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

I. Cases Receiving No Serum Therapy

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In a recent paper Tillett and Francis (1) reported the finding of definite and characteristic cutaneous reactions to certain purified chemical fractions of the pneumococcus in patients recovering from lobar pneumonia. In the cases studied, they showed that the type-specific polysaccharide (the soluble specific substance, or "S.S.S.") injected intracutaneously at the time of crisis or later produces a "wheal and erythema" type of response which is specific for the type of pneumococcus causing the disease and that fatal cases fail to give such responses. The acetic acid precipitable fraction of the pneumococcus, the so called nucleoprotein, when injected at about the time of crisis or later elicits a delayed tuberculin type of reaction in all cases of pneumonia without respect to the type of pneumococcus causing the disease. They showed that these cutaneous reactions are associated with the presence of agglutinins and precipitins in the serum of the patient.

These findings suggested that the type-specific skin reactions to the pneumococcus polysaccharides might be useful in prognosis. Furthermore, Tillett and Francis suggested that "persistence of infection even though specific antibodies are present, may inhibit the skin response," intimating that such reactions might be used as a simple test for complete recovery from the specific infection.

This paper is concerned with: (1) The cutaneous responses to

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repeated skin tests with the specific carbohydrates of Types I, II and III pneumococci and circulating specific antibodies at various intervals in 41 cases of lobar pneumonia that received no specific therapy. (2) Similar studies on a single occasion early or late in convalescence in 44 comparable cases and in 24 individuals without recent pneumonia.

Material and Methods

Repeated tests were done in 41 patients admitted to the medical wards of the Boston City Hospital during the winter and spring of 1930. These patients all had typical lobar pneumonia, clinically and radiographically. A pneumococcus of either Type I, Type II or Type III was obtained in each case from the sputum or the blood culture, or both. No specific antipneumococcic agents were used in the treatment of these cases. There were 30 recovered and 11 fatal cases. 15 of the the former had Type I, 7 had Type II and 8 had Type III infection; of the 11 fatal cases, 5 were caused by Type I, 4 by Type II and 2 by Type III pneumococci. A positive blood culture was obtained in only 2 of the recovered cases (J. S., Type I, and J. K., Type II (Fig. 1); whereas, 9 of the fatal cases had a pneumococcemia. The presence of complications will be mentioned later. The patients ranged in age from 13 to 58 years. Only 5 of the patients were females.

Skin tests were done on a single occasion 4 to 23 days after crisis in 35 cases of lobar pneumonia that received no specific antipneumococcic therapy, but were, in all respects, similar to the cases mentioned above. The sera of many of these cases were tested for antibodies during the acute disease and at the time of crisis, as well as at the time that the skin tests were performed. 9 other cases were studied on one occasion 5 to 14 months after recovery from pneumonia not specifically treated. 24 hospital patients with no recent history of pneumonia were studied. These included cases of peptic ulcer, chronic arthritis and blood and cardiac diseases. The ages of the latter group ranged from 13 to 77 years.

Skin Tests.—A 1:200 solution of each of the Types I, II and III purified specific carbohydrates was obtained from the Hospital of The Rockefeller Institute for Medical Research, through the courtesy of Dr. W. S. Tillett. 1:10,000 dilutions were prepared every few days from the stock solutions by diluting with physiological saline freshly made with redistilled water and then heating for 10 minutes at 100°C. to insure sterility. 0.1 cc. of each of these 3 solutions (containing 0.01 mg. of the carbohydrate) and of a control of the saline used in making the dilutions were injected intradermally on the flexor surface of the forearm. All 4 injections were repeated at various intervals during the acute disease and during convalescence. In a few instances where positive tests were obtained, higher dilutions up to 1:30,000,000 were used in order to determine the smallest amount with which a positive test could be obtained.

Antibody Determinations.—Serum was obtained from venous blood, drawn

shortly before, occasionally after, almost every test. The titer of agglutinins and of mouse protective antibodies for Types I, II and III pneumococci was determined in most instances. Agglutination tests were done by the method employed by Tillett and Francis (1). Protective antibodies were determined by the usual mouse method. Precipitin tests were done with the specific carbohydrates and with culture filtrates, but the results were irregular and are not reported.

Varieties of Cutaneous Reactions to the Specific Polysaccharides.—The intracutaneous injection of the type-specific carbohydrates usually produces no greater response than does the injection of an equal amount of physiological saline. The response occurring in reacting individuals recovering from lobar pneumonia has been described and illustrated by Tillett and Francis (1). Fairly wide variations in the appearance of the reaction were observed, in the course of this study, in different patients and at different times in the same patient. These variations were probably only differences in intensity, since mild reactions could be produced in patients who showed strong reactions to standard dosages by the injection of 0.0001 mg. The milder reactions consisted of a faint pink wheal, 6 to 10 mm. in diameter, which shaded into an area of faint erythema, 12 to 20 mm. in diameter. This type of reaction usually reached its maximum within 30 minutes and faded entirely before the end of 45 to 60 minutes. The intense reaction consisted of an almost white, edematous, sharply demarcated wheal irregular in outline giving the appearance of "pseudopods." 1 or 2 drops of serum occasionally oozed from the puncture wound of this type of case. This type of wheal was usually surrounded by an intense erythema, 4 to 6 cm. in diameter, the periphery of which faded into an area of faint mottling. Such striking reactions developed in 10 to 20 minutes and remained at their maximum intensity for 30 to 60 minutes, after which the wheal gradually blended with the surrounded erythema to form a fairly soft, usually non-tender, uniform swelling lasting 6 to 48 hours. The skin over the central portion of the swelling usually remained reddened. In rare instances ecchymosis apeared in the edematous area. Occasionally, although no reactions occurred on the day of the injection, erythema was observed 24 to 72 hours later at the site of injection of the carbohydrate which corresponded to the type of the infecting pneumococcus. Another test, performed upon noticing such a delayed reaction, elicited a typical immediate reaction with the same material.

Reactions were called doubtful when they were greater than that produced by the saline control but showed a wheal less than 0.8 cm. and a surrounding erythema less than 1.5 cm. in diameter.

Occurrence of Cutaneous Reactions to the Specific Polysaccharides

The results of all of the skin tests in the 30 recovered cases are charted in Fig. 1. Positive cutaneous reactions to the homologous type polysaccharides (derived from the same type as the pneumococcus causing the disease) were obtained in 17 of the 30 recovered cases. In

addition, 10 of the 17 patients who gave homologous positive tests also showed positive reactions to heterologous polysaccharides. In 2 other cases heterologous positive tests were observed but the homologous carbohydrates gave negative reactions. All the heterologous positive responses in cases due to Types I and III pneumococci were elicited with the Type II S.S.S. whereas the heterologous reactions in the Type

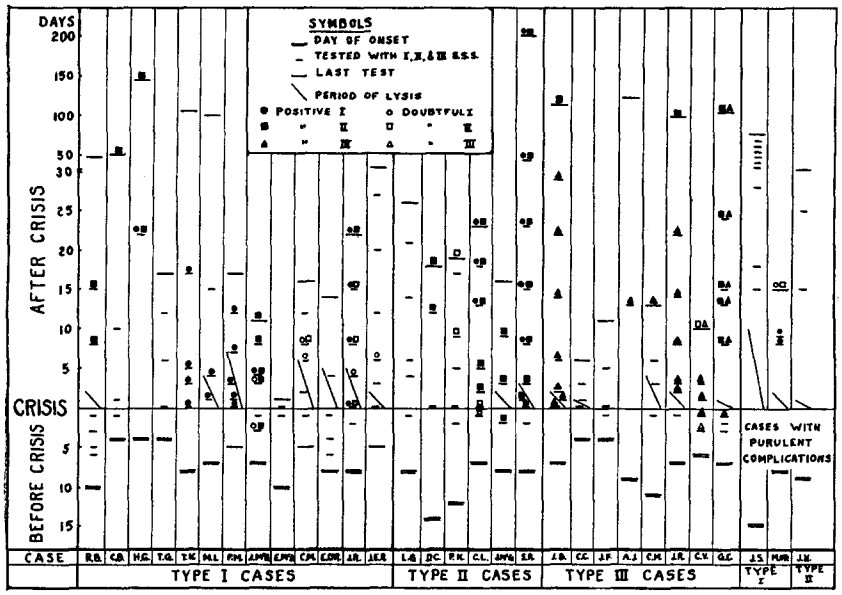

FIG. 1. Results of repeated skin tests with the specific carbohydrates of Types I, II and III pneumococci in 30 recovered cases of lobar pneumonia that received no specific therapy.

II cases occurred with the Type I polysaccharide. The type distribution of the cases and of the skin responses is summarized in Table I.

There were 3 cases among the 30 that recovered who had purulent pneumococcic complications at the time of the first test. One of these 3 patients, a case with Type I pneumococcus empyema, gave positive tests with the homologous S.S.S. In the other 2 cases, one having purulent Type I pneumococcus arthritis and the other having an empyema and purulent conjunctivitis from both of which Type II

pneumococci were obtained, the reactions to all the carbohydrates were negative. There were also 2 cases that had sterile pleural effusions during convalescence. One of these cases, a patient with Type I pneumonia, gave negative reactions to all tests, whereas the other, a Type III case, gave positive tests with both the Type III and the Type II S.S.S.

Of the 11 fatal cases, only one showed a positive homologous test. In this patient characteristic positive tests were elicited 36 hours and 12 hours before death. Cultures taken at these times showed, respectively, 500 and 700 colonies of Type II pneumococci per cubic centimeter of blood. Heterologous positive tests were not obtained in any of the fatal cases.

Occurrence of Protective Antibodies and Agglutinins

Protection of mice against the homologous type organism was demonstrated against 100 lethal doses or more in the sera of all of the 24 recovered cases in whom tests were done on more than one occasion. Homologous agglutinins were present after recovery in all but 2 of the 27 cases tested in dilutions of serum up to 1:64. The 2 patients who had no agglutinins also had negative skin tests with the homologous polysaccharides (C. B., Type I, and J. F., Type III). Protective antibodies for heterologous types of pneumococci were demonstrated in 19 of the 25 recovered cases in which such tests were carried out. In 3 additional cases, protection against 10 lethal doses or irregular protection was found. Heterologous agglutinins were present in the sera of 17 of the 28 patients tested after crisis. The findings in the recovered cases are summarized by types in Table I.

All 3 of the recovered cases having purulent complications and both patients with sterile pleural effusions had protection for the homologous and heterologous type pneumococci after the presence of the complication was established. Agglutinins were demonstrable in the sera of all of these patients for the homologous type organism and in one of the latter patients for an heterologous type.

Among the fatal cases, homologous agglutinins were demonstrated only once. This was on the day of death in a Type I patient who had been ill 22 days and had a persistent low grade pneumococcemia and

infected pleural fluid (reported by Lord and Persons (2)). Protective antibodies for the same type were present in this case at the same time and also on 2 other occasions during the previous week. No autopsy was obtained on this patient. Homologous protection against more

TABLE I

Summary of the Cutaneous Reactions, Agglutinins and Protective Antibodies in 30 Recovered Cases Repeatedly Tested

Infecting type	Type I tests		Type II tests		Type III tests	
	No. of cases tested	Cases with positive tests	No. of cases tested	Cases with positive tests	No. of cases tested	Cases with positive tests
Cutaneous reactions						
I	15	7	15	5*	15	0
II	7	2	7	4	7	0
III	8	0	8	0	8	6
Agglutinins						
I	14	13**	14	9	14	1
II	7	1	7	7	7	2
III	7	0	7	4	7	6
Protective antibodies						
I	14	14	14	11^{3}‡	10	3^{3}
II	6	3	6	6	4	2
III	5	3^{1}	5	5	4	4

* 2 of these failed to show homologous positive tests, all other cases having positive tests with an heterologous S.S.S. had positive homologous tests as well.

** 2 had agglutinins only in 1:2 dilution of serum.

‡ Raised numerals indicate cases having protection for 10 lethal doses or irregular survivals among the mice in the higher dilutions.

than 10 lethal doses was not found in any of the other fatal cases. Heterologous antibodies were never demonstrated.

Relation of the Cutaneous Reactions and of Antibodies to the Course of the Disease

Appearance of the Cutaneous Reaction to Specific Polysaccharides.—A positive homologous test was elicited in only 3 of 17 cases tested before

the day of crisis.[1] In 2 of these cases, the reaction was obtained on the day before crisis, and, in the 3rd, it was elicited 2 days before the crisis. By the day of crisis, 11 of 25 patients tested showed positive reactions to the homologous S.S.S. Whereas 12 of 25 patients tested by the end of the first week after crisis had already shown homologous positive tests, only 1 (J. McB., Type I) had a positive test with an heterologous S.S.S. within this period. This patient's heterologous positive reaction was observed when he was first tested 3 days before crisis. In general, the positive tests with the homologous polysaccharides were first demonstrable at about the time of crisis and, in some cases, while the temperature was still elevated and the patients appeared acutely ill. On the other hand, heterologous positive tests were first elicited 8 or more days later, and often quite late in convalescence. In particular, 3 patients first showed heterologous positive responses 2, 3 and 4 months after crisis at a time when the tests with the homologous carbohydrates were already negative in each instance.

Duration of the Skin Reactivity to Specific Polysaccharides.—The last positive test with the homologous polysaccharide was elicited less than 1 month after crisis in 15 of the 17 cases in which homologous reactions occurred (Fig. 1). Subsequent negative tests with the same types occurred in 9 of these 15 cases. Positive homologous tests were elicited in only 2 of the 8 patients tested after 3 months of convalescence. Reactions to heterologous polysaccharides, on the other hand, persisted in all but 1 of the 10 patients as long as observations continued; in 5 cases more than 3 months and in a 6th case 51 days after crisis.

The duration of the skin reactivity varied with the type of polysaccharide injected. Type II reactivity was more lasting than that elicited by the Type I and III carbohydrates. It will be seen that 6 of the 9 patients having positive reactions to the Type I polysaccharide gave negative responses subsequently and 2 of the remaining 3 were last tested only 22 days after crisis. Only 1 case (S.R., Type II) gave a positive reaction with Type I S.S.S. later than 2 months after recovery. Likewise, of the 6 cases reacting to Type III polysaccharide, only 3 were positive at the last test which, in 2 cases, was done less than

[1] In cases recovering by "lysis" it was found that the first day of the lysis period corresponded with respect to circulating antibodies to the day of "crisis," and it is so considered in the text and the accompanying figures.

2 weeks post-critically. In contrast to these results, only 1 of the 12 patients having Type II positive reactions failed to give a positive response at the time of the last observation. This suggests that the longer duration is not a characteristic of heterologous responses as such but, rather, a characteristic of the reactions to the Type II S.S.S. used.

Comparison of the Cutaneous Reactions and the Circulating Antibodies, and the Relation of Both to the Course of the Disease.—The time of

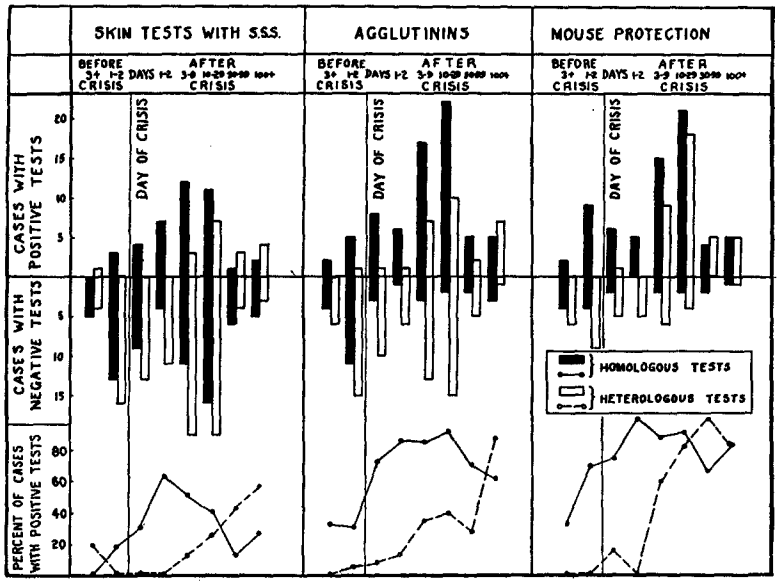

FIG. 2. Frequency with which cutaneous reactions, agglutinins and mouse protection were demonstrated at various stages of the disease in 30 recovered cases of lobar pneumonia receiving no specific therapy.

appearance of the skin reaction, of agglutinins and of protective antibodies for the homologous type could be compared in 24 cases. All 3 immune reactions were first demonstrated simultaneously in 5 cases; in 12 others, the protective antibodies and agglutinins appeared at the same time and before the skin reaction, when that was positive; and, in the remaining 7 cases, protective antibodies were demonstrated before either of the other reactions. Similarly, 19 cases could be compared

with respect to heterologous types. The skin reactions and the circulating antibodies were demonstrated simultaneously in 3 cases; protective antibodies and agglutinins were found at the same time and before the skin reaction in 6 cases; and, in 9 cases, protective antibodies appeared before either of the other reactions. In 1 case (J. McB., Type I), the appearance of the positive heterologous skin reaction preceded the finding of circulating antibodies. The frequency with which antibodies were found and positive skin reactions occurred at various intervals is represented graphically in Fig. 2.

When antibodies were present in any of the patients in this series they were demonstrated, in practically every instance, by the mouse protection test. The corresponding agglutinins were usually demonstrable, but this was not true in every case and often did not correspond at different times in the same case. All cases having positive cutaneous reactions with the type-specific polysaccharides had protective antibodies for the corresponding type of pneumococcus at the same time, but agglutinins were less regularly found. Figs. 3 to 6 show the results of skin tests and antibody determinations in 4 typical recovered patients.

From Fig. 2 it will be seen that agglutinins and protective antibodies, particularly the latter, appeared earlier than did the cutaneous reaction to the corresponding S.S.S., whether of the homologous or of the heterologous type. The persistence of heterologous antibodies over a period of over 3 months is again brought out rather strikingly.[2] The homologous agglutinins and protective antibodies persisted longer and in a larger percentage of cases than did the positive skin reactions with the corresponding polysaccharide.

Cutaneous Reactions and Circulating Specific Antibodies in Patients Tested on One Occasion

The results of the tests in patients tested once in convalescence and in those with no recent history of pneumonia are shown in Table II. It is seen that early in convalescence the cases which had previously received no skin tests frequently showed cutaneous reactions and anti-

[2] Two of the patients (S. R. and G. C. (Figs. 4 and 5)) were studied 12 and 13 months respectively after recovery. Cutaneous reactions and antibodies for more than 1 type were demonstrated in both of these patients.

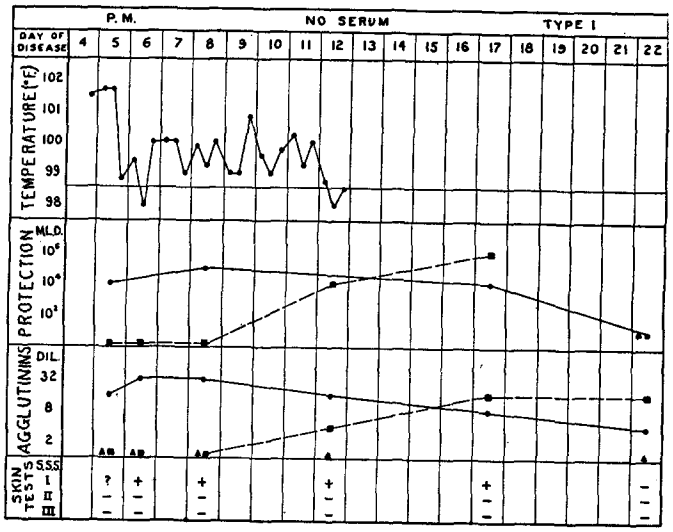

FIG. 3

FIGS. 3 to 6. Results of all of the tests performed in 4 different patients with lobar pneumonia receiving no serum therapy.

●——● Type I tests. + Positive.
■— —■ Type II tests. − Negative.
▲..... ▲ Type III tests. ? Doubtful.

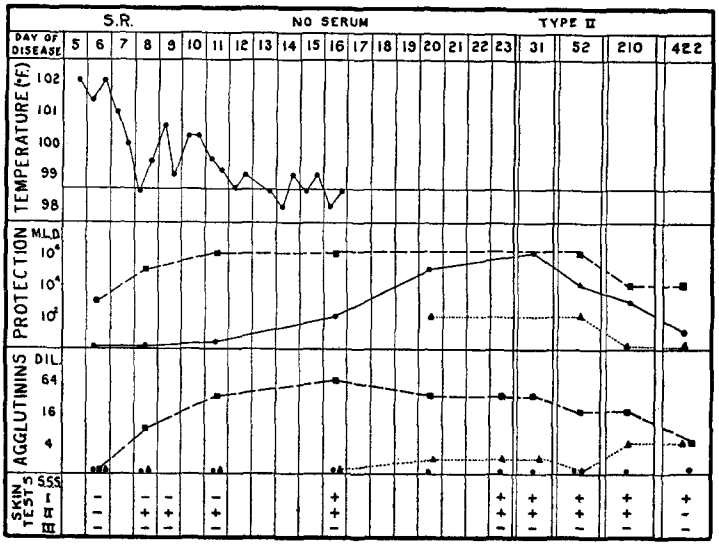

FIG. 4

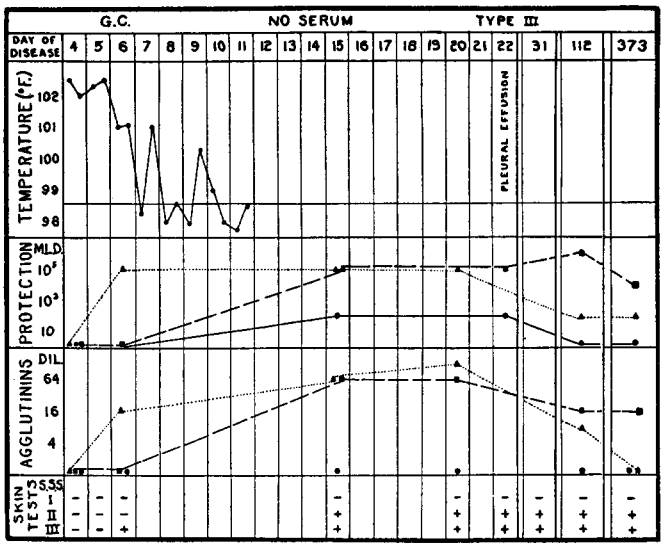
G.C.
NO SERUM
TYPE III
DAY OF DISEASE
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 31 112 373
TEMPERATURE (°F.)
102
101
100
99
98
PLEURAL EFFUSION
PROTECTION
M.L.D.
10⁴
10³
10
AGGLUTININS
DIL.
64
16
4
SKIN TESTS
S.S.S.
I
II
III

FIG. 5

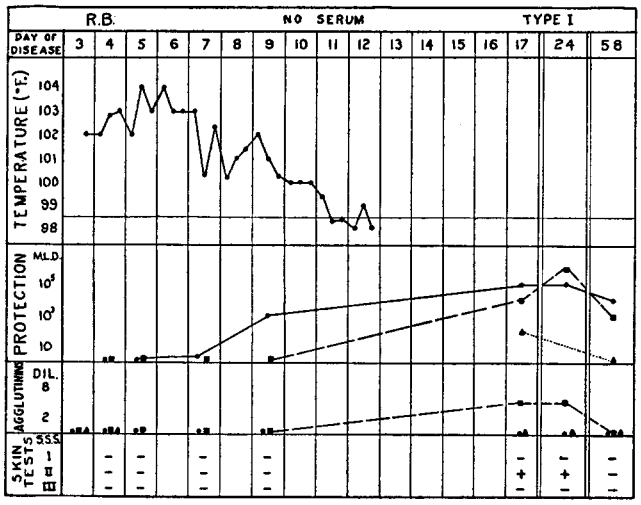
R.B.
NO SERUM
TYPE I
DAY OF DISEASE
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 24 58
TEMPERATURE (°F.)
104
103
102
101
100
99
98
PROTECTION
M.L.D.
10³
10²
10
AGGLUTININS
DIL.
8
2
SKIN TESTS
S.S.S.
I
II
III

FIG. 6

bodies specific for the infecting type of pneumococcus. A few patients showed heterologous skin responses and antibodies, but these were no more frequent than in the group of cases with no recent pneumonia. In the few instances where heterologous circulating antibodies were found at the time of the skin test, these were also shown to have been present during the disease. In the cases tested 5 to 14 months after

TABLE II

Summary of the Cutaneous Reactions, Agglutinins and Protective Antibodies in 3 Groups of Cases Not Previously Tested Intradermally

	Type of infection	No. of cases	Positive cutaneous reactions			Agglutinins present			Protective antibodies present		
			Type I	Type II	Type III	Type I	Type II	Type III	Type I	Type II	Type III
Group I											
Tested 4 to 23 days after crisis	I	13	7	1	1	9	0	2	10*	0	1
	II	12	2	10	2	1	11	0	2	8	3
	III	4	0	3	1	0	0	2	0	0	2
	Miscellaneous pneumococci	6	2	2	0	1	1	0	1	1	0
Group II											
Tested 5 to 14 months after crisis	I	5	0	1	0	0	0	0	1	0	1
	II	2	0	1	0	0	0	0	0	1	1
	III	2	0	0	0	0	1	0	0	1	0
Group III											
Cases without recent pneumonia		24**	4	10‡	0	0	0	0	0	5	1

* 2 additional cases had 10 M.L.D. and irregular survivals to 100 M.L.D.

** Protection tests done in 12 cases, 6 of which had positive skin tests.

‡ All of the cases having positive Type I tests also appear here.

recovery, typical positive cutaneous responses were often not associated with corresponding agglutinins or protective antibodies. This is in sharp contrast to the regular association of positive skin tests with corresponding antibodies in patients receiving repeated injections of S.S.S. The frequency of positive reactions in patients without recent pneumonia is also in sharp contrast to the almost complete absence of positive responses during the acute stage of the disease.

There were 5 patients with complications in the group tested early in convalescence.

One was a patient, who, 8 days after crisis, showed a positive cutaneous response to the Type I polysaccharide and circulating antibodies for the same type. On the same day, Type I pneumococci were cultured from pus obtained from the pleural cavity. The second patient was afebrile and apparently having a normal convalescence on the 9th day after crisis, at which time a positive Type I skin test was obtained and circulating specific antibodies for this type were present. 2 days later this patient had a rise in temperature, a positive blood culture was obtained and he died 2 days later. The organisms cultured from his blood, as well as those cultured at autopsy from the heart's blood, the involved lungs and pleural fluid, were Gram-positive, green-producing diplococci which were bile insoluble, grew in rough colonies and did not agglutinate with any of the specific sera available. This organism may have been a rough pneumococcus. 2 Type I patients had sterile pleural effusions; one of these had a positive homologous skin test. One other Type I patient in this group had an empyema; his skin tests were negative.

DISCUSSION

The observations of Tillett and Francis (1) that the protein-free, type-specific polysaccharide of the homologous pneumococcus elicits an immediate "wheal and erythema" type of reaction when injected into the skin of patients recovering from pneumococcus lobar pneumonia and that this reaction is associated with the presence of circulating specific antibodies has been confirmed. These authors found positive skin reactions in 100 per cent of 21 recovered Type I cases. In the present series only one-half of the Type I and two-thirds of the Type II and Type III cases that recovered showed skin reactions to the purified polysaccharide of the pneumococcus causing the disease, but these Type I cases are hardly comparable to those of Tillett and Francis, since most of their cases were treated with antipneumococcic serum.

The majority of fatal cases in this series failed to react to the homologous polysaccharides. Mention was made above of a patient who gave a characteristic skin response to the Type II S.S.S. at a time when he had massive blood invasion with Type II pneumococci. No mouse protective antibodies or agglutinins were found in this patient's blood serum. There is no adequate explanation for this peculiar occurrence, but two possibilities may be mentioned. In the first place, the antibody may have been fixed in the skin, although it had disappeared

from the blood stream. Secondly, this patient may have come in contact with some substances, such as yeasts (3), immunologically related to Type II polysaccharide, to which he had become partially immunized, and was therefore capable of reacting with the purified polysaccharide in the skin.

In the patients who had repeated skin tests with the specific pneumococcus polysaccharides antibodies were demonstrated to pneumococci heterologous to the type of infecting organism but corresponding to the types of polysaccharides used in the skin tests. Patients tested on only one occasion in convalescence did not show heterologous antibodies. Similar observations were recorded by Francis and Tillett (4) who suggested that these heterologous antibodies were associated with the previous intradermal injections. Direct experiments with normal individuals (5) have shown that the production of type-specific antibodies may be induced by single or repeated skin test doses of polysaccharides. It thus seems likely that the type-specific carbohydrates injected intradermally in small amounts are antigenic.

Although heterologous antibodies and sometimes heterologous skin tests developed for each of the 3 types in some of the cases, the heterologous serum antibodies and skin tests were by far most frequent for Type II. The explanation of this fact may depend on the properties of the solution of Type II S.S.S. used in this study which was possibly more actively antigenic than the Type I and Type III solutions. On the other hand, since "natural" antibodies for Type II pneumococci are present normally in nearly all adult human bloods (5), the basis for more effective immunization to Type II polysaccharide may be present in human subjects.

A study of Fig. 2 will indicate that the skin reaction is a less sensitive indicator of antibody production than is either the agglutination or mouse protection test. Furthermore, the large number of negative responses in patients who recovered without complications and the finding of positive tests in patients with persistent infection indicate the disadvantages of the skin test as a prognostic aid. The agglutination test, while demonstrable somewhat less frequently than mouse protection and, thus, a less delicate index of antibody formation, has the advantages of simplicity, of the absence of irregularities so often presented by the use of the mouse and of rapidity. When performed by

such methods as those of Arlyle Noble (6) and of Sabin (7), the agglutination reaction is as simple to perform as the skin reaction and is less time consuming.

SUMMARY AND CONCLUSIONS

1. A group of 41 non-serum treated patients with Type I, II or III pneumococcus pneumonia were studied during their disease and convalescence with respect to their skin reactions to specific pneumococcus polysaccharides and, in most instances, for the presence of circulating agglutinins and protective antibodies for all these 3 types.

2. One-half of the Type I and two-thirds of the Type II and Type III recovered cases gave the typical immediate "wheal and erythema" response to the homologous polysaccharide at or about the time of recovery. All cases tested showed protective antibodies and almost all showed agglutinins for the homologous pneumococcus. In the fatal cases, in general, positive cutaneous reactions and circulating antibodies were not obtained.

3. In cases of pneumonia receiving repeated cutaneous inoculations with various types of specific polysaccharide, antibodies for pneumococci differing from the infecting type but corresponding to the types of carbohydrate injected were present 1 week or later after such injections. These heterologous antibodies were most frequently demonstrated for Type II and were probably the result of immunization by means of the cutaneous injections.

4. Positive skin responses to homologous polysaccharides and corresponding circulating antibodies were demonstrated with similar frequency in the first 3 weeks after crisis in patients who had not previously received intracutaneous injections. In such patients heterologous antibodies were rarely found.

5. Typical skin reactions with the specific pneumococcus polysaccharides and mouse protective antibodies were demonstrated independently in a number of hospital patients who had had no recent history of pneumonia.

6. Some patients with demonstrable foci of persistent infection or with latent infections which later proved fatal showed positive cutaneous responses to the homologous type polysaccharide and circulating specific antibodies for the corresponding type.

7. The agglutination test, though less sensitive than the mouse protection test for determining the presence of antibody, has many advantages over the latter and is simplest to use in following the course of the untreated pneumonia.

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