exudate in the lung. It has been well established by various observers that, during the parenteral digestion of protein, substances are formed which may induce fever and symptoms of intoxication. Similar symptoms may be induced by the injection of peptone and other products of protein digestion into the circulation of animals. Most of the work that has been done in the production of fever by means of protein, however, has been carried out with foreign protein and not with the protein of the host. Moreover, at the time the resolu-

tion is probably greatest, that is, following the crisis, such symptoms are not present, although in most cases considerable amounts of the digested exudate are being absorbed. It is hardly likely that the specific and characteristic symptoms of pneumonia can be due merely to the absorption of the products of digestion in the local lesions.

Following the discovery of serum anaphylaxis in guinea pigs and its relation to protein intoxication in man, numerous efforts have been made to bring this into relation to the toxic effects seen in acute infectious disease. Friedberger has shown that if bacteria be treated with immune serum and then with complement, the supernatant fluid, after removal of the bacteria by centrifugalization, will be toxic when injected into a guinea pig, the animal dying within a few minutes with symptoms identical with those seen in anaphylactic shock. He has called the toxic substance produced outside the body in the manner stated " anaphylatoxin," and believes that it is identical with the substance formed within the body which produces the symptoms following the second injection of protein. He thinks that this experiment may offer the explanation for all bacterial intoxications. According to his theory in the period of incubation, during which the bacteria are already present but produce no symptoms, antibodies are being formed, and when these are present in sufficient numbers, the bacterial bodies begin to be split up and the substances so formed produce, not the acute symptoms which are speedily followed by death, because the bacteria are not present in sufficient numbers, but milder symptoms—fever, etc., a kind of chronic anaphylaxis. This can hardly explain, however, what occurs in pneumonia, in which all the evidence seems opposed to a long incubation period, the onset of the symptoms being sudden and apparently the immediate result of the infection.

The production of the so-called " anaphylatoxin " from pneumococci may readily be done, as we and also Neufeld and Dold have shown. Neufeld and Dold,[20] moreover, have shown that similar toxic substances may be obtained from bacteria by simple extraction in salt solution containing lecithin. Rosenow[21]

then showed that if pneumococci are merely placed in salt solution for forty-eight hours at 37° C. (98.6° F.), the extract so formed is toxic, and on injection intravenously into guinea pigs, acute symptoms and speedy death, like those seen in serum anaphylaxis, result. We have studied the effect of the injection of extracts obtained by autolysis in a very large number of guinea pigs,[22] and, in our experience, while occasionally sudden death is produced, this does not occur with great regularity.

Since the salt-solution extracts of pneumococci did not show as high toxicity as was anticipated, it was held possible that in the peritoneal cavity of an animal the solution of the bacteria might go on at a more rapid rate, from which cavity solutions might be obtained of greater and more constant toxicity. Guinea pigs were therefore inoculated intraperitoneally with large doses of pneumococci. As soon as possible after death, the peritoneal cavity was washed out with salt solution. The cells and bacteria were then removed from this solution by centrifugalization and the supernatant fluid was used for intravenous injection into healthy guinea pigs. Of eleven animals so treated, eight showed immediate symptoms like those seen in anaphylaxis, and four of these died within a few minutes with typical features of anaphylactic death and with characteristic necropsy findings.

From the experiments it is evident that the development of the toxic substance is more constant and striking in the peritoneal cavity of the guinea pig than it is in the test-tube. In the animal body, however, conditions are complex and it is difficult to know whether the toxic substance is specific or bears any direct relation to the infectious agent. We therefore tried to obtain solutions of the pneumococcus bodies by other means. Making extracts in chloroform and in ether did not yield solutions that could be readily studied. We next studied the solution of pneumococci obtained by means of bile. In making the solutions a 2 per cent. solution of sodium cholate in normal salt solution was employed. The effect of the intravenous injection of bile extracts of pneumococci has now been tested in a very large number of guinea pigs and rabbits. In a large proportion of cases death with acute symptoms resembling those in ana-

phylactic shock occurs. When smaller amounts of the extract are injected, or when the toxicity of the extract is less, the animals die in from two to twelve hours. Such animals usually show more or less pulmonary œdema and hemorrhages, and small hemorrhages are present in the peritoneum and diaphragm and in the walls of the stomach and intestines.

It is probable, from the effects produced, that the substances operative here are the ones that produce the effects in " anaphylatoxin " and in the salt-solution extracts. In the latter case it has been assumed by Rosenow that the toxic substances result from the digestion of the bacterial protein by the ferments contained in the bacterial cell. The proof of this, however, does not seem convincing. The fact that the solution of the pneumococci in cholate solutions may occur within one-half hour at 4° C. (39.2° F.) is evidence that in this case the active substance is not the result of ferment action. In a recent communication Jobling and Strouse[23] have presented good evidence to show that the lysis of pneumococci in salt solution is probably not merely the result of ferment action. All these experiments indicate that the bodies of pneumococci contain substances which are toxic when they are set free by the solution of the bacterial bodies. They therefore present evidence in favor of the well-known endotoxin theory of Pfeiffer.

During the past few years this theory of the origin of toxic substances has been largely neglected, owing to the interest in the theories of Vaughn and Friedberger, according to which the intoxication in all forms of infection is caused by substances which are intermediate products in the digestion of protein. Vaughn goes so far as to state that the substances producing the symptoms are identical in all infections and that the different symptom-complexes are dependent, not on the nature of the intoxicating substance, but on other conditions. It would hardly seem, however, that the intoxicating substance causing the rapid pulse and rapid, labored respiration and violent delirium of pneumonia is identical with the intoxicating substance in typhoid in which there is a relative slowing of the heart and low, muttering delirium. Though the intoxication

may be due to the products of protein digestion, it does not necessarily follow that the substance is the same in all cases, as the bacterial proteins must differ enormously in composition.

While the obtaining of toxic substances from the bodies of pneumococci is of great interest, it is quite evident that this, in itself, does not contain the proof that we are dealing with the substances responsible for the intoxication in pneumonia. In order to present such evidence, further knowledge is required of the nature of the substance and especially of its relation to pneumococcus immunity.

Certain facts have already been established in regard to this toxin. Its study has been greatly facilitated by the fact that when added to washed sheep-corpuscles hæmolysis occurs. So far as studied, the toxic effects are caused by the same substance which produces hæmolysis, since the two properties are influenced by the same measures and vary in equal degree. One of the most important facts that has been determined in regard to this toxin is that the toxic and hæmolytic properties vary with the virulence of the organism employed. Extracts from non-virulent cultures, so far as studied, are not toxic. The substance which is responsible for the formation of methæmoglobin in the body and the discoloration of blood in cultures, however, does not seem to be present in this toxin. The toxin is destroyed by heating one-half hour at 56° C. (132.8° F.). This may explain why the injection of pneumococci killed by heat produces no effect in the animal injected. It loses its toxicity when kept for twenty-four hours at 37° C. or for two or three days on ice. It may be dried, in which condition its toxic properties disappear much more slowly. It does not pass readily through a Berkefeld filter and it is precipitated by colloidal iron solutions. Many attempts have been made to neutralize its action by the use of dyes, by nucleic acid, nucleates, glycocoll, glycuronic acid, etc., substances which are considered to attach themselves to toxic substances in the body and thus to render them non-toxic. None of these experiments have been successful. The only substance so far found which is able to neutralize the effect of the toxin is cholesterin. When cholesterin is mixed with the

toxic substance and kept at 37° C. for fifteen minutes, the toxic effect, as shown by injection into animals, and also the hæmolytic effect, is lost. When the toxin is mixed with cholesterin and immediately injected into the animal, however, or when the toxin is first injected and is immediately followed by the cholesterin, the toxic effects cannot be prevented. Nor can the toxic effects be prevented by injecting the cholesterin before the toxin. The most important results in this study have been obtained in the attempts to produce antiserums to these toxins, and of these I shall speak later.

Whatever may be the mechanism by which intoxication is brought about, have we any evidence as to the determining factors in the final outcome, that is, as to why the patient recovers or dies? The results of our blood-cultures would seem to indicate that the occurrence of septicæmia plays an important part in the death of the patient. A study of the virulence of the cultures from the blood also seems to show that the intoxication is greater and the prognosis worse when the organisms have a high virulence than when they have a low virulence. Moreover, our clinical experiments seem to indicate that the progressive extension of the local lesion is of bad prognostic import; that the failure of the body to erect a limiting barrier to the local extension of the disease is an important factor in the fatal outcome. At any rate, it is certainly true that in most fatal cases, on examining the lung, one sees, not a sharply localized lesion, but an extending lesion frequently involving several lobes. This progressive extension seems to bear some relation to the virulence of the organism. With organisms of low virulence, the body is able to resist the infection, as regards both the spread of the local lesion and the general infection.

We have made quite extended studies to learn something of the nature of the general resistance of the body to the pneumococcus infection and its effects. It would seem that in pneumonia with its sudden crisis—one of the most startling and dramatic events with which the physician has to deal—an ideal opportunity would be offered to learn the nature of the process of recovery. It must be borne in mind, however, that only in

certain cases does such a critical change in the patient occur. Of about 10,000 cases collected by Musser and Norris[24] the temperature fell by crisis in only about half. In the other cases it is difficult to determine with accuracy just when the change in the patient's condition occurs. It is therefore a mistake to think that in pneumonia we have a sudden change from susceptibility to resistance. More likely the process is a gradual one, and the marked change in the patient's condition occurs when the resisting factor, which increases gradually, reaches a degree sufficient to be effective. This factor of resistance may not be a single one, but the result may be due to a summation of several factors.

It has been suggested that the crisis represents a kind of anaphylactic reaction. It is known that following serum anaphylaxis there occurs a period during which the animal is in a refractory state. If the intoxication in pneumonia is due to peptone-like substances derived from the bacterial protein, it is possible that the crisis is a form of cumulative shock, following which the patient is refractory. Little is known, however, concerning such prolonged anaphylactic intoxication, and the nature of antianaphylaxis is still so obscure that it does not seem profitable to dwell longer on this theory, attractive as it is.

The fact that the crisis usually occurs in about seven days is strongly suggestive that the reaction is a true immunity reaction, since it is about in this time that antibodies appear in the blood in their maximum concentration, as we know from artificial immunization.

The view that recovery in pneumonia is due to the production of immune substances presupposes that at the end of an attack of pneumonia the patient is immune. We know from experience, however, that this is not so, or if immunity is present, it is of very short duration. I have seen a patient return to the hospital with a typical attack of pneumonia two days after discharge from a previous attack. Moreover, it is well known that a person may have repeated attacks; in fact, one attack seems to render a person more susceptible. It is quite possible, however, that the relative natural immunity of man requires

only a very slight assistance in the shape of acquired humoral immunity in order to render the body able to overcome the infection, and following this the immune bodies may very quickly disappear from the blood. The attempts to demonstrate the appearance of known immune substances in the blood during and following an attack of pneumonia have not previously been very successful. An increase of the ordinary bactericidal substances which act in conjunction with complement has not been proved. Most observers have found that the pneumococci grow quite well in the blood-serum of patients recovering from pneumonia, even in the serum of immunized animals.

It has been asserted that by combining the leucocytes and serum of pneumonia patients, or by using the defibrinated blood, definite differences may be demonstrated between the blood of normal persons and that of patients during or following the crisis. None of these experiments seem to me free from objections. There does not seem to be sufficient evidence for the conclusion that the recovery is due merely to an increase of opsonins, though Clough,[25] who has studied the phagocytic activity of the serum obtained after crisis or lysis in a series of eleven cases, found in six of these definite power of the serum to bring about phagocytosis of virulent pneumococci. In two of these cases the serum was tested before crisis and showed no such action. In his experiments, with one exception, the phagocytic activity was limited to the homologous strain. It has been stated by Rosenow that a difference exists as regards phagocytic activity between the leucocytes of patients with pneumonia and those of normal persons, though the results of others (Tunnicliff and Eggers) do not confirm these conclusions. Wolff[26] has attempted to show the increase of phagocytic power in the blood of pneumonia patients by making a composite curve combining the number of leucocytes with the opsonic index. We feel, however, that the errors in the usual opsonic technic are too great to justify his conclusions.

Of more importance are the experiments showing an increased protective power for mice of the blood of patients after recovery from pneumonia, as tested against known lethal doses

of pneumococci. Neufeld has shown that while normal human serum had no protective action, that obtained from certain patients following the crisis had a definite effect. Strouse,[27] and Seligmann and Klopstock,[28] however, failed to demonstrate such changes. Studies on this question were therefore undertaken by Dochez[29] on a series of cases. The method used was the following: Specimens of the patient's serum were obtained on various days both before and following the crisis. When possible, the organism against which the serum was to be tested was cultivated from the patient, either from the blood or sputum. In case the pneumococcus, when isolated, was of low pathogenicity, the virulence was raised by successive animal passages until a dose of 0.000001 c.c. of a broth-culture was sufficient to kill. Twenty-four-hour broth-cultures fresh from animals were used for infection, and the serum and varying quantities of the culture were mixed in the barrel of a syringe and immediately injected intraperitoneally. The appearance of protective substances in the blood could then be detected, as shown by the protocol from one such experiment (Table 1).

TABLE 1.—Protective Power of Serum of an Untreated Patient with Lobar Pneumonia at Varying Stages during the Disease

Quantity of Culture in c.c.	Quantity of Serum in c.c.	Virulence; No Serum	Control; Normal Serum	Serum Obtained								
				Three Days Before Crisis	Two Days Before Crisis	One Day Before Crisis	Three Hours After Crisis	Two Days After Crisis	Four Days After Crisis	Five Days After Crisis	Seven Days After Crisis	Eight Days After Crisis
0.1	0.2	—	—	—	24†	24†	32†	29†	42†	42†	32†	24†
0.01	0.2	—	—	—	42†	20†	75†	42†	42†	27†	—	32†
0.001	0.2	27†	42†	42†	29†	42†	66†	*	*	*	42†	42†
0.0001	0.2	42†	42†	66†	42†	66†	*	*	48†	42†	44†	42†
0.00001	0.2	50†	42†	32†	42†	42†	*	*	*	42†	66†	45†
0.000001	0.2	45†	43†	42†	47†	66†	*	*	*	*	45†	96†

* Animals protected as shown by survival. † Number of hours before death of animal injected.

The serums from fourteen cases of pneumonia were so studied. In ten of these the serums were tested against homologous organisms. Of these ten cases all but one showed at some time the appearance of protective substances in the blood. Of the

serums from four cases tested against stock cultures, only one showed any protective power. The amount of protection was never very high, though in some instances 0.2 c.c. of serum were able to protect against one thousand times the minimal lethal dose. The time of appearance of the protective substances varied somewhat, though in seven instances protective substances either appeared for the first time or showed a marked increase in amount at the time of crisis or, in case of lysis, during the period when the symptoms were abating. In two cases the serum taken during the period of defervescence exhibited little or no power of protection, even against homologous strains, and it was not until some time later, in one case sixteen days, that the presence of protective substances in the blood was demonstrated.

Clough[24] later carried out a similar set of experiments, and in nine out of twelve cases the serums after crisis or lysis showed definite protective power against homologous strains. The technic used differed somewhat from that employed by Dochez and the results were not so striking, but show well that in most cases the serum acquires definite protective power.

These experiments are of great importance as showing, first, that in many cases, at least, protective substances appear in the blood of patients recovering from lobar pneumonia, and second, that these protective substances in many cases are active only against the race of organism concerned in the infection. These experiments, however, do not yet establish that crisis or recovery in pneumonia is due alone to the development of these protective substances in the blood. As already stated, in certain cases they cannot be demonstrated. It is altogether probable, however, that they play some part in the final outcome. As to the nature of the substances which are most active, it is impossible at the present time to state.

Probably recovery from pneumonia occurs when the growth of the organisms is inhibited and their toxic effects neutralized. It is impossible to state which comes first. It is conceivable that if the toxic effects of the bacteria are neutralized, the body is readily able to cause their destruction, since it is possible that

pathogenicity depends entirely on toxicity. There is some evidence, as I shall show, that immune serums are antitoxic. With the present technic it has not been possible to demonstrate increase of antitoxic power of the patient's serum during the crisis.

In the immune-body theory of the crisis, the local lesion is left entirely out of consideration. It is quite evident that in pneumonia we are dealing, not merely with a septicæmia, but with a condition in the lung which has a very important bearing on the termination. The involved portion of the lung forms a solid mass in which are growing numbers of micro-organisms. In each alveolus are fibrin, leucocytes, red blood-corpuscles and bacteria, and in the spaces free serum. Now it is known that as the process advances, the number of leucocytes becomes greater and greater. Resolution finally occurs almost certainly as a result of this increase and associated breaking down of the leucocytes, and with this the setting free of ferments which bring the fibrin into solution. The fact that this does not occur earlier is due to the overbalancing of the leucocytic ferments by the antiferments of the serum, and the lytic ferments become active only when the relation between leucocytes and serum is in favor of the former. It is conceivable that recovery only ensues when such a balance occurs and when, with the solution of the fibrin, tension is relieved and there is an outlet for the exudate. Instead of the surgeon inserting a knife, nature does the work by injecting a ferment.

It is quite probable, moreover, that during resolution other factors than the purely mechanical are at work. With the solution of the exudate, numerous substances are formed which have a direct destructive action on the bacteria. Such substances as the soaps of fatty acids, which are known to have such a destructive action, have been demonstrated in the resolving lung by Lamar.[30] Moreover, it is well known that during the growth of pneumococci outside the body, substances are formed in the culture medium which themselves are destructive. It is quite probable that such substances are being formed in the lung and they may aid in bringing about destruction of the

pneumococci. Against the view that crisis depends mainly on resolution of the exudate, however, may be brought the very evident and conclusive objection that they do not necessarily occur synchronously. Resolution may be long delayed, or resolution may be occurring in one part of the lung while the process is advancing in another.

That leucocytes play some part in recovery is rendered probable by the experiments of Klein and Winternitz.[31] They have shown that when rabbits are treated with benzene, a leucotoxic substance, the animals rapidly succumb to pneumococcus infection, whereas when they are treated with toluene, which is a similar substance but which has no effect on the leucocytes, no decreased resistance is seen. Whether the chief function of the leucocytes consists in limiting the local infection, in which they undoubtedly play a rôle, or in aiding in the development of a general immunity is not indicated by these experiments. Clinicians, however, have long been of the opinion that a low leucocyte content of the blood is unfavorable.

It is not unlikely that in recovery all of the factors mentioned play a part. The destruction of the bacteria in the lung lesion may depend on local factors quite different from those responsible for the destruction of the bacteria in the circulating blood. From present knowledge it would appear that the growth of bacteria in the blood is the most serious part of the pneumonic process, and it seems that this, at least, is influenced by the appearance of circulating anti-bacterial substances.

METHODS OF CURE

It has been known since 1891 that susceptible animals may be rendered resistant to the action of pneumococci by the injection of increasing and properly spaced doses of pneumococci, beginning with the dead organisms. Moreover, it was early shown that if a very small amount of the serum of the immunized animal is injected into a second animal, this animal for a short time is also immune. These experiments are so striking and fundamental that it is no wonder that various attempts have been made to prepare and use such serums

therapeutically. The clinical results, however, have not been convincing. Certain observations made principally by Neufeld and his collaborators, and other observations made in our own laboratory, suggest reasons why such results have not been satisfactory and methods for overcoming the difficulties.

Opinions have differed as to whether or not an immune serum produced by the injection of a given race of pneumococci into an animal is effective against all races of pneumococci. The first accurate studies on this problem were made by Neufeld and Händel.[32] They tested a so-called univalent serum against various races of pneumococci. While this univalent serum was protective in mouse experiments against fifteen strains studied by them, against other strains the serum had practically no effect. They decided that these atypical strains were not *Serum-fest* in the ordinary sense of the term, since the serum obtained during convalescence from one of the patients, from whom one of these organisms had been isolated, protected mice against the homologous strain and also against one of the other atypical strains, but did not protect against the typical strain. They then produced an immune serum against one of the atypical strains to see whether all atypical strains could be affected by this immune serum, but found this not to be true. In their further studies they found that the second immune serum, which they called *Serum Franz*, protected against only three of the atypical strains isolated by them, but failed to protect against three other strains. These latter three strains they further showed to be individual in their reactions. Neufeld and Händel did not have access to a large number of patients with pneumonia from whom to obtain cultures, and could not determine the frequency of occurrence of atypical types, nor could they make extended studies on grouping of the organisms on a biological basis, though from their studies the possibility of making such a grouping was most evident.

With the opening of the Hospital of the Rockefeller Institute in October, 1910, patients suffering from lobar pneumonia were admitted for treatment and study, and an extended study was commenced of the pneumococci obtained in these cases. An

immune serum was prepared by injecting a horse with a culture of pneumococcus obtained from Professor Neufeld, the same race he had employed in the production of his immune serum. The protective power of this serum for mice was tested against a number of races of pneumococci cultivated from a series of cases of pneumonia. A report by Dochez[33] gave a preliminary report of this study, indicating that this serum protected against only about half the races studied. It was therefore evident that if such a serum were employed therapeutically, an effect could be expected at the most in only about half of the cases treated.

Experiments[34] were then undertaken to determine whether it would be possible to make a biologic classification of pneumococci obtained from cases of pneumonia, based on their reaction to different serums in protection experiments. Rabbits were therefore immunized to each of the races which were not acted on by the horse-serum, which we have called Serum 1, and the protection afforded by these different rabbit serums against all the other races tested. A considerable number were found to show cross-protection, that is, a serum prepared by injection of one of the number acted on all the races of this group. A horse was then immunized to one of this group and the serum is called Serum 2. In this way it has been possible to divide the pneumococci obtained from cases of pneumonia into four groups. In Group 1 are included all the races against which Serum 1 is effective. In Group 2 are included all those against which Serum 2 is effective. Whether the races included in this group correspond with the organisms described by Neufeld as acted on by his immune serum *Franz* is not known at present. In Group 3 are placed all the organisms of the so-called *Pneumococcus mucosus* type. These organisms have very large capsules and produce a sticky exudate in animals. In Group 4 are included all the races against which Serums 1 and 2 are not effective and which, from their other properties, do not belong in Group 3. Animals may readily be immunized to any member of this Group 4, and the serum of the immunized animal is protective against the race used for immunization. In no instance, however, has this serum been found effective against any other

race of this group or against the organisms of the other groups. So far as cultural and morphologic characters are concerned, no constant group differences have been discovered between the members of Groups 1, 2 and 4. By means of the agglutination reaction, however, it has been found possible to group them in exactly the same manner as by protection experiments.

As previously stated, the members of Group 3 differ from the others somewhat in their morphologic and pathogenic characters. They differ further in the fact that while animals may be very highly immunized to them, the serum of such animals possesses no protective power; they induce active but no passive immunity. Studies have been undertaken by Hanes to learn

TABLE 2.—AGGLUTINATION OF PNEUMOCOCCUS MUCOSUS

Organism No.	Immune Serums Nos.								Normal Rabbit Serum
	19	26	42	54	68	96	I	II	
19	+	+	+	+	+	+	−	−	−
26	+	+	+	+	+	+	−	−	−
42	+	+	+	+	+	+	−	−	−
54	+	+	+	+	+	+	−	−	−
68	+	+	+	+	+	+	−	−	−
96	+	+	+	+	+	+	−	−	−
I	−	−	−	−	−	−	+	−	−
II	−	−	−	−	−	−	−	+	−

on what factor this failure to produce passive immunity depends. It was found that the serum of the immunized animals not only does not protect, but also has no agglutinating power. It has been known that certain encapsulated bacilli also fail to be agglutinated by immune serum. Porges,[35] [36] however, has shown that such bacilli are agglutinated by the serum of immunized animals, provided the bacilli are previously treated so as to destroy their capsules. This method was therefore employed by Hanes[37] in studying these cocci. Six typical races of *P. mucosus* obtained from cases of pneumonia were studied. The bacteria were treated with dilute hydrochloric acid and heated for fifty minutes at 80° C. (176° F.). The fluid was

then neutralized and the bacteria so treated tested for agglutination. Controls were made with members of Groups 1 and 2 treated in the same way. The results were definite and striking. Agglutination of all the six races of *P. mucosus* occurred promptly with all six immune serums obtained by inoculating each of a series of rabbits with one of these races. No agglutination of Pneumococcus 1 or 2 occurred with any of these serums, and *P. mucosus* was not agglutinated by either Serum 1 or 2. (See Table 2.)

These experiments show that, so far as tested, all the organisms of the *P. mucosus* type belong in one biological group differing from those of the other groups. In order to show the relation of these organisms to streptococci, the method of complement-fixation was employed. With this method there occurred a considerable amount of cross-fixation among the various races of pneumococci, including *P. mucosus*, but no cross-fixation was observed in testing the complement-fixing powers of *Streptococcus mucosus* or *S. pyogenes* serums. It therefore seems evident that *P. mucosus* is really a variety of pneumococcus, and that biologically it forms a distinct variety of this organism.

Further studies of the various members of the *P. mucosus* group, to see if any were affected by the immune serum *in vivo*, were all negative. None of the serums were able to protect mice, even against the homologous organism. These experiments and also observations of Gruber and Löhlein seem to indicate that the failure of such serums to protect is in some way related to the formation of the thick, mucoid capsules by these organisms. As soon as the bacteria commence to grow in the body, capsules are formed which prevent the action of the immune serum. By the methods employed by Dochez and Hanes, it has been possible to study the races of pneumococci obtained from a series of cases of pneumonia. The classification by protection and agglutination experiments of sixty-two organisms so obtained gave results as shown in Table 3. In every instance in which an organism could be placed by protection in one of the groups described, the agglutination reaction has corresponded.

The races placed in Group 4 have been called heterogeneous, since each race, so far as studied, appears independent, and no grouping of the members on a biologic basis, by means of protection or agglutination, is at present possible. Table 4 shows the results of the study of agglutination with these races.

Gillespie[38] has also made a study of the various races, using the method of agglutination of bacteria by acid, as introduced by Michaelis. The results also show certain group differences in the agglutination of the various races.

Recent observations by Rosenow indicate that by certain methods it is possible completely to change the characters of the organisms of the entire streptococcus-pneumococcus group, so

TABLE 3.—Classification by Protection and Agglutination

	Number	Per Cent.
Group 1	35	47
Group 2	13	18
Group 3 (P. mucosus)	10	13
Total typical	58	78
Group 4 (heterogeneous)	16	22
Total heterogeneous	16	22
Total number	74	

that one may be transformed into the other, even *S. hæmolyticus* into a typical pneumococcus and *vice versa*. It has long been thought that the various closely related bacteria must originally have had a common source and have become differentiated by processes of adaptation. It is remarkable, however, that the changes can occur in such a short period of time as shown by Rosenow, even though they are subjected to extreme changes in environment, as has apparently been done. Some experiments performed in my laboratory several years ago by Strouse indicated that sudden mutations might appear in this group. Important as these experiments are, they do not have an immediate bearing on the pneumonia problem, except as regards the origin of the infection.

As regards the course of the disease and the possibility of specific methods of cure, the possibility of transformation of type of the organism concerned is not significant. In all the studies of organisms obtained at different times from the same case, in no instance have there been any indications of a change in type; the type first isolated has always subsequently been found. Moreover, many of these strains have now been cultivated for a long time outside the body, both in artificial mediums and in repeated passages through animals, some of them for several years, and they have in all cases retained their original characteristics. The results of the present year are not included in Table 3. This year the largest number of cases has been due to organisms of Type 2. It is possible that the prevalence of cases due to the various types varies from year to year and in different places, which may explain the variation in mortality observed in different times and places. The mortality of our limited number of cases due to organisms of different types is shown in Table 5. The most striking fact is the low mortality due to organisms of Type 4. Further observations may possibly change our ideas with regard to the relative severity of cases due to organisms of the different types.

TABLE 4.—STRAINS OF HETEROGENEOUS GROUP TESTED AND RESULTS OF AGGLUTINATION

Serums Pneumococcus	Pneumococcus Strain Numbers													
	1	2	34	36	37	38	52	55	60	62	71	76	82	102
1	+	0	0	0	±	0	0	0	0	0	0	0	0	0
2	0	+	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	+	0	0	0	0	0	0	0	0	−	0
37	0	0	0	0	+	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	+	0	0	0	−	0
71	0	0	0	0	0	0	0	0	0	0	+	0	−	0
76	0	0	0	0	0	0	0	0	0	0	0	+	0	0
82	0	0	0	0	0	0	±	0	0	0	0	0	+	0
Polyvalent, 34, 38, 62	0	0	0	0	0	+	0	+	0	+	0	0	−	0

In addition to the fact that there are immunological differences in the pneumococci concerned in pneumonia, there is

probably another reason why the use of immune serum has not proved efficacious in the past. The method of employment of such immune serum has been to use small doses, from 10 to 20 c.c., usually given subcutaneously. Neufeld and his assistants, by titrating immune serum against varying doses of pneumococci and making injections into mice, have concluded that in order to obtain protective power a certain proportion of serum in relation to body-weight is required. This concentration they have called the *Schwellenwert* or threshold concentration, and from experiments on mice they estimate that for man the dose of serum employed by them must be at least 77 c.c. Undoubtedly, however, this *Schwellenwert* must vary enormously under different conditions, depending on the virulence of

TABLE 5.—MORTALITY IN PATIENTS INFECTED WITH VARYING TYPES OF ORGANISMS

Infection Type	No. Patients	Patients Died	Per Cent.
1	34	8	24
2	13	8	61
3	10	6	60
4	15	1	7
Total.....	72	23	32

the organism, the time the serum is given, etc. In any case their experiments indicate that the serum must be given in very much larger amounts than has hitherto been done, in order to obtain curative results. They concluded that such an antibacterial serum does not obey the law of multiple proportions. This is undoubtedly true for the conditions employed by them, namely, injections made separately in different parts of the body, and is also true for the conditions present in the therapeutic injections in man. Dochez has shown, however, that when the serum and cultures are mixed before injection, such a serum does obey the law of multiple proportions up to a certain point, but there is a maximum degree of infection against which no amount of serum, however large, is able to protect.

It would therefore seem that one of the factors of the protective mechanism must be supplied by the body, and that, when the infection is very great, a sufficient number of immune bodies may be supplied by the administration of serum, but the body cannot react to a sufficient extent adequately to supply this second factor. This suggests that in order to obtain results from serum, it should be administered early, before the infection has reached too extreme a grade, beyond which no amount of serum can be effective, and also offers a possible explanation of the fact that in certain cases, such as the one which I shall mention, Case 4, the serum seems to have absolutely no effect. The nature of this additional factor is not known. If, as previously stated, the serum owes much of its effect to its bacteriotropic power, the number and activity of the leucocytes may be the additional factor. That this factor may be stimulated is shown by the results of artificial immunization, whereby very much larger doses of bacteria are resisted than can be protected against by immune serum.

These experiments have indicated that for the successful employment of immune serum in pneumonia, it must be employed fairly early and in large doses, and a serum must be used which is effective against the variety of organism causing the infection. We have been able to produce a serum of very great efficiency against organisms of Type 1, and one effective against organisms of Type 2. So far it has been impossible to produce a serum effective against *P. mucosus,* and, for the reasons stated, it would only be practical to treat cases of pneumonia due to organisms of Type 4 with homologous serums. This is of less importance, however, since the cases due to these organisms are apparently of mild grade.

To employ the serum effectively in cases due to organisms of Types 1 and 2, it has been necessary to devise a method for quickly determining in each case the type of organism concerned. This has been done and the following method is employed. When a patient with pneumonia is admitted to the hospital, a culture is immediately made from the blood and also one from a portion of sputum coughed up from the lung, or,

when this is not obtainable, a culture is made directly from the lung by the insertion of a needle. This procedure seems to be without danger. When there are large numbers of organisms in the sputum, a culture may be obtained most rapidly by injecting the washed sputum into the abdominal cavity of a mouse. After four or five hours the peritoneal cavity may be washed out with salt solution and the cells thrown down in the

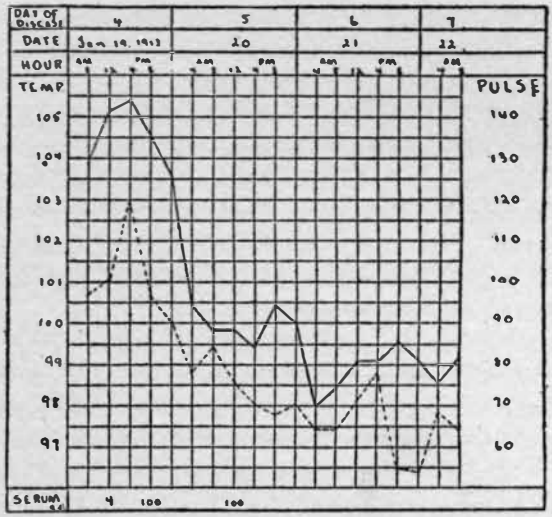

FIG. 1.—Case 1, E. S., No. 988, aged 20 years, admitted January 18, 1913, on the third day of the disease. Physical signs showed involvement of the lower left lobe and also signs of beginning involvement in the lower right lobe. Blood culture was negative. Pneumococci obtained from sputum were of Type I. The patient was quite ill on admission, respirations were labored and there was cyanosis. His condition was markedly improved on the day following the first administration of the serum, as shown by the temperature and pulse curves. Recovery was uneventful except that slight signs in the lungs persisted for several weeks.

centrifuge; a suspension of the organisms is thus obtained. In whatever way the culture is obtained, the agglutination test is at once applied. If the organism fails to agglutinate with either Serum 1 or Serum 2, it is, of course, useless to undertake serum treatment. If, however, one of the serums agglutinates the organism, treatment may be commenced at once with the appropriate one.

So far it has been possible to treat only a comparatively

small number of cases. Twenty-three cases have been treated with the serum. Of these, fifteen were due to organisms of Type 1 and eight to organisms of Type 2. The method of administration of the serum was the following: When admitted, the patient was given 0.5 c.c. of serum subcutaneously to discover if hypersensitiveness existed. As soon as the type of organism was determined, from 50 to 100 c.c. of the serum, diluted one-half with salt solution, were injected intravenously. The condition of the patient served as a guide in the later treatment. Usually the serum was not administered oftener

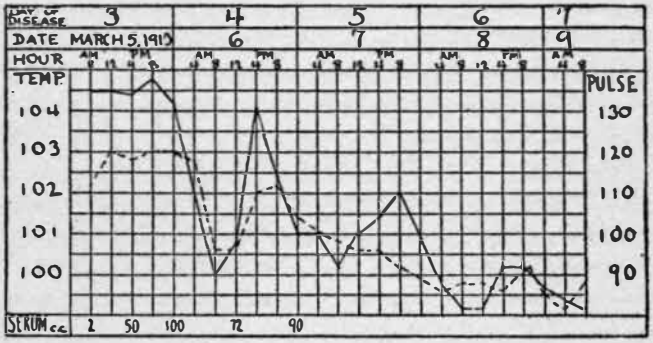

FIG. 2.—Case 2, B. G., No. 1175, aged 36 years, admitted March 4, 1913, on the second day of the disease. There was slight involvement at the base of the right lobe. Blood culture was positive. Agglutination test showed Type 1 organism. Treated with serum on day following admission. The signs of involvement in the right lower lobe became more distinct, but there was no apparent extension of the involvement beyond this lobe during the course of the disease. Following injection of the serum the patient's general condition improved. The patient complained of some urticaria and joint pains beginning on the twelfth day.

than every twelve hours. The patients treated received totals of from 190 to 460 c.c. of serum, except one, who received a total of 700 c.c. of serum. The patients treated were all seriously ill. They were treated in series. Every case infected with a pneumococcus of Type 1 or Type 2 was treated. Of the fifteen cases due to Pneumococcus 1, all of the patients recovered but one, and of the eight cases due to Type 2, two patients died. One of these patients objected to the treatment and would not allow its continuation, so it was not thoroughly carried out. When we consider that the mortality among the

untreated patients infected with Types 1 and 2 is very high (Table 5), the result is certainly not discouraging. It must also be remembered that so far most of our cases have been admitted late in the disease. Treatment was commenced on the third day in six cases, on the fourth day in five cases, the fifth day in six cases and the sixth day in six cases. If treatment can be commenced early, it is probable that the results will be even better than they now are. It is to be hoped that during this winter a large number of cases may be treated early in the disease. Effective treatment in the cases due to Types 1 and 2 should cut down the total mortality due to pneumonia very materially, as it has already done in our hands. I prefer at present, however, not to lay the main stress on the mortality statistics, since these are not large enough to be conclusive, but to refer to other criteria which we possess as to the efficacy of the serum.

Let us first consider the effect on the clinical course of the disease. Following practically all the injections, a reaction has occurred. The temperature usually rises and then falls, but does not necessarily remain low. In two instances the rise of temperature has been marked. In the other cases the rise of temperature following the injection was only a degree or so. In all the cases except the fatal ones, the serum has apparently had an ultimate favorable effect in lowering the temperature and shortening the course of the disease, though, of course, this is a very difficult matter of which to be absolutely sure. In no case was one injection of the serum sufficient to bring on a crisis.

Figures 1, 2, 3 and 4 show the effect of treatment on the temperature curves in certain of the cases. It is manifest that wrong impressions may be produced by the exhibition of temperature curves unless all the curves of a series are given. To avoid this difficulty, so far as possible, however, a curve from each group of cases is shown. Figure 1 represents the curve of a case in which the serum apparently had a marked effect, the temperature falling promptly and in a striking manner. Figure 2 indicates a temperature curve in a case in which

the temperature fell following the administration of serum, but several doses were necessary before the temperature remained low. Figure 3 shows the temperature curve in a case in which there were apparent effects of the administration of serum, but after the administration of serum was discontinued, the temperature curve rose and only fell after further large doses of serum were administered. In Figure 4 is given a temperature curve in which the serum had apparently no effect and the patient died on the seventh day.

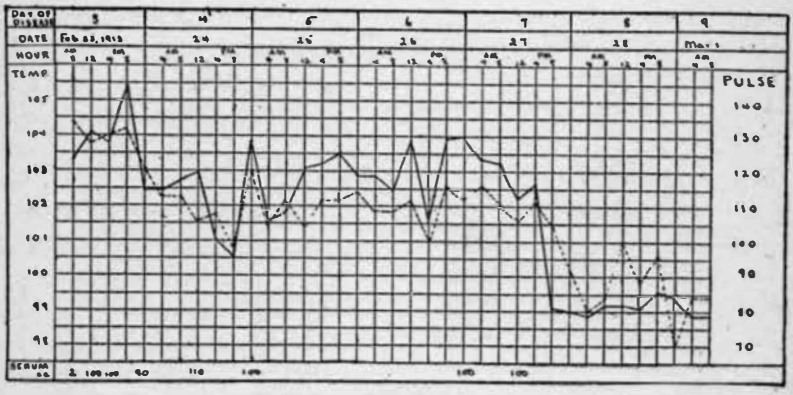

FIG. 3.—Case 3, M. L., No. 1091, aged 30 years, admitted February 22, 1913, on the second day of the disease. Blood culture showed a growth of pneumococcus, Type 1. Physical signs indicated involvement of the right lower lobe with slight involvement of the left lower lobe. The patient's condition improved following the treatment and the signs in the lungs became less well marked. On the sixth day, however, the patient's condition was again worse, the temperature was higher and the pulse more rapid, so that treatment was commenced again, following which the patient's condition markedly improved and he made a rapid recovery except for symptoms due probably to serum sickness on the twentieth to the twenty-fifth days of the disease.

All the patients seemed to feel better following the injection of the serum, and in a number of cases the apparent lessening in the degree of intoxication was very manifest. When the treatment was commenced early, no extension of the involvement of the lung occurred. On the other hand, there was no special tendency in the treated cases for the lung lesion to resolve rapidly. If anything, there seemed to be a tendency for resolution to be delayed in these cases. This has been noted by others in certain cases treated by serum.

More important than the foregoing criteria, however, as indicating an effect of the serum, are the following observations, since they have depended solely on objective procedures: First to be mentioned is the effect of the serum on the organisms in the blood. In ten cases pneumococci were isolated from the blood before the treatment was commenced. In all cases blood-cultures were made before each treatment; and in all of these ten cases after one treatment and before the second (or within

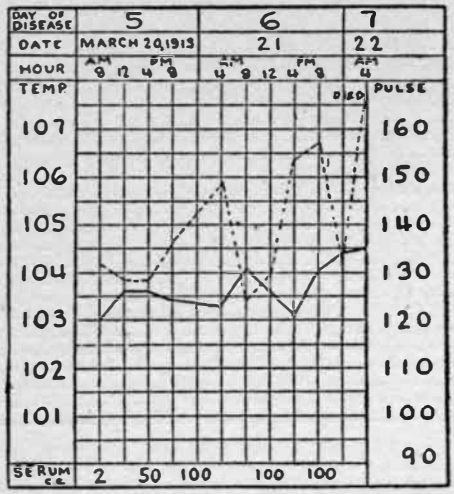

FIG. 4.—Case 4, F. W., No. 1090, aged 36 years, admitted March 19, 1913, on the fourth day of the disease. The patient was extremely ill on admission. There was involvement of the right upper and lower lobes. Blood culture and culture from the lung showed the presence of pneumococcus, Type I. There was some irregularity of the heart. Following treatment on the fifth day, the patient seemed better. The blood culture was negative. The pulse continued rapid and showed an extrasystolic irregularity, however, and the patient showed a very marked cyanosis. In spite of the active serum treatment, the patient died on the seventh day.

from eight to twelve hours) the blood had become sterile. The conclusion seems justified, therefore, that one large dose of active serum given intravenously is sufficient to sterilize the blood. It also seems certain that if the organisms are not present in the blood, the administration of the serum will prevent their invasion. Second, in a previous study of the protective substances in the blood-serum of patients with pneumonia, it has been shown that, as a rule, the appearance of

protective substances in the blood, when demonstrable, coincides rather sharply with the period of critical fall in temperature and the disappearance of symptoms. Before the crisis they are not present in the blood in any measurable degree.

A similar study has been made by Dochez of the protective substances in the serum in a number of the cases of pneumonia treated with serum. In all the cases studied, it has been possible to demonstrate the appearance of such substances in considerable amounts in the serum very shortly after the administration of one dose of the immune serum, even when this serum has been administered early in the disease, at a period when such protective substances are otherwise never present (Table 6). These

TABLE 6.—PROTECTIVE POWER OF SERUM B. G.; TREATMENT COMMENCED ON THE THIRD DAY

Quantity of Culture in c.c.	Virulence; No Serum	Quantity of Serum in c.c.	Serum Obtained			
			Third Day, Before Treatment	Third Day, 6½ Hours After Treatment	Fourth Day, After Treatment	Ninth Day, Five Days After Last Treatment
0.001	—	0.2	16†	*	*	† Days
0.0001	—	0.2	20†	† 5 Days	*	*
0.00001	24†	0.2	24†	*	*	*
0.000001	28†	0.2	24†	*	*	*

*Animal protected as shown by survival. †Time in hours before death of animal injected.

substances persist, and in case they play a part in the mechanism of recovery, as was concluded from the previous study, it is evident that their appearance indicates a favorable action of the immune serum.

The results obtained, therefore, from the clinical and laboratory study of this series of cases of pneumonia treated by the injection of large amounts of appropriate serum, seem to indicate that a method has been devised for the successful specific treatment of at least a portion of the cases of acute lobar pneumonia. Studies on the treatment of pneumonia by the intravenous injection of the Neufeld-Händel immune serum have been made by Beltz,[39] Weitz[40] and Geronne.[41] In none of these

series, however, were studies made of the type of organisms concerned in the infection of the cases treated, and in all of the cases the amount of serum administered was too small, judging from our own experience, to be of value.

The mode of action of the immune serums is still somewhat obscure. It is quite evident that there is an antibacterial action, inasmuch as the bacterial invasion of the blood is prevented. The action on the local lesion, however, is less evident. It is probable that here the organisms can less readily be reached by the serum, though apparently in most cases the growth of the bacteria in the margins of the lesion has been inhibited, as shown by prevention of spread of the process. In addition to the antibacterial action, the clinical cases show a definite change as regards intoxication. It is possible, of course, that this is entirely associated with the destruction of the organisms. Certain experimental work, however, has indicated that the serum may possess some antitoxic effect.

When the immune horse-serum is added to the toxin obtained by dissolving pneumococci in bile, it is found that such a serum has a well-marked effect in inhibiting the hæmolysis of sheep corpuscles by this toxin. When it is added to the toxin in doses of 1 c.c. of serum to 4 c.c. of toxin and placed at 37° C. (98.6° F.) for one-half hour, the effect of the toxin when injected into guinea pigs is diminished or entirely prevented. These effects of the immune horse-serum are much less specific, however, than are the protective or antibacterial effects, since the Serum 1 acts on both Toxins 1 and 2, though most markedly on Toxin 1. Serum 2 also shows a similar diminution in specificity in antitoxic action as compared with antibacterial action. These experiments offer some evidence that part of the effect of the immune horse-serum is antitoxic, admitting, of course, that the toxic substances obtained from the bacterial bodies are responsible for the intoxication.

An effort has been made to obtain a pure antitoxic serum by the injection of the toxin alone into animals. Rabbits have been immunized by the repeated injection of this toxin and a sheep also has been highly immunized. The sheep-serum and also the

immune rabbit-serums show antitoxic power, as indicated by antihæmolytic action and also by neutralizing effect on the toxin, as tested by injection into guinea pigs. The effects, however, are less marked than those of the antibacterial horse-serum. These antitoxic serums are also protective against the living organisms, as shown by tests on mice. The protection, however, while fairly high, is less well marked than that of the horse-serums. The protection is not so specific as that of the horse-serum, since the serum produced by the injection of toxin prepared from an organism of Type 1 is not only protective against this organism, but also, though to a less extent, is protective against organisms of Type 2.

The interpretation of these experiments is attended with much difficulty. It is possible that these antitoxic serums may show protective power only because living organisms were introduced, since in the preparation of the toxin one cannot be positive that all organisms have been destroyed. These antitoxic serums, however, possess no power to cause agglutination, and this fact, together with their lessened specificity, suggests that we are dealing with serums which owe their power to other properties than those of the antibacterial serums. The experiments are of importance, moreover, since they indicate that immunity may be obtained against the substances contained in the bile extracts, and since the essential criterion of a toxin in the Ehrlich sense is that immunity may be obtained to it. Much more work will have to be done before such antitoxic serums should be employed therapeutically.

It is probable that in the future it will be possible to obtain the same therapeutic effects by the injection of much smaller amounts of serum than are now employed. Work now being carried on by Avery shows that the immune substances are all contained in the globulin fraction of the serum, and methods are now being devised for the concentration of the serum, so as to avoid the injection of a very large part of the serum protein which contains no immune substances. In this way it will probably be possible to avoid serum sickness, which has occurred in a number of our patients in from ten to twelve days after

the administration of the large amounts of horse-serum. This serum-sickness, while causing some discomfort to the patients, is not of any serious import, so far as we know.

It may be possible later to produce polyvalent serums that are efficacious. At present, however, and until the value of the special serum in the cases due to organisms of Groups 1 and 2 is unquestionably determined, it does not seem to be advisable to make such attempts. The objection is frequently raised that this method of treatment is very complicated. One may reply to this that so is the treatment of appendicitis.

At the present time I can do no more than mention the efforts along other lines that have been made to produce curative results by specific measures. Most important studies were made by the late Professor Hiss in the treatment of bacterial inflictions by means of leucocytic extracts. So far as concerns pneumonia, the results of experiments on animals are not very convincing, but the brief clinical report of cases of pneumonia treated, as stated in the article published since his death, seems extremely favorable and promising. It is to be hoped that study along this line will be continued.

Lamar has devised a method for the local treatment of pneumococcus infections. He has shown that immune serum has a much greater effect on pneumococci treated with sodium oleate solutions than on cocci simply washed in salt solution. This action of the soap, however, is inhibited if the serum be added first or mixed with the soap solution before treating the bacteria. The inhibiting action of serum, however, may be prevented by the addition of small amounts of boric acid, as Liebermann and von Fennyvessy have shown. By combining the soap, serum and boric acid in proper concentrations, Lamar has found a mixture that is much more efficacious in the local treatment of experimental pneumococcus infections than is serum alone. The treatment of local infections, as meningitis, with such a mixture, using serum effective against the race of organisms concerned, should be tried in all suitable cases. It is doubtful, however, whether such a mixture can be employed intravenously.

A final possible method which may be rendered practical in the treatment of pneumonia is along the lines of chemotherapy, as laid down by Ehrlich. It has been generally held that such a method of treatment may be of value in protozoan infections, but not in diseases due to bacteria. Morgenroth[42] and his co-workers, however, have shown that a derivative of quinine—ethylhydrocupreine—has a specific action on pneumococcus infections in mice, and Wright has shown that this drug is bactericidal for pneumococci in the test-tube. The drug has been employed clinically, but cases of amblyopia developing have indicated that the toxic dose in man too closely approaches the curative dose to permit the safe administration of the drug. It is possible, however, that with further study, its toxic properties may be reduced without lessening its curative effect.

CONCLUSIONS

Much obscurity still exists concerning the mode of natural infection in pneumonia, though by animal experimentation many facts in regard to it have been discovered.

The symptoms in pneumonia are probably due to toxic substances derived from the bacterial cells.

The outcome is dependent on the virulence of the organisms concerned and on the ability of the body, first to limit the local infection, and second, to prevent the invasion of the blood by the organisms, as on the latter the outcome of the disease mainly depends.

Leucocytes probably play a part in the resistance, certainly as regards the local spread, and probably also to some extent as regards the general infection.

The most important part in prevention of the general infection is probably played by immune substances contained in the serum. Such substances are present in the serum of immunized animals.

Pneumococci differ in regard to their immunological reactions and on these they may be divided into several groups.

In order to use immune serum effectively in treatment, as in prevention, it is necessary to employ the serum effective against

the group of organisms to which the special organism causing the infection belongs.

Immune serums effective against two of the most important groups have been produced. This treatment has been carried out in a limited number of patients with promising results.

It is probable that the methods of application of such serums will be improved, and it is possible that the method may be combined with other measures directed toward other factors which are important for the outcome. In any case, facts regarding the nature of the disease are being disclosed, and the outlook, at least for lessening the ravages of this dreadful disease, is encouraging.

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