

INTRACUTANEOUS REACTIONS IN LOBAR PNEUMONIA *

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In 1915 Clough¹ reported intracutaneous reactions in lobar pneumonia patients. He prepared his antigen as follows: Cultures used were made from the lungs in cases of fatal pneumonia, with no statement as to type. From twenty-four to thirty-six hour old cultures in 5 per cent. glucose broth plus calcium carbonate were decanted off the carbonate, centrifuged, washed with salt, recentrifuged, the sediment taken up in a few drops of distilled water, dried, weighed, ground for three hours with sterile sand, extracted with 10 c. c. saline for each gram of dried material, incubated for eighteen hours; centrifuged at high speed for several hours until no more sediment came down; used in this form or precipitated with absolute alcohol. One gram of the dried culture residue yielded about 0.15 gm. dried alcoholic precipitate.

He used two methods of inoculation: first, allowing a drop of 1 per cent. solution of his antigen to dry on a scarification as for a von Pirquet tuberculin test; second, injecting a 0.25 per cent. solution intracutaneously, causing a painful inflammatory reaction subsiding after twenty-four hours. There was no appreciable increase in severity or time of the reaction in the pneumonias over the controls. The more dilute solutions gave discrete papules from 0.5 to 2 cm. in diameter with an ill-defined area of hyperemia. The actual measurements of the papules averaged a little larger in the pneumonias, but this was only slight and inconstant. He found no difference in reactivity early, at crisis, or later in convalescence. As to Clough's work, then, it may be said that he used a rather elaborately prepared antigen with practically negative results.

Weil² criticizes Clough's antigen for being too strong so that it produced reactions independent of any immunologic response. He used forty-eight hour old cultures on Loeffler's serum medium suspended in distilled water, from 2 to 3 c. c. to a tube, shaken, incubated two hours, and then heated to 60 C. for one hour. In a few early experi-

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1. Clough, P. W.: Johns Hopkins Hosp. Bull. **24**:295, 1913.

2. Weil, R.: J. Exper. M. **23**:11, 1916.

ments a Type III organism was used "with success," otherwise Type I was used exclusively. For the test, a sufficient amount of antigen was injected to produce a small wheal, that amount being between 0.1 and 0.2 c. c. All showed an immediate superficial ill-defined blush which was considered merely traumatic. In the negatives this faded within a few hours and nothing more developed. In the positives, within twenty hours a fairly well circumscribed area of erythema had developed with slight infiltration and elevation of the skin surrounding the point of puncture. This may be a true papule, persisting for forty-eight hours or even longer. Weil obtained no reaction during the course of the disease, but "after the subsidence . . . a considerable percentage of cases do present reactions." These exceptionally appeared twenty-four hours after crises, but more commonly later even after two, three or more weeks. The controls "may or may not present a reaction dependent presumably on their previous sensitization by the pneumococcus or an allied organism." A few cases of pneumonia gave no reaction at any time. In summary, Weil obtained positive reaction in a "considerable percentage of cases" after crisis, and in some controls. The reaction which he considered positive reached its maximum within twenty hours, though it might persist for two days or more. He used a relatively simple incubated suspension of pneumococci in distilled water.

Steinfeld and Kolmer³ prepared an antigen in much the same way, except that they suspended in saline instead of distilled water and omitted the incubation. Separate preparations of Types I, II and III were made. The final dilution was such that 1 c. c. contained 2 billion cocci, that is 200 million were injected for a test. They found that at the end of twenty-four hours all tests showed a zone of hyperemia. By forty-eight hours a definite papule had appeared in the positives, with an erythema greater than 1 cm. in diameter and accompanied by slight edema. This reaction persisted for four to five days, and gradually cleared. There was no instance of a pustule formation. In the negatives the erythema had largely cleared by forty-eight hours. Of nineteen cases of clinical lobar pneumonia, six gave positive reactions with one or more antigens. But in none did the type or types found in the sputum exactly correspond with those shown by the skin reaction. For instance, one case showed Type I in the sputum and reacted to Types I and III; another case showed Types I and II in the sputum and reacted to Types II and III, etc. The earliest reaction appeared on the ninth day of the disease, or three days after crisis, and the latest on the thirty-ninth day after onset. "A large number of tests with healthy persons and patients suffering from chronic ailments not involving the chest" were negative. The

3. Steinfeld, E., and Kolmer, J. A.: *J. Infect. Dis.* **20**:344, 1917.

authors conclude that there is no constant relation between the reaction in the skin and the type of organism found in the sputum, and think it probable that the allergic reactions to pneumococcus protein are of a more general character than the agglutination reactions.

In 1918 Weiss and Kolmer⁴ report an intracutaneous test with a preparation of "pneumotoxin" as opposed to the "pneumoprotein" of the former tests. Perhaps the most important difference in their antigen over those used previously is that at no time is the product heated to a temperature which would endanger the thermolabile substances. They centrifuged eighteen hour broth cultures of Type I pneumococcus, washed once with saline, took up in 5 c. c. saline and dissolved the cocci in 1 c. c. of a 2 per cent solution of sodium choleate. The total volume was made up to 30 or 40 c. c., centrifuged to remove undissolved pneumococci and 0.2 per cent. tricresol added. The "control fluid" was treated as above, except for the inoculation with pneumococci. The minimal lethal dose for a 250 or 300 gm. guinea-pig was found, and the antigen diluted so that 0.1 c. c. contains one twentieth the minimum lethal dose (M. L. D.). This amount was injected intracutaneously for the test. The preparation deteriorated rapidly and was made fresh every day. The authors found the reaction in "all respects similar to that described for the Schick test with diphtheria toxin." Cases just prior to the crisis showed a strongly positive reaction marked by vesiculation. Others showed a definitely circumscribed area of edema and erythema gradually fading, leaving a scaling zone of brownish pigmentation. The "control fluid" and heated "pneumotoxin" remained negative. Pseudoreactions involving the entire arm with diffuse erythema and a definite area of edema never persisted longer than forty-eight hours. Of thirty-eight cases of "acute lobar pneumonia" all reacted positively; of sixteen cases of "lobar pneumonia convalescents" five reacted positively; of five "doubtful" cases ("alcoholism and tuberculosis with possible superimposed pneumonia"), one gave a positive reaction; and of twenty-three controls all were negative.

In summary, they state that the tests with "pneumotoxin" were elicited as early as the fifth day (the earliest under observation) and as late as the thirteenth day of the disease. In general, the test was positive throughout the toxemia.

Further in the paper the authors took up the question of the type specificity of this reaction. The sputum from ten cases which reacted positively to their type I "pneumotoxin" was examined for type organism. Six of these showed Type IV and four showed Type I. They consider that "sensitization to the toxin presumably takes place with its liberation (by action of normal enzymes on pneumococci normally

4. Weiss, C., and Kolmer, J. A.: J. Immunol. 3:395, 1918.

localized in the lung alveoli) at the time of the prolonged chilling due to exposure." They feel that the method is not as yet of value in serologic type diagnosis.

In these two papers Kolmer shows that his heated saline suspension of pneumococci gives positive reactions after crisis which are not specific for type, and that his unheated bile salt solution of pneumococci gives positive reactions before as well as after crisis which are also not specific for type. It is rather a surprise after having compared this latter reaction to the Schick test in diphtheria, to find that the presence rather than the absence of a reaction is considered specific. The authors' theory of early sensitization against their "pneumotoxin" is certainly open to question when we consider the absence of any sensitization necessary in the case of the positive Schick reaction, and also the lack of specificity in the toxic radicle as shown by Vaughan's work.⁵

EXPERIMENTAL WORK

It was hoped that by the study of a considerable series of pneumonia cases and normal and diseased controls, using antigens made from the various types of pneumococci (homologous and heterologous) prepared in various ways, additional information might be obtained concerning the rôle played by allergy in pneumonia with especial reference to crisis. Furthermore, it was hoped that by varying the method of preparation and dose of antigens prepared from the various types of pneumococci, it might be possible to obtain antigens sufficiently delicate to indicate the type of infection present. If this reaction should prove to be positive in the early stages of the disease, it might furnish an earlier, quicker and simpler means of type determination than can be obtained by the present method of mouse inoculation, thus making it possible to institute specific serum therapy in suitable cases with less loss of time than is at present possible.

Cultures and Their Sources.—The cultures ⁶ used in the preparation of antigens were:

5. Vaughan, V. C., and Wheeler, S. M.: *J. Infect. Dis.* 4:476, 1907.

6. The cultures used were obtained as follows: IA from Dr. F. T. Lord. IB, IIA, IIB, IIIA and IIIB from Dr. G. B. White, State Biological Laboratory, Forest Hills. IVB and IVC from Miss E. A. Beckler, Bacteriological Laboratory, State House, Boston, isolated Dec. 13, 1920. The remaining cultures were isolated in the Pneumonia Laboratory of the Department of Preventive Medicine and Hygiene. The following were obtained from blood cultures taken on patients on the Special Pneumonia Service at the Boston City Hospital on the dates as indicated: IC, Jan. 15, 1921; ID, January 24; IF, February 24; IG, February 18; IH, February 28; IJ, March 15; IIC, February 15; IIID, February 27, and IVE, January 24. The following cultures were isolated from the sputum of similar patients: IE, February 8, and II, March 22. The following cultures were isolated from the sputum of patients outside Boston as indicated: IVA, Foxboro State Hospital, and IVD, Framingham.

Type I: 10 strains, designated I A, I B, etc.

Type II: 2 strains, designated II A and II B.

Type III: 4 strains, designated III A, III B, etc.

Type IV: 5 strains, designated IV A, IV B, etc.

Methods of Preparing Antigens.—The cultures used were from eighteen to twenty-four hour old growths in 100 c. c. of a 1 per cent. glucose beef infusion broth, neutral to phenolphthalein, which was found to correspond to a hydrogen ion concentration of from 8.0 to 8.2. On this medium there was homogeneous clouding of the broth with an abundant sediment in the bottom of the flask. The morphology of the organisms used was typically that of pneumococcus, gram-positive, bile soluble, and the type was repeatedly verified by agglutination with type serum from the New York State Board of Health and the Rockefeller Institute. All growths were examined microscopically for contamination, and serologically to confirm the previous type determinations.

For all the antigens, the first steps were identical. The growth was centrifuged, and the sediment was washed twice with sterile salt solution, centrifuging after each washing. Throughout the work the centrifuge was run at about 3,000 revolutions per minute. The time interval for centrifuging and shaking electrically was one-half hour.

The finished antigen was sealed in ampules containing sterile glass beads so that if desired the suspension could be shaken thoroughly before use to insure uniformity of material. Whenever the antigens were heated to 56 C. special care was taken that the entire ampule was completely submerged in the water bath. Before using the antigens were plated on whole rabbits' blood agar to test sterility, and in the simpler preparations bacterial counts were made according to Wright's method⁷ as commonly used in standardizing bacterial vaccines. The ampules were kept on ice until used. No antigen was used more than a week after being put on ice. Both the opalescent supernatant fluid and also the entire opaque suspension of organisms were used. Before using the antigens were diluted with sterile saline solution and doses varying from 10 to 1,000 million bacteria or their equivalent were injected in 0.1 c. c. volume.

With the earlier antigens the strains (except Type IV) were kept separate. Thus a patient with a known Type I pneumonia might be inoculated with four strains of Type I, one strain each of Types II and III, and a polyvalent Type IV, the whole constituting one test. No especial merit was found in this method, the few homologous strains used giving no better results than the heterologous strains, and since it subjected the patients to multiple inoculations, the strains of each

7. Wright, A. E.: *Lancet* **2**:1556, 1900; **2**:11, 1902.

type were pooled. The Type IV antigen was later omitted since in specific reactions the two nonspecific types furnished ample controls.

Antigen 1.—Cultures I A and I B, II A and II B, III A and IV A, IV B and IV C were used. The sediment after the second washing was taken up in from 20 to 30 c. c. of saline solution and heated to 56 C. for one hour. The ampules were then vigorously shaken, and put on ice.

Antigen 2.—Prepared as Antigen 1, except that it was incubated one week before being used. At the end of that time the sediment formed on standing showed on smear much amorphous gram-negative material including faint staining cocci with individual gram-positive cocci and rare diplococci.

Antigens I C and I D.—These were prepared as Antigens 1 and 2 from cultures I C and I D. They were used autogenously and on a few other patients.

Antigen 3.—Prepared as Antigen 1 with the following cultures: I A and I B; II A and II B; III A and III B; and IV A and IV C.

Antigen 4.—Same as Antigen 3, except that it was incubated one week.

Antigen 5.—Same as Antigen 4, except that it was incubated two weeks before using.

Antigen 6.—After the second washing with saline solution, the sediment was taken up with distilled water instead of saline solution. Cultures used: I A, I B and I C; II A; III B; IV A, IV C, IV D and IV E.

Antigen 7.—Same as Antigen 6, except that it was incubated one week.

Antigen 8.—After the second washing with saline solution, the sediment was taken up in 10 c. c. of a 10 per cent. bile solution,⁸ and incubated for one hour. Attempts were made to obtain suitable animal membrane for separation of the bile salts from the dissolved pneumococcus substances by dialysis, but it was found more feasible to obtain the separation by precipitation of the protein with four volumes of absolute alcohol. This was thoroughly shaken, centrifuged and the sediment again mixed with absolute alcohol. After recentrifuging, the sediment was dried overnight in partial vacuum over sulphuric acid. The residue was weighed, ground with sufficient sodium chlorid to make the final solution physiologic and taken up by adding distilled water drop by drop while grinding until 0.1 c. c. contained 1/150 mg dried sediment.⁹ (Later injections up to 1/10 mg. were used.)

8. Bacto-oxgall was used.

9. Gay, F. P. and Minaker, A. J.: J. A. M. A. **70**:215 (Jan. 26) 1918.

This was heated to 56 C. for one hour. For this antigen all the strains of each type were pooled. The cultures used were: I A, I B, I C, I D and I E; II A, and II B; III A, III B and III C.

Antigen 9.—After the second washing with saline solution, the sediment was taken up in a small amount of saline solution and precipitated with four volumes of absolute alcohol. This was centrifuged and taken up a second time in absolute alcohol. After again centrifuging the sediment was extracted by thoroughly shaking with ether. The sediment was dried for one hour in the incubator, weighed, and ground with sufficient salt to make the final solution physiological, and taken up in distilled water, added slowly while grinding. The dilutions were such that the antigen contained 20/150 mg. per 0.1 c. c. This was at first diluted twenty times before using but was eventually used full strength. The antigen was heated to 56 C. for one hour. In this preparation the strains of each type were pooled. The cultures used were: I A, I B, I C, I D, I F, I G, and I H; II A and II B; III A, III B, III C, and III D.

Antigen 10.—The most important difference between this and the foregoing antigens is that it was at no time raised above 37.5 C. The strains of each type used were pooled and were as follows: I B, I C, I D, I G, I H, I I and I J; II B; III C and III D. After the second washing with salt solution the sediment was divided into known amounts and kept on ice. The day of the tests, sufficient of sterile solution of 2 per cent. bile plus 0.25 per cent. tricresol was added to make 0.1 c. c. contain the equivalent of one twentieth of the growth of pneumococci on 60 c. c. of broth.¹⁰ An incubated solution of 2 per cent bile plus tricresol was used as control.

In summary, the first seven antigens were simple suspensions of pneumococci in saline solution or distilled water. Antigens 1, 3 and 6 were not autolyzed in the incubator, while antigens 2, 4, 5 and 7 were incubated one week or more. In antigens 8 and 9 the pneumococci were treated with bile and alcohol, and alcohol and ether, respectively. Thus the pneumococcus protein was subjected to considerable violence. Antigen 10 differed from all the others in that it was never raised above 37.5 C., while the others were all held at 56 C. for one hour. In the bile solution whatever endotoxins the pneumococci contain should be liberated.

Tests.—A "test" consisted of multiple inoculations. In the first seven antigens each strain of the different types was made up separately, with the exception of Type IV strains which were pooled and served

10. Cole, R.: J. Exper. M. **16**:644, 1912; **20**:346, 1914. Avery, O. T., Chickering, H. T., Cole, R. and Dochez, A. R.: Acute Lobar Pneumonia, Prevention and Serum Treatment, Monograph No. 7, Rockefeller Institute for M. Res., 1917. Weiss, C.: J. M. Research **34**:103, 1918.

as a control for the reactions which might be specific for type. Thus, with these antigens, a Type I pneumonia might receive from one to four different strains of Type I antigen, besides one Type II, one Type III and the pooled Type IV antigen. As it was very exceptional to obtain a reaction with one strain and not with all of the same type used (this occurred only in Case 7, which on the thirty-eighth day of the disease responded positively to the homologous strain and negatively to the heterologous strains of Type I), this method was abandoned later, since it subjected the patient to the discomfort of multiple inoculations.

Antigens 8, 9 and 10 were made from pooled strains of each of the fixed types. Type IV was no longer used, since in specific type reaction the two nonspecific type antigens acted as ample controls. Thus with these antigens a "test" consisted of three inoculations, one for each of the three fixed types.

With each new antigen various dilutions of each strain or pooled type were injected in an effort to obtain the titre of the antigen for both the specific type reactions and also for those reactions which did not show type specificity. These titres were, unfortunately, found to be very close to each other, and varied within considerable limits with each preparation and individual.

As stated earlier, tests were performed with both the opalescent supernatant fluid and the whole thoroughly mixed opaque antigen. In general, the supernatant fluid gave fewer reactions that were nonspecific for type, but it was also feared that in the same dilutions it might also elicit specific type reactions with less constancy.

The tests were made on the flexor surface of the forearms. A 26 gage needle was used and 0.1 c. c. was injected intracutaneously. When properly done a bleb appeared which persisted for some minutes; there was pitting at the hair follicles, and no bleeding at the point of inoculation.

Readings.—The tests were read, as a routine, after two, eighteen, twenty-four, thirty, forty-eight and seventy-two hours. Additional readings were made on the positive reactions.

Reactions Appearing with Only One Type of Pneumococcus.—The reactions in this group were at their height in from twenty-eight to thirty-two hours. These showed a deep red, indurated, papular center measuring from 2 to 4 cm. in diameter. Around this there was a somewhat lighter areola measuring up to 8 cm. in diameter. In forty-eight hours the papule still persisted, usually duller in color, the areola had entirely faded and in about half the cases was replaced by a zone of pigmentation. The papule in some cases persisted to the sixth day after the test. In one case there was very fine scaling. In some cases this type of reaction showed nothing after eighteen and twenty-

four hours. In others there was the reaction described below which was fading in twenty-four hours. The reaction to but one type of the antigen used was, in general, specifically positive for the type of infection, and will be referred to hereafter as specific type reaction.

Reactions with Two or More Types.—These reactions were at their height in eighteen hours. They showed no distinct differentiation between central papule and surrounding areola, and were usually indefinite in outline, fading gradually into the surrounding normal skin. The erythema was often mottled and of large dimensions up to 10 or 12 cm. in the longest axis which was always parallel to the long axis of the arm. On fading, unless they later showed specific type reaction, there was no pigmentation or scaling. By twenty-eight hours there remained only a small area about 0.5 cm. across of local redness around the point of inoculation. This reaction was always elicited with more than one of the types of the antigen used and generally with all when all types were used in the same strength. In an individual showing this reaction the number of types in which it appeared seemed, in general, to depend on the strength in which the type antigen was used and in no case on its specificity for the type of infection present. As will be discussed later these reactions were considered as a response to some substance possessed in common by all types of pneumococci or to some property of the antigen not even specific for the entire pneumococcus group. This reaction which was elicited in common by all types of pneumococci will be referred to hereafter as the common reaction.

In an effort to increase the number of specific type reactions, the strength of the antigens was varied with the object of reducing the common reactions to a minimum and yet retain sufficient strength to elicit the reaction to a single specific type.

Special reference must be made to the reactions obtained with Antigen 10 in which the thermolabile toxic substances from the pneumococcus were not destroyed. When used in the strength recommended by Kolmer⁴ and in weaker dilutions, severe local reactions were elicited, showing papules becoming pustular with wide zones of erythema and induration up to 12 or 14 cm. in their longest dimension. This was at its height in about thirty hours when the erythema faded, leaving no pigmentation or scaling in any case. The pustule persisted for weeks. This reaction was well marked in both controls and pneumonia cases, the most severe being in a normal control. The control injection of bile and tricresol showed only a small local papule, but none of the violent erythematous and edematous reaction.

Results.—During the course of the work, 124 persons were tested, as follows: (1) Lobar pneumonia, 104 cases; forty-seven had a Type I infection (specific antipneumococcus serum being used in twenty of

these and not used in twenty-seven); eight had Type II infections; seven had Type III pneumonia, one had Types II and III, twenty-nine had Type IV, and in twelve the type was not determined (no atypical Type II cases presented themselves); (2) controls, twenty persons, of whom sixteen were patients showing for the most part acute febrile conditions other than lobar pneumonia, and four were apparently normal. On these 124 persons, 223 tests were performed, divided about equally between the ten antigens. The earliest test was performed on the third day of the disease and the latest on the eighty-first day. The number of tests on each individual varied from one to five.

Of the 104 cases of lobar pneumonia tested, eleven (10.5 per cent.) gave specific type reactions, forty-six (42.3 per cent.) gave a common reaction and fifty-two (50 per cent.) gave no such reaction. Of the twenty controls, none gave a specific type reaction, nine gave a common reaction and eleven gave no reaction.

The eleven cases which gave specific type reactions were distributed among the types of infection as follows:

TABLE 1.—CASES MANIFESTING SPECIFIC TYPE REACTIONS

Type of Pneumonia	Number of Cases Tested	Number Showing Specific Type Reaction	Per Cent. Specific
I	47	6	12.7
II	9	2	22.2
III	8	1	12.5
IV	29	2	6.8

This reaction was specific for the type of infection in the patient as determined in the blood or sputum, or both, except with Antigen 9. With this antigen, reactions were elicited in three patients, in each case with the Type II preparation; while with Types I and III preparations the reactions were consistently negative. Of these patients one showed a Type II pneumococcus in the sputum, one a Type I and one a Type IV. In the last two cases blood cultures were persistently negative and repeated examinations of the sputum failed to show a Type II organism. In the Type I patient, the test was repeated with the same antigen five days later and was negative. In the cases of Type I and IV pneumonias, the reaction with Type II antigen was obviously nonspecific as to type of pneumococcus. Although this Type II preparation of Antigen 9 gave frequent negative tests in other cases, it is not unlikely that its apparent specific reaction in one case may be accounted for on the law of chance. Thus all three reactions might well be considered as nonspecific for type, and as a response to some form of protein common to all types of pneumococci, though in that case the Type I and III preparations should also have shown positive reactions, or to some other factor in the antigen rather than

to specific type sensitization in the patient. None of these three patients gave a specific reaction with any of the other antigens used.

Deducting these three reactions from Table 1, we may reconstruct it as follows:

• TABLE 2

Type of Pneumonia	Number of Cases Tested	Number Showing Specific Type Reaction	Per Cent. Specific
I	47	5	10.6
II	9	1	11.1
III	8	1	12.5
IV	29	1	3.5

Although the number is small, it may be said that in these tests, no one of the fixed types of pneumonia shows any marked preponderance over the others as regards the ease with which intracutaneous reactions, specific for the type of infection, could be elicited.

The relation between the specific type reactions in Type I cases and treatment with specific antipneumococcus serum is shown in Table 3.

TABLE 3.—SPECIFIC TYPE REACTIONS AND SPECIFIC SERUM TREATMENT

Total of Type I pneumonias tested.....	47		
Total of Type I pneumonias with specific reactions.....	6		
Treated	Total Tested	Specific Reactions	Per Cent. Specific
With serum	20	2	10
Without serum	27	4	14.8

Thus, instead of finding more ease in eliciting this reaction following specific serum therapy, as might be expected from the report¹¹ of the earlier appearance of antibodies in such cases, a somewhat smaller proportion of these reactions appeared among cases so treated than among those receiving no serum.

Of the eleven cases showing specific type reactions, seven gave one such test with one or more negatives; three gave two with one or more negatives, and one gave three with one negative test. There was a total of sixteen specific type reactions out of thirty-seven performed on these eleven patients. The earliest specific test was performed on the sixth day of the disease, or the day of the termination of crisis; the latest was on the thirty-eighth day, or twenty-nine days after crisis. The only reaction of this kind obtained before crisis was on the eighth day of the disease, or three days before crisis. This, however, was not specific for the type of organism found in the patient's sputum. Dividing the disease into weekly periods, the specific type reaction occurs as shown in Table 4.

11. Knox, J. H., Moss, W. L. and Brown G. L.: J. Exper. M. **12**:562, 1910.

TABLE 4

Week	1st	2d	3d	4th	5th	6th
Of the disease.....	1	6	3	2	3	1
After crisis	7	1	4	1	1	.

One reaction occurred before and one during crisis. Thus the largest number of these reactions appeared during the second week of the disease or the first week after crisis. With the crisis a phenomenon of allergy, this is what would be expected since the seven to fourteen day interval is that commonly required for antibody formation (vaccinia¹² and serum disease¹³) after which period there is a gradual reduction in the concentration of antibodies, and since directly after the clinical manifestation, the antibodies may be in their highest concentration,¹⁴ as it is on these antibodies that the antigen must depend for its reaction.

In these eleven cases the various antigens were used as shown in Table 5.

TABLE 5

Number of antigen.....	1	2	3	4	Ic	5	6	7	8	9	10
Total tests	4	5	3	4	3	6	2	3	1	4	4
Specific tests	0	5	0	3	1	2	1	1	0	3	0

It will be remembered that Antigens 2, 4, 5 and 7 were identical with Antigens 1, 3 and 6, respectively, except that the former were autolyzed in the incubator for a week or more, while the latter were put directly on ice. Thus out of eighteen tests with autolyzed antigens, eleven (61.1 per cent.) gave specific type reactions; while out of nine tests with nonautolyzed antigens only one (11.1 per cent.) so reacted. The superiority of the former type of antigen is well illustrated in Case 11 in which autolyzed antigens gave specific type reactions on the thirteenth and twenty-ninth days, and no such reaction on the twenty-third day. It is very unlikely that in the interval of sixteen days between the positive tests, the patient lost his specific type sensitiveness and regained it again, but rather that the antigen used during this interval was not suitable to elicit such a reaction. In this connection, it is significant that when one case responded specifically on the thirty-eighth day of the disease to an autolyzed antigen of culture I C

12. Rosenau, M. J.: Preventive Medicine and Hygiene, New York., D. Appleton & Co., 1918.

13. Von Pirquet, C., and Schick, B.: Die Serumkrankheit, Leipsig, Deuticke, 1905.

14. Chickering, H. T.: J. Exper. M. **20**:599, 1914. Dochez, A. R.: J. Exper. M. **16**:655, 1912. Ricketts, H. T.: Infection, Immunity and Serum Therapy, Chicago, A. M. A. Press, 1906. Rosenow, E. C.: J. Infect. Dis. **3**:683, 1906. Tunnicliff, R.: J. Infect. Dis. **8**:302, 1911. Wolf, H. E.: J. Infect. Dis. **3**:731, 1906.

(homologous) the identically prepared, but nonautolyzed, antigen gave no reaction.

Of the antigens in which the pneumococci were subjected to considerable violence (Antigens 8, 9 and 10) No. 9 was the only one showing any reaction to a single type and the lack of type specificity of these reactions has already been discussed.

In the light of Weil's² work, the common reactions are especially worthy of analysis. Of the 104 cases of lobar pneumonia tested, forty-six (42.3 per cent.) showed the reaction with one or more antigens, and of the twenty controls, nine (45 per cent.) showed such reactions. They were elicited by the various antigens as shown in Table 6.

TABLE 6

Antigens	1	2	3	4	5	6	7	8	9	10
Reactions in pneumonias.....	5	4	6	7	3	2	0	2	7	17
Reactions in controls.....	1	3	0	1	0	2	0	2	0	3

The earliest reaction of this type appeared on the sixth day of the disease and the latest on the forty-sixth day. In relation to crisis, one appeared twenty-four hours before, two during, and the remainder after crisis.

These reactions were at their height in eighteen hours, and were fading in from twenty-four to thirty-six hours. They showed no differentiation between central induration and areola. Though a central macule, sometimes just palpable about the point of inoculation, might persist for forty-eight hours or even longer, there was no pigmentation or scaling. Weil describes what he considers a positive reaction to the pneumococcus protein as follows:

Within the twenty hours following the injection further changes may occur at the site of injection. A fairly well circumscribed area of erythema, with slight infiltration and elevation of the skin surrounding the point of puncture, may develop. If the infiltration is marked, a true papule results. These changes may persist for forty-eight hours or more. . . . The normal individuals, or the diseased controls, may or may not present a reaction, depending presumably on their previous sensitization by the pneumococcus or an allied organism.

It would seem, then, that the reactions obtained by Weil and those described here as common reactions were the same. Weil is not disturbed by similar reactions in controls and feels that they are specific for the pneumococcus protein. When a history of lobar pneumonia cannot be obtained, he feels that an abortive and unrecognized attack may be used as an explanation of sensitization. Another explanation is that suggested by Gay⁹ for his "meningococcin" reaction in normals with negative cultures, namely, that the presence of the organisms on the mucous membrane in the past was sufficient to

sensitize. It is impossible to check up Gay's suggestion, because the discovery of a carrier state in the past is beyond present day bacteriologic technic. At least, it can be said that a test in which 45 per cent. of the controls react is of no service diagnostically.

SUMMARY

Of 104 cases of lobar pneumonia, eleven (10.5 per cent.) gave one or more intracutaneous reactions to only one type of pneumococcus used, while forty-six (42.3 per cent.) reacted to two or more types. Of twenty controls none showed the single type reaction, while nine (45 per cent.) showed the multiple type reactions.

These two reactions are sharply differentiated both as to time and character. The reactions elicited to a single type of pneumococcus were (with the exception of those from Antigen 9) specific for the type of organism isolated from the patient. The reactions, elicited by multiple types of pneumococci in 42.3 per cent. of the cases of lobar pneumonia and in 45 per cent. of the controls were not specific for the type of pneumococcus causing the disease. To determine whether they are specific for pneumococcus protein or not a control antigen made from other bacteria should be used.

In 10 per cent. of the cases treated with Type I antipneumococcus serum, specific type reactions were obtained, and in 14.8 per cent. not so treated there were similar reactions. No one of the fixed types showed any marked preponderance of specific type reactions. The longest period over which the specific type reaction was obtained was seventeen days. The largest number of the specific type reactions occurred during the second week of the disease (six cases) and the first week after crisis (seven cases).

With antigens prepared from simple saline suspensions of pneumococci, 61.1 per cent. of the tests, performed on the patients showing the specific type reactions, were positive when the antigen used had been autolyzed in the incubator for a week or more, and 11.1 per cent. of the tests with nonautolyzed antigen were positive.

Of the antigens in which the pneumococci were treated with bile, alcohol, etc., only one gave reactions to a single type and these were probably all nonspecific for the type of infection present.

No reactions comparable to those reported by Weiss and Kolmer with their "pneumotoxin" were obtained with a similar preparation, nor was there any specific absence of reactions as might be expected from an analogy to the Schick test.

CONCLUSIONS

1. Intracutaneous reactions specific for the type of pneumococcus causing lobar pneumonia may be obtained in certain cases.

2. The reaction has not been demonstrated sufficiently early to be of service in directing specific serum therapy.
3. The largest number of reactions occur during the period when, on the assumption of allergy, the highest concentration of antibodies would be expected.
4. The sensitization responsible for the specific reactions may persist more than two weeks.
5. The most satisfactory antigen for obtaining specific type reactions is made by autolyzing saline solution or distilled water suspensions of the various types of pneumococci.
6. A reaction, differing from that which is specific for type in time and character, may be obtained in a considerable number of cases of lobar pneumonia and controls. This appears with more than one of the pneumococcus type antigens and is in no way specific for the type of organism causing the infection. Whether this reaction is specific for a common factor in all types of pneumococcus protein or whether it is in no way specific for the organisms composing the antigens has not been demonstrated.