

## IMMUNOLOGICAL STUDIES IN PNEUMONIA.

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The immunological processes of pneumonia have been studied within recent years by many observers, among whom may be mentioned Klemperer,<sup>1</sup> Römer,<sup>2</sup> Neufeld and Haendel,<sup>3</sup> Wadsworth,<sup>4</sup> Dochez,<sup>5</sup> and Chickering.<sup>6</sup> It has been established that a certain type of immune body, namely, the agglutinins, tends to appear in the blood shortly after recovery from the disease, and to persist therein for a variable period of time. During the course of the disease this antibody has only exceptionally been found. In addition, specific protective substances have been found in convalescent serum.

During the past winter we have approached the problem with the help of a method not previously employed in the study of this disease. Essentially, the method consists in passively sensitizing guinea pigs with serum taken from cases of pneumonia at various stages of the disease, and during convalescence. Passive sensitization of guinea pigs with human serum had previously been practised by Bruck<sup>7</sup> and Schloss in the study of food idiosyncrasies. The application of the method to the study of infectious diseases has been attempted in the case of tuberculosis.

<sup>1</sup> Klemperer, G., and Klemperer, F., *Berl. klin. Wchnschr.*, 1891, xxviii, 833.

<sup>2</sup> Römer, Experimentelle und klinische Grundlage für die Serumtherapie der Pneumokokkeninfektion der menschlichen Cornea (*Ulcus serpens*), Wiesbaden, 1909.

<sup>3</sup> Neufeld, F., and Haendel, *Arb. a. d. k. Gsndtsamte*, 1910, xxxiv, 166, 293.

<sup>4</sup> Wadsworth, A., *Am. Jour. Med. Sc.*, 1904, N. S., cxxvii, 851; *Jour. Exper. Med.*, 1912, xvi, 54.

<sup>5</sup> Dochez, A. R., *Jour. Exper. Med.*, 1912, xvi, 665.

<sup>6</sup> Chickering, H. T., *Jour. Exper. Med.*, 1914, xx, 599.

<sup>7</sup> Bruck, C., *Arch. f. Dermat. u. Syph.*, 1909, xcvi, 241.

One of the most serious difficulties associated with this method lies in the fact that human serum is extremely toxic to guinea pigs. After a considerable amount of preliminary investigation we finally adopted the use of inactivated serum, as recommended by Schloss<sup>8</sup> and others. For the sake of uniformity we have, in all except the first studies, regularly injected 4 cc. of inactivated serum into the subcutaneous tissues. After a period of time varying from two to six days, the guinea pigs have been tested to determine whether or not they presented an induced hypersusceptibility to the pneumococcus. The method of performing this test differs from that hitherto observed in this type of experiment. The routine test, as usually performed, consists in the introduction of the antigen either into the peritoneum or into the veins of the sensitized animal. In the study herein described that method was not practicable.

The antigen was prepared in the following manner. Forty-eight hour cultures on Loeffler's serum medium (ox serum) were emulsified in distilled water, using 2 to 3 cc. of water for each tube, according to its size. The emulsion was shaken and autolyzed at 37° C. for two hours, and then at 60° C. for one hour.<sup>9</sup> With a very few exceptions, the organism employed was of Type I. In a few of the earlier experiments a Group III type (*Pneumococcus mucosus*) was used with success. It seems probable that this anaphylactic reaction is more general for the whole pneumococcus family than has been found to be the case in agglutination and protective experiments. This autolytic extract when injected intravenously into guinea pigs is primarily toxic, but the toxicity varies with different animals to a considerable extent, so that the reaction produced in the anaphylactic experiment is unreliable and confusing. As an alternative, the induced hypersusceptibility of the guinea pigs was tested by the Dale<sup>10</sup> method. This method consists in removing the uterus of the sensitized animal and suspending it in a bath of Locke's fluid. The uterine preparation traces a rhythmical curve, which changes strikingly upon the addition of antigen to the bath fluid. There is an immediate, or slightly delayed, response in the nature of a sharp

<sup>8</sup> Schloss, O. S., *Am. Jour. Dis. Child.*, 1912, iii, 341.

<sup>9</sup> Dochez, A. R., and Gillespie, L. J., *Jour. Am. Med. Assn.*, 1913, lxi, 727.

<sup>10</sup> Dale, H. H., *Jour. Pharmacol. and Exper. Therap.*, 1912-13, iv, 167.

contraction, which may be followed either by prompt relaxation or by tetanus of the muscle. In testing the pneumococcus autolysate, it is necessary to keep in mind certain important factors. In the first place, the antigen of itself possesses in a certain degree the property of stimulating a contraction, even when added to the normal uterus. In this respect, however, it does not differ from normal sera, such as horse serum or ox serum, which have the same effect upon the normal guinea pig uterus. An essential feature of the method therefore consists in the use of the antigen in such amounts as do not, of themselves, suffice to induce contractions in the control uterus.

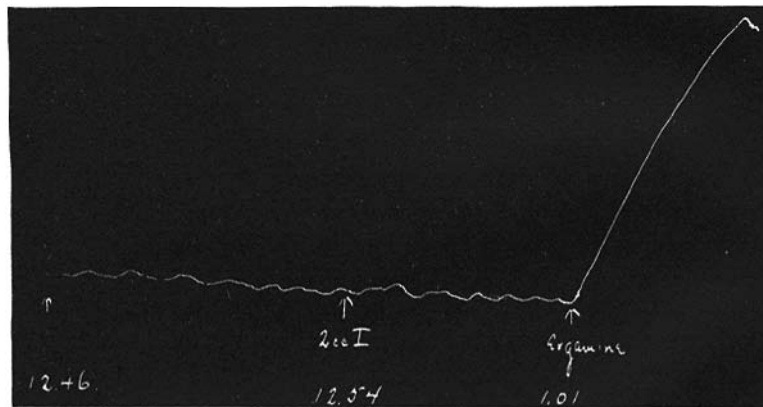
In the present study we have made a practice of using approximately one-quarter of that amount which just fails to induce a response in the normal uterus. As a rule, 1 cc. of extract, which has always been uniformly prepared throughout the series, is added to a bath containing 150 or 200 cc. of fluid. It is a necessary precaution to test each sample of autolysate, in order to be sure that its primary toxicity is not greater than is assumed for this amount. Occasionally it has been found necessary to diminish the amount of antigen used in the tests on account of an unexpectedly high primary toxicity. The other technical details of the method are not such as to require special discussion in this connection.

#### OBSERVATIONS.

Blood has been taken and tested from a series of cases which presented themselves, clinically, as pneumonias, as well as from a series of controls which were either normal individuals or cases of other forms of disease; such as typhoid, rheumatism, etc. In every case of pneumonia the sputum was cultured in order to determine the etiological organism, and in some cases blood cultures as well were made; in all, 20 cases of pneumococcus, 2 of *Streptococcus mucosus*, 2 of streptococcus, and 1 case of *Bacillus mucosus* were determined. In 9 cases the organism was not definitely determined.<sup>11</sup>

<sup>11</sup> The sputum examinations and the blood cultures were carried out for the Mt. Sinai cases by Dr. H. Celler, to whom we wish to express our indebtedness. He reported on sputa only when the plates presented practically pure cultures of the organism.

Leaving out of consideration the cases of questionable etiology, none of those cases which were bacteriologically identified as other than pneumococcus infections, with the exception of one case of *Streptococcus mucosus* infection, gave a positive response. Of the twenty cases of pneumococcus infection, there were only two which did not at some stage of their course present a positive response. Of the questionable cases, in which the bacteriological diagnosis could not be obtained, there were four in which the clinical pictures seemed, with certainty, to exclude the possibility of a pneumococcus infection. In none of these four was a positive result obtained. In



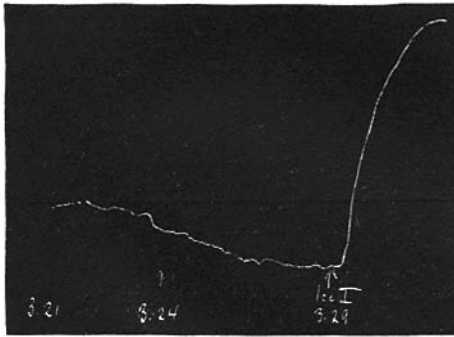
TEXT-FIG. 1. Guinea pig 583. Received 4 cc. of inactivated serum derived from a case of acute articular rheumatism. Tracing, taken on fourth day, shows no reaction to pneumococcus antigen, although there is a sharp response to ergamine.

the other cases of that group it was not possible to decide, with any degree of certainty, whether or not the infection was due to pneumococcus. The results obtained in these cases were in some instances positive, and in others negative.

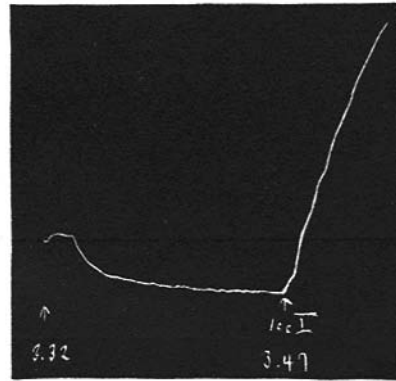
The time of occurrence of reaction differed from that observed by others in the study of the agglutinins. In all of the cases in which it occurred, it appeared before the temperature had reached normal. In only two of these cases was it also present during convalescence, and then in far less marked degree. The reaction was present within

two days of the onset of the disease in one case, within three and four days in certain other cases. When found, it persisted almost unexceptionally up to the time of crisis; or if the disease ended by lysis, it persisted throughout the lysis. Two of the cases ended fatally; in one of these it was found throughout the disease; in the other, which died upon the day of admission, it was not found. Those two cases in which it persisted after crisis did not appear to differ according to any clinical criteria from those in which it disappeared. In none of the control cases was the reaction induced.

The conditions which have been described may fitly be illustrated by a number of typical tracings. Text-fig. 1 shows a tracing taken from



TEXT-FIG. 2.

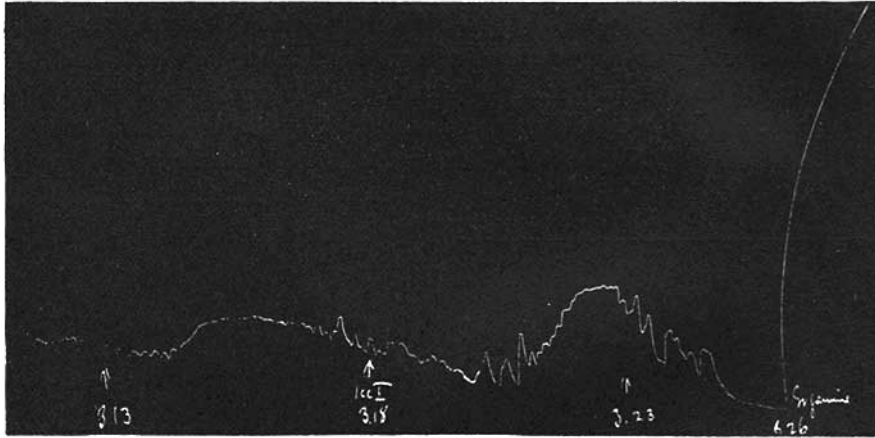


TEXT-FIG. 3.

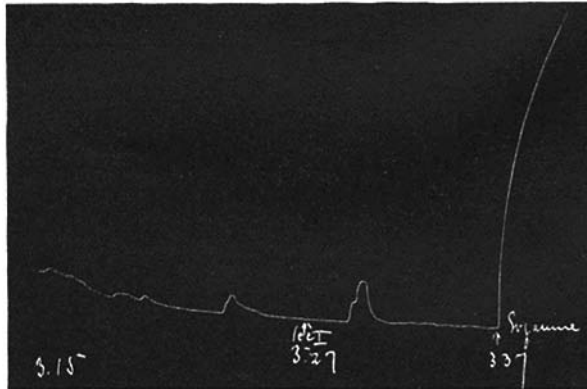
TEXT-FIGS. 2 AND 3. Guinea pig 565. Received 4 cc. of inactivated serum derived from a case of acute lobar pneumonia due to pneumococcus, five days after onset. Tracings, made on fifth day, show a sharp response to antigen.

the uterus of a guinea pig which had received 4 cc. of the inactivated serum of a patient suffering from an attack of acute articular rheumatism. The temperature at the time the blood was taken was 103° F. It is evident that there is no response to the application of the pneumococcus antigen. Text-figs. 2 to 7 illustrate the course of a case of lobar pneumonia. The patient was admitted to Mt. Sinai Hospital on the fourth day of the disease. The onset had been acute and was marked by a chill. Upon admission, there were characteristic signs of consolidation of the middle and lower lobes of the right

lung. The temperature was 105.4° F., and the leucocyte count 15,200, of which 85 per cent were polynuclears. The temperature varied only slightly for four days, and on the fifth day fell by uninterrupted crisis. Recovery was uneventful from the crisis. The clinical picture was that of a lobar pneumonia of moderate severity.



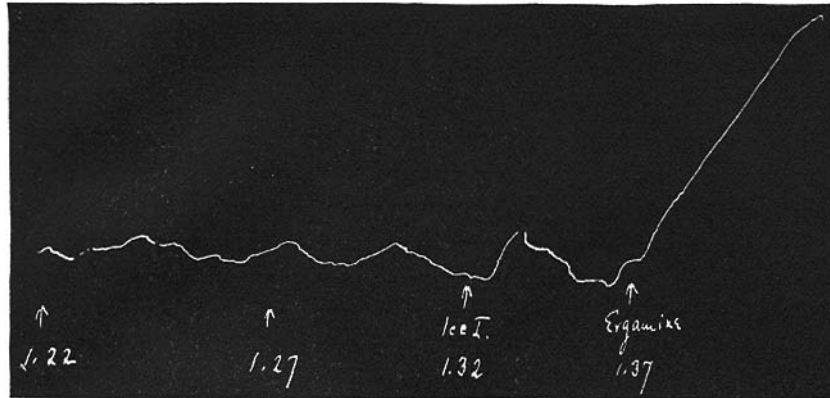
TEXT-FIG. 4.



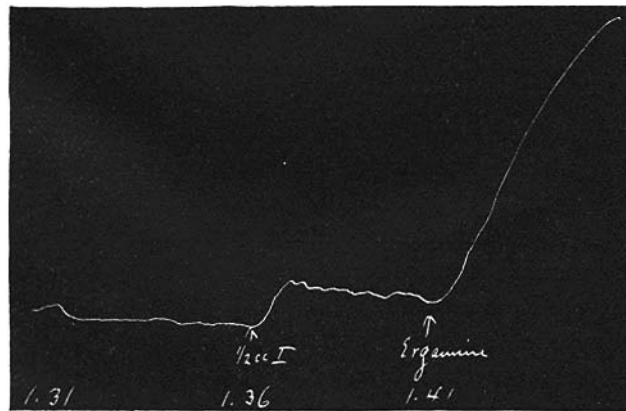
TEXT-FIG. 5.

TEXT-FIGS. 4 AND 5. Guinea pig 572. Received 4 cc. of inactivated serum derived from same case during crisis. Tracings made on third day. There is no response to antigen. Ergamine produces a sharp contraction.

Pneumococci were found in the sputum, but not in the blood. Blood was taken for the anaphylactic tests on three occasions; namely, during the height of the disease, during the crisis, and on the third day after the crisis. On each occasion the blood was subjected to the same procedure. The serum was allowed to separate from the clot,



TEXT-FIG. 6.



TEXT-FIG. 7.

TEXT-FIGS. 6 AND 7. Guinea pig 588. Received 4 cc. of inactivated serum derived from the same case three days after crisis. Tracing taken on fourth day. One horn shows a minimal response to the antigen. Both horns respond well to ergamine.

and was then centrifuged to free it of cells. It was then inactivated for one-half hour at 56° C. Of this serum, 4 cc. were injected subcutaneously into a female guinea pig weighing between 250 and 300 gm. After an interval of from three to five days the guinea pig was killed, and the uterine horns were immediately removed and suspended, according to Dale's method, in a bath containing 200 cc. of Locke's fluid. The reaction to the pneumococcus autolysate was tested, and if this was negative, ergamine was subsequently added to establish the normal reactivity of the muscle. The figures show that the blood taken during the course of the disease had effectively sensitized the uterus to the pneumococcus antigen. The blood taken during the crisis and after the crisis failed to induce this result, although the muscle evinced a normal reactivity towards ergamine.

Of interest in connection with our own results are those described by Seligmann.<sup>12</sup> This author reports that he attempted passive sensitization of guinea pigs with postcritical and precritical serum, but that he failed to achieve any positive result. He gives no detailed protocols of his experiments, but describes his method, which differs in practically every detail from that employed in the present study. He sensitized by means of the intraperitoneal injection of 5 to 6 cc. of the human serum. We have almost regularly found this dosage fatal for the guinea pig, and therefore inactivated the serum and gave it subcutaneously. The interval between passive sensitization and the toxic injection in Seligmann's experiments was only one day, whereas we allowed three or more days for the absorption of the human antibodies. His second injection consisted of a suspension of a pneumococcus culture, while we used the soluble autolysate. Finally, he employed the intravenous injection, whereas we tested by the Dale method. Without further analysis, it must be clear that any one of these differences in technique would suffice to account for the divergence in results.

#### DISCUSSION.

The question which immediately arises naturally involves the nature of the substances in the sera concerned in the production of

<sup>12</sup> Seligmann, E., *Ztschr. f. Immunitätsforsch.*, 1911, ix, 79.



this reaction. There is practically no doubt that they must be grouped among the immune substances, the so called antibodies. There are no other substances, so far as we know, which passively sensitize a guinea pig's uterus. Apparently they are specific in character. Whether or not they have protective value is a problem which has not been approached. The fact that their incidence in the disease differs strikingly from that of the agglutinins and the lytic antibodies, suggests the likelihood that they may be different from those substances. In fact, there is some ground for the belief that hemolysins, agglutinins, and precipitins may not be identical with anaphylactic antibodies, judging from the fact that the first group of substances does not always run parallel with the second in amount.

Such a conclusion, however, does not necessarily follow. It is possible that exactly the same substance acts as agglutinin in the test-tube and as sensitizing substance in the guinea pig, and that the apparent difference in the effects observed by the two methods is due simply to the coexistence of varying amounts of antigen. According to this view, agglutination is inhibited in the precritical serum through the presence of antigen, a factor which does not, however, inhibit passive sensitization. The postcritical serum, on the other hand, might contain less antibody, so as to impair its sensitizing value, but might at the same time be almost free from antigen, so as to permit of the demonstration of agglutinins in the test-tube.<sup>13</sup>

The clinical material on which this work is based was obtained chiefly from the service of Dr. Libman at the Mt. Sinai Hospital, and in part also from the service of Dr. Brill at the Mt. Sinai Hospital, and from the service of Dr. Coleman in Bellevue Hospital, for whose cooperation we desire to express our thanks. We are also indebted to Drs. Cole and Dochez of The Rockefeller Institute Hospital for pneumococcus cultures and sera.

<sup>13</sup> The experiments on which this theory is based are included in a series of papers by Weil, R., *Jour. Immunol.*, 1916, i (in press).

## CONCLUSIONS.

The serum derived from human cases of pneumonia has been used to sensitize guinea pigs passively. As antigen an autolysate of a pneumococcus culture was employed. By means of Dale's method it has been possible to show with considerable regularity that the blood contains sensitizing antibodies during the course of pneumonia, but none after the crisis. Patients suffering from other diseases have failed to give this reaction, as have also normal individuals.