SPECIFIC CUTANEOUS REACTIONS AND CIRCULATING ANTIBODIES IN THE COURSE OF LOBAR PNEUMONIA*

II. Cases Treated with Antipneumococcic Sera

By MAXWELL FINLAND, M.D., and W. D. SUTLIFF, M.D.

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston)

(Received for publication, July 7, 1931)

The results of skin tests with the type-specific polysaccharides (soluble specific substance or S.S.S.) of Types I, II and III pneumococci in cases of lobar pneumonia receiving no specific therapy and in a group of patients suffering from other diseases are reported elsewhere (1). The general correlation of the positive skin reactions to the homologous S.S.S. with recovery from lobar pneumonia offered a possible method of observing and controlling specific antipneumococcic serum therapy. The present study deals primarily with repeated skin tests in cases of lobar pneumonia treated with specific immune sera and a correlation of the results of these tests with circulating specific antibodies. Another similar group of patients were studied for their skin reactions and circulating antibodies on a single occasion some time after recovery.

In preliminary experiments it was found possible to "produce" a positive skin test. This was accomplished by the intravenous injection of therapeutic immune serum in a normal individual who had no history of pneumonia and who gave negative skin tests with the 3 types of specific pneumococcus polysaccharides. The skin reaction was elicited only by the specific carbohydrate of the pneumococcus corresponding in type to that of the antibody injected. It was demonstrated after 5 cc. of concentrated Type I antibody (Felton) and ap-

* This study was aided in part by grants from the William W. Wellington Fund of the Harvard Medical School and from the Influenza Commission of the Metropolitan Life Insurance Company.

peared immediately after the injection of this amount. The reaction was again elicited after 6 hours, but not after 30 hours. Some lots of serum failed to give this response with the same or larger amounts. It was also found possible in cases of lobar pneumonia to elicit positive cutaneous responses within a few minutes after the injection of various amounts of specific antisera.

Materials and Methods

The patients upon whom these studies were made were, in general, similar to those used in the previous study (1) except that the present cases were treated with specific antipneumococcic sera. In all, 31 cases were studied. Felton's bivalent (Types I and II) concentrated antibody solution was given to 17 Type I and 11 Type II cases; 3 of the former and 2 of the latter terminated fatally. Convalescent sera from homologous type cases were given to 1 Type II patient and to 2 patients with Type III pneumonia. All 3 of these patients died. The age of the patients ranged from 13 to 76 years, 4 being under 20 and 2 over 60. All but 4 were males. Only 3 of the 28 recipients of Felton's concentrated antibody solution had a positive blood culture (J. O'B., Type I, and B. M., Type II, (Fig. 1) and a fatal Type II case) whereas all 3 of the convalescent serum recipients showed pneumococci in their blood. Serum administration was begun in these cases from 36 hours to the 8th day after the onset of the disease and all were acutely ill at the time the first dose was given.

Skin tests were performed and agglutinins and protective antibodies determined as described in a previous paper (1). Control skin tests with the polysaccharides were done and samples of the patients' blood were obtained 30 minutes or more before the first injection of serum. Subsequently, skin tests were performed and blood samples were obtained before further injections of serum and at intervals during convalescence.

Immune Sera.—A description of the materials and methods employed in the administration of serum to the Type I cases is given elsewhere (2). The concentrated sera used in these cases was furnished by the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Public Health through the courtesy of Dr. Benjamin White. The concentrated antibody solution used in the treatment of the Type II cases of this series was prepared and supplied by Dr. Felton (3) of the Department of Preventive Medicine and Hygiene of the Harvard Medical School and contained 2000 to 3000 units (4) of mouse protection per cubic centimeter against Types I and II pneumococci. The convalescent serum recipients were studied in the same manner as the other patients. The methods used for preparing and giving the convalescent sera in these cases are included in the clinical report by Beebe and Sutliff (5). Skin tests were done on the donors at the time that their blood was obtained, and their sera were tested for antibodies.

Varieties of Cutaneous Response in Serum Treated Cases.—All the various degrees

of response observed in the cases receiving no specific therapy (1) were seen in this group except that reactions with edema of several hours duration were less common. Skin tests which showed no reaction for 30 minutes or more after injection, or skin tests, which, once positive, had already faded, sometimes gave a typical response within a few minutes following a subsequent dose of serum. In one instance (J. K., Type II), only the last of 7 skin tests done at 1 to 2 hourly intervals during the 1st day of treatment showed a positive Type II response. The next skin test performed 16 hours later was positive with Types I and II S.S.S., and was followed, 2 minutes after the injection of the polysaccharide, by the appearance of blotchy wheals and erythema formations at the site of all previous injections of Types I and II S.S.S.

Results in Cases of Type I and Type II Pneumonia Treated with Bivalent (Types I and II) Concentrated Antibody Solution (Felton)

First Appearance of Positive Cutaneous Reactions.—Observations were made before treatment in 20 of the 28 patients receiving Felton's serum. In none was a positive response elicited to any of the 3 polysaccharides injected. The first skin test after the beginning of serum therapy was positive with the carbohydrate homologous to that of the disease in 14 of the 23 recovered cases. This positive response was elicited in 10 of these 14 cases within 24 hours after the first dose of serum was given. Of the 9 recovered cases whose first test after the institution of serum treatment was negative, 5 gave a positive homologous response within 11 hours after the 1st dose, in the 6th the second test done the next day was positive, and in the 3 remaining patients a positive homologous response was never elicited.

The reaction to both the Type I and the Type II polysaccharides usually became positive at the same interval after serum administration regardless of the type of the infecting pneumococcus. The few exceptions may be seen by a study of Fig. 1 on which are represented graphically the results of all the skin tests done in the recovered cases treated with the concentrated antibodies.

Relationship between the First Appearance of the Positive Cutaneous Response and the Amount of Serum Administered.—A positive response to the homologous S.S.S. was elicited in 10 of 13 patients tested after receiving amounts of serum up to 40 cc., and in 8 other patients at the time of the first test after the injection of serum, when more than 40 cc. had already been given. 2 cases that showed doubtful or negative

responses after small doses showed positive reactions after 93 and 85 cc., respectively. The frequency with which positive skin tests were obtained after various doses of serum and at various intervals after its administration is shown graphically in Fig. 2.

Duration of the Skin Reactivity in Serum Treated Patients.—The frequency with which positive tests were elicited diminished rapidly

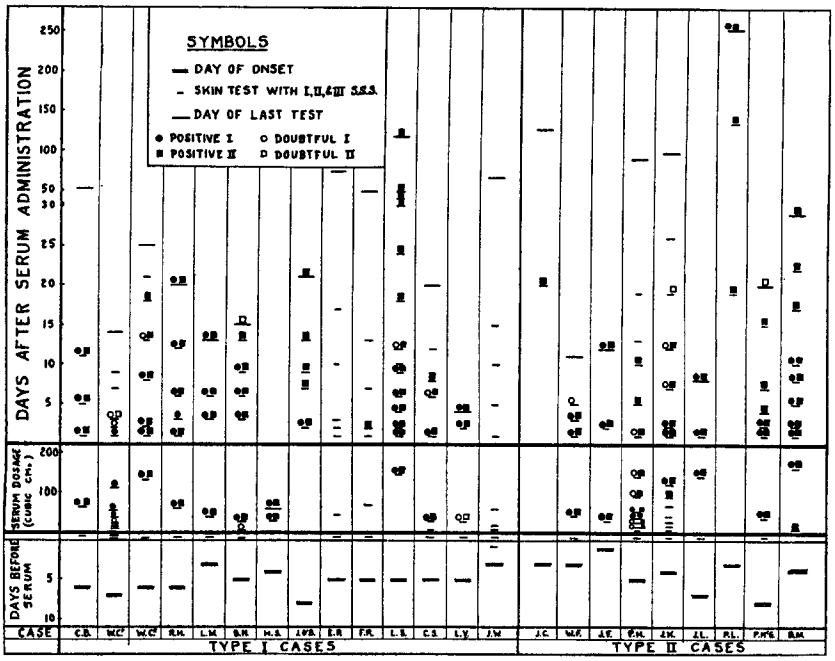

FIG. 1. Results of repeated skin tests with the specific polysaccharide of Type I, II and III pneumococci in 14 Type I and 9 Type II recovered cases of lobar pneumonia receiving bivalent (Type I and II) concentrated pneumococcic antibodies (Felton).

after the 3rd day following serum administration, as shown graphically in Fig. 2. Only 2 of 9 cases in this series on whom tests were done later than 1 month after recovery gave positive cutaneous responses to S.S.S.: a Type I case on whom a positive Type II test was elicited at the end of the 4th month and a Type II case on whom both Type I and Type II S.S.S. gave positive reactions more than 8 months after

serum treatment. This diminution in the frequency of positive tests as convalescence progressed was more striking for the reactions elicited by the Type I S.S.S. than for those obtained with the Type II carbohydrate, regardless of whether the infecting organism was Type I or II.

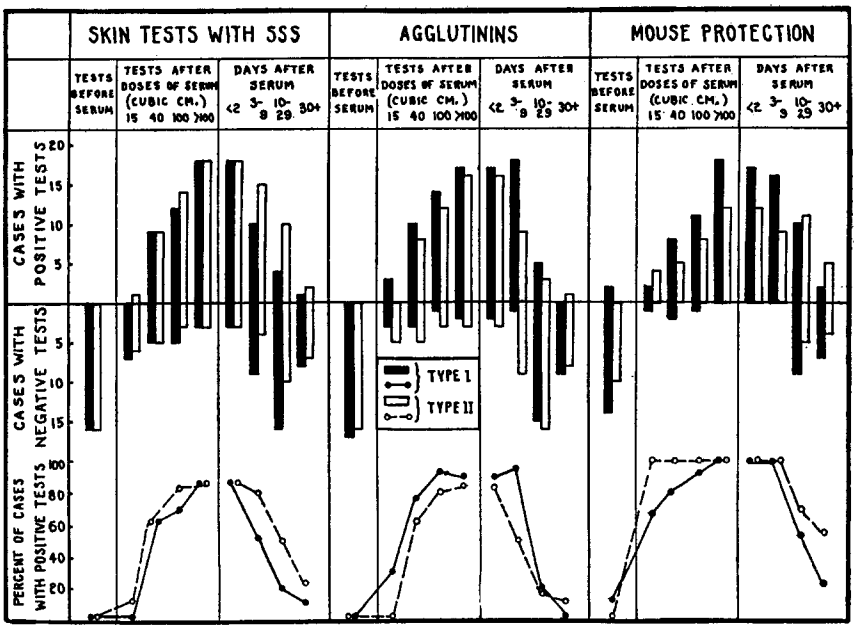

FIG. 2. Frequency of cutaneous reactions, agglutinins and mouse protection at various intervals in 23 recovered cases of lobar pneumonia treated with bivalent (Type I and II) concentrated pneumococcic antibodies (Felton).

Skin Tests in Fatal Cases and in Cases Recovering with Complications.—A positive test was elicited in only 1 of 5 fatal cases receiving concentrated antibodies.

This was obtained in a Type II patient with the homologous S.S.S. 48 hours after the last dose of serum was given and 20 hours before the patient's death. One of the fatal cases, from whose sputum Type I pneumococci were recovered at the time of admission to the hospital, was given a total of 335 cc. of concentrated serum in repeated doses over a period of a week and failed to give a positive response to any of the cutaneous tests. Blood cultures in this case were repeatedly negative.

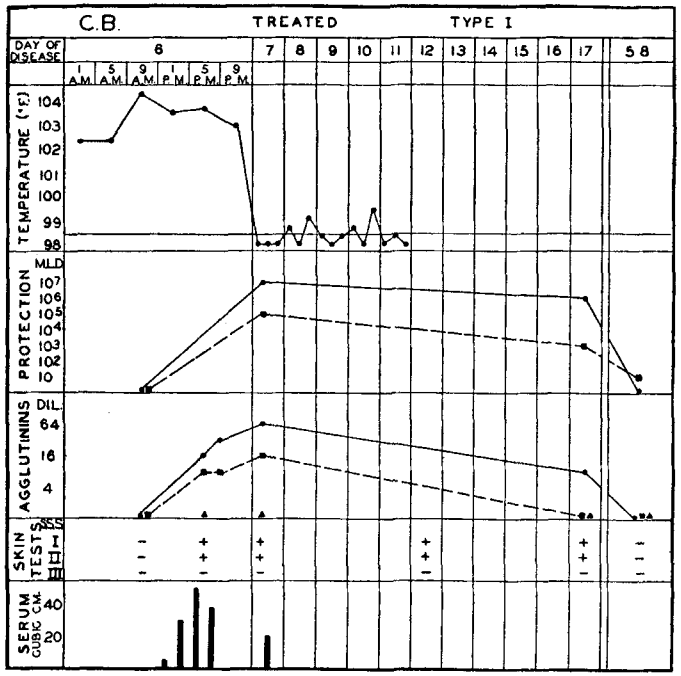

FIG. 3

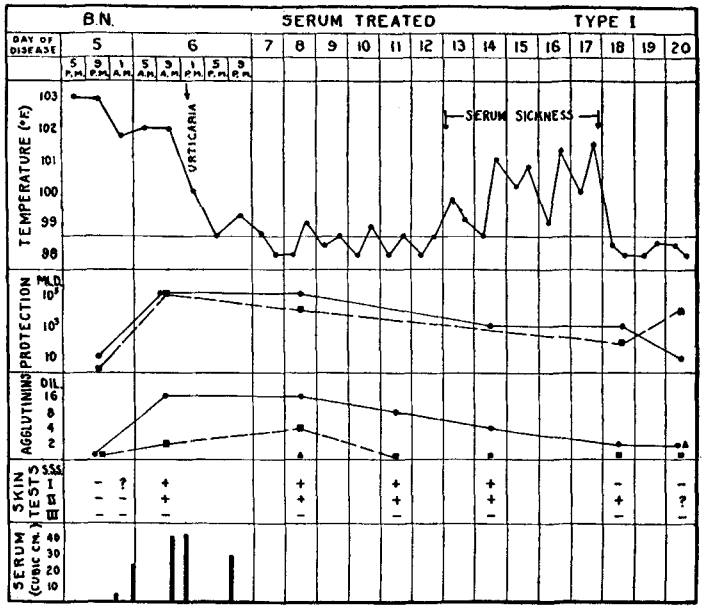

FIG. 4

FIGS. 3 to 6. Results of all of the tests done in 4 serum treated cases of lobar pneumonia. Cases shown in Figs. 3 to 5 received Felton's serum; the case shown in Fig. 6 received convalescent serum.

+ Positive.
− Negative.
? Doubtful.
0 No growth.

●———● Type I tests.
■— —■ Type II tests.
▲.....▲ Type III tests.

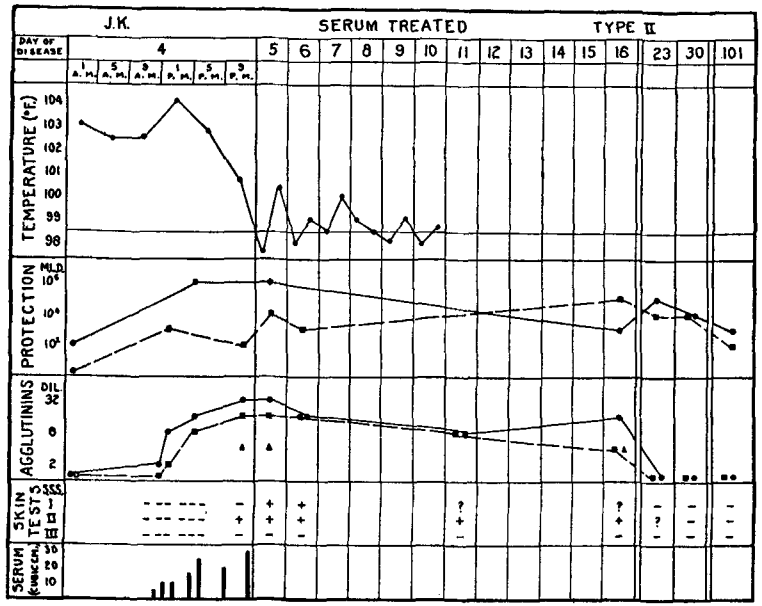

FIG. 5

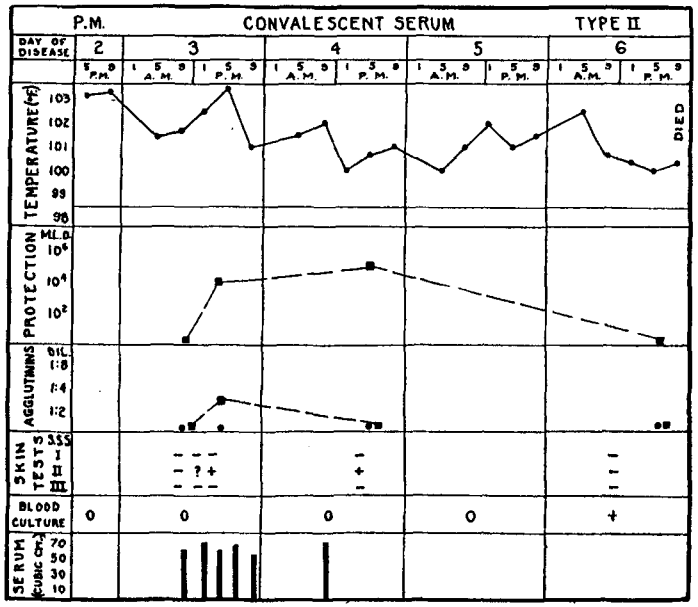

FIG. 6

Cultures at autopsy from each of the affected lobes of the lung, from several abscesses in these lobes and from both parotid glands, which were also abscessed, showed pure cultures of *Staphylococcus aureus*, but no pneumococci.

There was only 1 case in the series that developed a purulent complication (J.O'B., Type I, see Fig. 1). This patient had positive Type I and II tests on the 2nd day after serum was given, but 5 days later the Type I test was negative. He had a purulent infection of the left shoulder joint from which Type I pneumococci were recovered. 2 patients with sterile pleural effusions (W. C. and L. S., Type I) showed the usual positive responses.

Comparison of the Skin Response and Circulating Antibodies in Serum Treated Cases.—Agglutinins were studied in the sera of almost all patients for all 3 types before and after serum administration. Tests for protective antibodies against Types I and II were carried out in 17 patients and for the homologous type alone in 7 other cases. None of the patients tested showed agglutinins for any of the types before the onset of serum therapy. In only 2 patients (J. K., Type II, and F. R., Type I) were protective antibodies (against 100 lethal doses of Type I in each case) found before treatment. Fig. 2 shows graphically the frequency with which cutaneous reactions, agglutinins and protective antibodies were demonstrated at various intervals.

In 7 cases in which the antibodies were not first demonstrated simultaneously, protective antibodies tended to appear first or at the same time that agglutinins were demonstrated, and the positive cutaneous responses, were subsequently elicited. Later in the disease, the order in which the antibodies disappeared was not as regular as in the cases receiving no serum. In general, protective antibodies tended to persist longer than agglutinins or skin reactivity, but 2 instances (R. H., Type I, and B. M., Type II) were observed in which positive skin reactions were obtained without protective antibodies or agglutinins for the corresponding type. There was but one instance in which agglutinins were present in the absence of corresponding protective antibodies. The sera from many cases had protective antibodies without corresponding agglutinins. 5 cases were shown to have protective antibodies 2 months or more after recovery; all of these had Type II protection, and 2 had, in addition, Type I protection (J. K., Type II, and F. R., Type I).

The results of all the tests in 3 typical patients are shown graphically in Figs. 3 to 5.

Variations in the Skin Response Following the Administratin of Various Lots of Serum.—The data on this subject are necessarily meager inasmuch as several lots of serum were used and only a small number of cases treated with each lot. That different lots may vary in their effect on the production of positive skin tests is suggested by experiences with 2 lots.

Lot CP_7, containing 3500 units of mouse protection against Type I and 1500 units against Type II, was given to 4 Type I patients and 1 Type II patient all of whom recovered. Positive tests with Types I and II S.S.S. were obtained with the first test following serum administration in 4 patients, only 1 of whom had received more than 35 cc. at that time. One Type I case had a positive reaction to Type I S.S.S. after 35 cc., but a positive Type II test was never obtained. Lot CP_{13}, containing 3000 units against Type I and 1500 against Type II, was given to 3 Type I patients. 2 patients received 45 and 75 cc., respectively, yet failed to give positive reactions to either Type I or Type II S.S.S. The 3rd patient is not included in this series, as complete immunological studies were not made. He was treated with 180 cc. of the material on the 4th day of the disease, but failed to show any clinical improvement from the serum and died within 24 hours.

Cutaneous Reactions in Cases of Type II and Type III Lobar Pneumonia Treated with Homologous Human Convalescent Serum

3 patients received convalescent sera obtained from patients about 1 week after recovery from the homologous type lobar pneumonia. All the donors gave positive cutaneous reactions to the corresponding type S.S.S., and had demonstrable agglutinins and mouse protective antibodies in their sera.

One of the recipients (P.M., Fig. 6), a Type II case who had negative skin tests, no demonstrable circulating antibodies and a negative blood culture before serum was given, showed a positive homologous skin test 1 hour after 130 cc. of serum was given. At this time he also had demonstrable protective antibodies and agglutinins for Type II pneumococci in his serum. 2 days later, no further serum having been given, his blood culture became positive for the first time, his skin tests were negative and no circulating antibodies were demonstrable, and the patient died. 2 other patients, with Type III infection, received 75 and 310 cc. of serum, respectively, but in neither was a positive response to Type III S.S.S. elicited, nor were circulating antibodies demonstrated on repeated tests. Both of these cases also ended fatally. The patient receiving the larger doses had a steadily increasing

bacteremia in spite of the repeated doses of serum. He had, before serum, a strongly positive skin reaction to Type II S.S.S., and protection against 100 lethal doses of Type II pneumococci was demonstrated in his serum at that time. The Type II positive skin reaction was repeatedly elicited and remained strongly positive until 4 hours before death, at which time no wheal appeared but erythema of the same extent, but less intense, resulted. Type II agglutinins were not found and Type II protective antibodies were not demonstrated after serum was given.

TABLE I

Cutaneous Reactions with S.S.S. and Circulating Specific Antibodies in 3 Groups of Cases Treated with Felton's Serum

		No. of cases	Positive skin tests			Protection present			Agglutinins present		
			Type I	Type II	Type III	Type I	Type II	Type III	Type I	Type II	Type III
Tests performed 10 to 22 days after serum administration	Cases previously tested with S.S.S.	19*	4	12	0	11	12	—	8	6	0
	Cases not previously tested	9**	5	6	0	9	8	—	5	2	0
Tests 5 to 14 mos. after serum	No previous tests	11‡	4	6	0	0	0	0	0	0	0

— No tests done.

* Type II protection tests were done in only 15 of these cases.

** One of the cases of empyema had positive reactions to Types I and II S.S.S.

‡ Protection tests were done in only 5 patients, all of whom had positive skin tests. The 4 patients having positive Type I reactions had positive Type II skin tests as well.

Single Skin Tests and Antibody Studies after Serum Administration

It has previously been shown that the intracutaneous injection of the type-specific pneumococcus polysaccharides may result in the production of antibodies specific for the type of carbohydrate injected (1, 6). In order to learn whether this factor played a significant rôle in the serum treated cases discussed above, 20 cases receiving Felton's serum were studied at a single occasion after serum had been given.

These cases may be divided into 2 groups. The first group was studied for skin reactions and specific antibodies 10 to 22 days after serum administration. This group included 6 cases having Type I pneumonia (2 of these had empyema at the time they were studied),

2 with Type II and 1 with a miscellaneous pneumococcus infection. The second group was studied 5 to 14 months after recovery. This group consisted of 11 Type I recipients of Felton's serum, including 2 patients who had empyema and 2 others who had sterile pleural effusions following their disease. The number of cases showing positive tests in these 2 groups are shown in Table I. In this table is also included a group of the cases previously described that received repeated skin tests and were again tested from 10 to 22 days after serum.

In the group of cases tested for the first time during the 2nd or 3rd week after serum administration the incidence of positive skin reactions, of agglutinins and of protective antibodies is not significantly different from that found during the same period in the serum treated cases who had previously received several skin tests with the specific carbohydrates.

DISCUSSION

The observations here presented demonstrate the possibility of "producing" and maintaining for some time a positive response in the human subject to the cutaneous injection of the purified, protein-free, type-specific carbohydrate of the pneumococcus. This is accomplished by the intravenous administration of the corresponding immune serum derived either from the horse or from the human patient after recovery from lobar pneumonia. Previous observations by Cole (7), and more recently by Sutliff (8), and by Lord and Persons (9), have indicated that demonstrable agglutinins and protective antibodies may be produced and maintained in the blood of patients with lobar pneumonia by the administration of sufficient amounts of potent immune sera. These observations are here confirmed and extended to include the specific cutaneous response to the pneumococcus polysaccharides.

The purified type-specific polysaccharide, although only a part of the pneumococcus antigen, reacts specifically with antibodies produced by the whole antigen (10, 11). Tillett and Francis (12) have further observed that the carbohydrate may induce a reaction in the skin of patients recovering from lobar pneumonia and that this reaction is specific for the type of infecting pneumococcus and is associated with the presence of agglutinins and precipitins in the sera of the patients. Attempts to stimulate the production of antibodies by the purified carbohydrates have, however, failed (11, 13). These substances have,

therefore, been considered non-antigenic and the term "haptenes" applied to them by Landsteiner (14, 15), has been generally accepted. More recently, however, it has been shown by Francis and Tillett(6) and in a previous communication (1) that these purified carbohydrates injected in minute amounts into the skin of patients ill with or recovering from lobar pneumonia and receiving no specific antisera are capable of stimulating the production of antibodies specific for the type of carbohydrate injected. Further observations (16) have shown that this is possible in normal individuals, suggesting the antigenic character of these bacterial carbohydrates when used in this manner.

The duration of the immune reactions (Fig. 2) in serum treated patients receiving repeated intracutaneous injections of polysaccharides appears to be distinctly shorter than in the corresponding patients previously reported (1). In the former group such reactions were rarely found 1 month after recovery, whereas in the latter homologous and heterologous antibodies persisted for several months.

These 2 groups of cases were in every respect comparable except for serum administration. It may thus be inferred that the passive introduction of antibodies in patients receiving specific polysaccharides intracutaneously interferes with antibody production of the carbohydrates. This is similar to the well recognized failure of individuals to develop effective active immunity in other diseases following the intensive therapeutic use of antibacterial or antitoxic sera.

The results here presented show that, in most instances, cases terminating fatally after the administration of specific serum fail to give positive skin responses. This failure to react apparently cannot always be interpreted as indicating a persistence of the type-specific pneumococcus infection. That the failure of these fatal cases to react may be non-specific is suggested by the absence of positive reactions to the polysaccharides corresponding to the heterologous antibodies injected and by the failure of one patient to give a positive response to either the Type I or the Type II polysaccharide although he had a massive infection with *Staphylococcus aureus* and pneumococci could not be recovered from his organs at postmortem. Inhibition of heterologous skin reactions in other acute infections has been reported by others (17). The almost complete absence of cutaneous reactions in these individuals is probably not due to the inability of the patients'

skin to react, for typical reactions to the Type II polysaccharides occurred in 3 cases terminating fatally (1 of these is reported in a previous paper (1)) and such patients were, in some instances, shown to respond to small doses of histamine with the characteristic wheal and erythema.

Cases which failed to give characteristic positive reactions did not all terminate fatally. The failure to induce positive cutaneous responses by the injection of antisera in patients, in whom clinical benefit is otherwise obvious, may depend on the character of the serum, some sera being more or less deficient in the quality of inducing cutaneous responses, or on the responsiveness of the patient. Similar lack of reactivity in some normal individuals was observed by Coca and Grove (18) in connection with the passive transfer of atopic reagins.

The presence of foci of infection has not been constantly associated with the absence of skin responses. About one-half of the surviving patients with well established foci of pneumococcus infection persisting after recovery from pneumonia, both with and without serum treatment, showed cutaneous responses after these foci were demonstrated.

To attempt to define the curative dose of antipneumococcic sera on the basis of immune reactions in the patient is hazardous. The general correlations, however, between the occurrence of positive skin reactions and recovery, and between the dosage of immune sera and the appearance of the positive skin reaction are sufficiently regular to be at least suggestive. In the majority of the patients in this series who recovered and showed positive reactions, such reactions first appeared and were maintained after doses of 40 cc. of Felton's concentrated antibodies. It is thus suggested that the effective curative dose of most of the preparations used, perhaps only in the milder cases, is in the vicinity of this amount.

The relationship of the skin test to agglutinins and protective antibodies has been discussed in a previous communication (1) and nothing further need be added here.

SUMMARY AND CONCLUSIONS

1. Characteristic cutaneous responses to the type-specific protein-free carbohydrates of both Type I and Type II pneumococci have been

"produced" in cases of lobar pneumonia due to either of these types by the intravenous injection of concentrated bivalent (Types I and II) antipneumococcic sera (Felton).

2. A positive cutaneous response to the specific polysaccharide of Type II pneumococci has been passively transferred from human cases convalescing from this infection to a patient suffering from pneumonia due to this organism.

3. The cutaneous responses to the type-specific polysaccharides and circulating antibodies were studied in 51 cases of lobar pneumonia. Positive cutaneous reactions were, in most instances, associated with recovery, even when purulent complications were present. Failure to elicit a positive reaction was usually followed by a fatal outcome.

4. The positive reactions in patients who were treated with concentrated sera and recovered were most often elicited within 24 hours after the first dose and after a total of 40 cc. had been given.

5. The positive skin reactions obtained after the administration of specific antisera were associated with the presence of mouse protective antibodies and agglutinins in the sera of the patients.

6. The immune reactions in serum treated cases receiving repeated inoculations with the specific carbohydrates disappeared more rapidly than in similar cases receiving no antiserum. It is suggested that the administration of antisera in some way interferes with the production of antibodies by the intracutaneously injected carbohydrates.

REFERENCES

1. Finland, M., and Sutliff, W. D., *J. Exp. Med.*, 1931, **54,** 637.
2. Sutliff, W. D., and Finland, M., *J. Am. Med. Assn.*, 1931, **96,** 1465.
3. Felton, L. D., *J. Infect. Dis.*, 1928, **43,** 543.
4. Felton, L. D., *J. Am. Med. Assn.*, 1930, **94,** 1893.
5. Beebe, R. T., and Sutliff, W. D., *New England J. Med.*, 1930, **203,** 823.
6. Francis, T., and Tillett, W. S., *J. Exp. Med.*, 1930, **52,** 573.
7. Cole, R., *J. Exp. Med.*, 1917, **26,** 463.
8. Sutliff, W. D., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25,** 292.
9. Lord, F. T., and Persons, E. L., *J. Exp. Med.*, 1931, **51,** 151.
10. Avery, O. T., and Heidelberger, M., *J. Exp. Med.*, 1925, **42,** 367.
11. Avery, O. T., and Tillett, W. S., *J. Exp. Med.*, 1929, **49,** 251.
12. Tillett, W. S., and Francis, T., *J. Exp. Med.*, 1929, **50,** 687.
13. Avery, O. T., and Morgan, H. J., *J. Exp. Med.*, 1925, **42,** 347.
14. Landsteiner, K., and Simms, S., *J. Exp. Med.*, 1923, **38,** 127.

15. Landsteiner, L., *Biochem. Z.*, 1919, **93,** 106; 1920, **104,** 270.
16. Sutliff, W. D., Finland, M., and Jackson, H., Jr., *J. Clin. Invest.*, 1931, **10,** 660.
17. Mitchell, A. G., Wherry, W. B., Eddy, B., and Stevenson, F. E., *Am. J. Dis. Child.*, 1928, **36,** 721. (Also others cited by these authors.)
18. Coca, A. F., and Grove, E. F., *J. Immunol.*, 1925, **10,** 445.