CUTANEOUS REACTIONS IN PNEUMONIA. THE DEVELOPMENT OF ANTIBODIES FOLLOWING THE INTRADERMAL INJECTION OF TYPE-SPECIFIC POLYSACCHARIDE

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In a previous communication (1) the occurrence of cutaneous reactions to the polysaccharides and proteins of Pneumococcus during lobar pneumonia was reported. The investigation disclosed the interesting fact that the capsular carbohydrates of Pneumococcus were capable of inciting a reaction when injected intradermally. The character of the response and the conditions under which it was obtained are briefly summarized as follows: The characteristic reaction is the development of an *immediate wheal and erythema* at the site of injection; a positive reaction was first elicited at or about the time of crisis; it was produced only by the polysaccharide homologous in type to that of the Pneumococcus causing the disease; at the time of reaction type-specific antibodies for the homologous organism were demonstrable in the patient's serum.

In contrast to the polysaccharides, the so-called nucleoprotein fraction of the Pneumococcus evoked a *delayed* response which reaches its height in 24 hours. It simulated in appearance the tuberculin reaction. The protein reaction generally appeared first early in convalescence and tended to become progressively more marked during the course of recovery. Circulating antibodies for the nucleoprotein were present in approximately equal concentrations during the periods of acute illness and recovery. The protein reaction bore no specific relation to the type of Pneumococcus producing the infection.

The present report includes further observations on cutaneous reactions to pneumococcus fractions and deals especially with the appearance of antibodies for more than one specific type of Pneumococcus

in the serum of convalescent patients. The individuals whose sera presented these unusual reactions had received repeated intradermal injections of the pneumococcus carbohydrates. The possible relationships between the repeated injection of the specific substances and the antibody formation will be discussed.

Materials and Methods

A. Skin Test Materials

1. Type-Specific Capsular Polysaccharides.—The type-specific polysaccharides were obtained in purified form from Types I, II and III Pneumococcus by the method of Heidelberger and Avery (2). They are chemically and serologically distinct and have been identified with the capsular material of the cell. The Type II and Type III polysaccharides are nitrogen-free; that of Type I contains 5 per cent nitrogen as part of the sugar molecule (3). The polysaccharides were diluted in physiological salt solution to a concentration of 1:10,000 and 0.1 cc. containing 0.01 mg. of the specific soluble substance was injected intracutaneously. Skin tests were repeated at intervals throughout the disease and convalescence.

2. Nucleoprotein.—The nucleoprotein was prepared by the method of Avery and Morgan (4) from an R strain derived from a Type II S organism. The material was standardized on the basis of nitrogen content and 0.01 mg. of protein in 0.1 cc. of physiological saline was used in all tests. These tests were repeated at approximately weekly intervals during the patient's stay in hospital.

B. Titration of Sera for Antibodies

At intervals during the acute illness and convalescence samples of serum were obtained and tested for the presence or absence of circulating antibodies.

1. Type-Specific Antibodies.—The presence in the serum of type-specific antibodies was determined by (a) agglutination, and (b) passive protection of mice.

a. Type-Specific Agglutinins.—0.5 cc. of varying dilutions of the serum to be tested was mixed with 0.5 cc. of a heat-killed suspension of type-specific pneumococci. Final readings were made after the mixtures had been incubated for 2 hours in the water bath and then placed in the ice box overnight.

b. Passive Protection of Mice.—The capacity of patient's serum to protect mice against infection was tested by the usual technique. 0.2 cc. of serum diluted to 0.5 cc. with salt solution was employed. So far as possible each serum was tested against pneumococci of Types I, II and III. The cultures used were invariably fatal for mice in doses of 0.0000001 cc.

2. Antibodies for Nucleoprotein.—Antibodies for the nucleoprotein material were determined by the precipitin reaction as previously described (1).

A study of the cutaneous response to the injections of the type-specific polysaccharides of the Pneumococcus and of the occurrence of circulating antibodies has been made in 74 cases of lobar pneumonia. The results confirm earlier observations and may be briefly summarized as follows: Positive reactions were elicited only with the homologous polysaccharide at crisis or shortly thereafter; homologous type-specific antibodies were demonstrable in the serum of each reactive patient. In no instance was a positive skin reaction to a polysaccharide obtained in the absence of circulating antibodies for the type of Pneumococcus from which the polysaccharide was derived. Table

TABLE 1

The Incidence of Cutaneous Reactions to the Homologous Polysaccharide in Convalescent Patients

Type of infecting organism	No. patients	No. positive reaction	Per cent giving positive reactions
I	21	21	100
II	17	10	58.8
III	9	4	44.4

13 recovered cases of Group IV—no reactions to polysaccharides of Types I, II, III.

5 recovered cases of Type II (atypical)—no reactions to polysaccharides of Types I, II, III.

9 fatal cases of various types—no reactions to polysaccharides of Types I, II, III.

I shows the relative frequency of positive reactions to the homologous type of polysaccharide in patients convalescent from Type I, II or III pneumonia. All but 4 of the Type I cases were treated with anti-pneumococcus Type I serum.

From Table I it can be seen that positive cutaneous reactions were obtained in all of the Type I cases and in approximately 50 per cent of the Types II and III patients. The polysaccharides of Types I, II and III elicited no response in the 13 Group IV and the 5 atypical Type II patients. The absence of reactivity in the 9 fatal cases agrees with earlier experiences in that in patients with lethal termination no reaction was obtained. 12 patients convalescent from Type II or Type III pneumonia also failed to react but in 8 of them no reaction

would be expected since their sera contained no circulating antibodies; the sera of the remaining 4 patients contained circulating antibodies but no reaction to the polysaccharide occurred. Thus, of the entire group of patients included in Table I a positive skin reaction was expected in 39 by virtue of the fact that they were recovered cases of Type I, II or III infection whose sera contained type-specific antibodies. Positive reactions were obtained in 35 of them.

The cutaneous response to the nucleoprotein fraction of the Pneumococcus was studied in the same group of cases. The results have confirmed the validity of the earlier conclusions. The delayed protein reaction appeared first during convalescence. The reactivity of the patient did not appear to be influenced by the titer of circulating antibodies for the protein fraction.

Occurrence of Antibodies to Heterologous Types of Pneumococcus

Other observers have repeatedly demonstrated the occurrence of circulating antibodies for the homologous type of Pneumococcus at or about the time of crisis. The present investigation confirms these results and, in addition, discloses the fact that at the time when type-specific antibodies are first demonstrable in the blood the patient becomes reactive to the cutaneous injection of the homologous type-specific polysaccharide. The reactions have been specifically limited to the homologous types. In the present study repeated tests were made with the type-specific polysaccharides of Types I, II and III with a view to determining the length of time through which the reactive capacity might persist. In view of the fact that positive results at the time of recovery were strictly limited to the homologous type, it was rather surprising to observe after repeated tests that a positive skin reaction was obtained in certain instances with a polysaccharide of heterologous type as well. With the appearance of the skin reaction to the heterologous polysaccharide, circulating antibodies for the heterologous type of Pneumococcus were demonstrable in the patient's serum, in addition to those for the homologous type. The chief interest in this report, therefore, is concerned with the development of specific antibodies for more than one type of Pneumococcus in patients who have repeatedly received intradermal injections of type-specific carbohydrates. These "additional" antibodies were

characterized by the fact that they were heterologous in type to that of the infecting organism and were not demonstrable at crisis but appeared 1 to 3 weeks after the patient's recovery. These features were disclosed as a result of what at first appeared to be a discrepancy in specificity of the cutaneous reaction. The results can be more clearly brought out by describing in detail the course of events in one patient.

M. A., a girl of 14 years, suffering from Type II pneumonia was admitted on the second day of disease. No circulating antibodies were demonstrable in the patient's serum at this time. Crisis occurred on the tenth day. On the thirteenth day skin tests with the polysaccharides of Types I, II and III revealed a positive reaction only to the Type II material. The serum taken shortly thereafter contained circulating antibodies for Type II Pneumococcus alone. 8 days after the first test the skin tests were repeated and at this time positive reactions occurred both to Type II and Type I polysaccharides. At this time her serum contained antibodies for Type I as well as for Type II Pneumococcus. (See Chart 1.)

Because of the appearance of heterologous antibodies and skin reactions a series of patients was studied in order to determine the frequency with which the phenomenon occurred. Skin tests and antibody determinations were made at varying intervals in 18 patients. Of these, 3 were cases of Type I pneumonia, 7 of Type II, 3 of Type III and 5 of Group IV. At or about the time of crisis antibodies for the homologous type of Pneumococcus appeared in all but 2 of the Types I, II and III patients. In the Group IV cases tests were not carried out with the homologous organism. However, in no instance were heterologous antibodies demonstrable at that time. As convalescence progressed, repetition of skin tests and antibody determinations revealed that there had *developed*, since they were previously absent, specific antibodies and a positive skin reaction for at least one of the heterologous types of Pneumococcus. Table II shows the intervals between the appearance of homologous and heterologous antibodies in relation to the day of disease and recovery.

From Table II it can be seen that in 10 of the 18 patients heterologous antibodies were detected during convalescence; in 3 Type I patients, 4 Type II, 2 Type III, and 1 of Group IV. In the great majority of cases the presence of circulating antibodies for the heterologous type was first indicated by a positive skin test to the polysac-

TABLE II

Differences in Time of Appearance of Homologous and Heterologous Antibodies in Patients Receiving Skin Tests with Type-Specific Polysaccharides

Patient	Antibodies to homologous type		Antibodies to heterologous types				Day of crisis
	Type	Day observed	Type			Day observed	
L. H. (serum), No. 7165	I	3			III	17	6
M. P. (serum), No. 7263	I	8		II		14	5
F. R., No. 7253	I	9		II		22	7
M. C., No. 7183	II	14	I			19	5
G. M., No. 7248	II	10	I			19	9
E. C., No. 7269	II	11	I			18	9
M. A., No. 7261	II	15	I			22	10
H. R., No. 7262	III	6		II		19	
			I			26	8
K. R., No. 7143	III	5	I	II		21	4
A. P., No. 7211	IV	Not determined	I			26	
					III	44	7
M. C., No. 7189	II	15	0	0	0		8
I. S., No. 7195	II	9	0	0	0		6
C. Q., No. 7225	II	0	0	0	0		7
J. F., No. 7171	III	0	0	0	0		10
G. B., No. 7178							8
R. F., No. 7256	IV	Not determined	0	0	0		9
T. R., No. 7197							5
G. R., No. 7236							8

charide of that type. The type of Pneumococcus for which heterologous antibodies developed was in 7 instances Type I, in 4 Type II, and in 2 Type III. In 3 cases specific antibodies for 2 heterologous types appeared. Although the homologous antibodies were detectable at the time of crisis, those for heterologous types were usually noted in the second week of convalescence, the earliest being 9 days after recovery, the latest 37 days after recovery. The period of time during which antibodies were detectable in the serum and skin reactivity remained positive varied from a few days to several months.

These observations led to the hypothesis that the mechanism involved in the production of antibodies for heterologous types of Pneumococcus was associated with the intradermal inoculations of the type-specific

TABLE III

The Incidence of Type-Specific Antibodies in the Control Series of Patients

Type of infecting organism	No. cases	Antibodies for homologous type of Pneumococcus	Antibodies for heterologous types of Pneumococcus
I	6	5	1*
II	6	6	1*
III	2	2	0
Group IV	7	Not determined	1**

* Type III antibodies present during disease and recovery.

** Type II antibodies present during disease and recovery.

polysaccharides. In order to obtain more information on this point the next 21 pneumonia patients admitted to hospital were studied as controls. In this series no injections of the specific polysaccharides were made but the serum was tested at intervals for circulating type-specific antibodies to Pneumococcus Types I, II and III. In the Group IV cases tests were not made for antibodies reactive with the infecting organism. In most of the 21 cases homologous antibodies were observed at the time of recovery. However, in striking contrast to the group of patients which had received repeated injections of the specific soluble substance, no patient in the control series *developed* antibodies for a heterologous type. In only 3 patients (1 each of Types I, II and Group IV) antibodies for a type other than that of the infectious agent were observed. In these three instances the presence

of the heterologous antibodies was detected in low titre early in the acute illness and the concentration of the antibodies did not increase during convalescence. They, therefore, bear no apparent relation to the present infection but are presumably attributable to a preexisting state. This interpretation is substantiated by the fact that some normal individuals possess in their serum type-specific antibodies.

Table III presents the distribution of the types of Pneumococcus in the control group of patients and the number in whom circulating antibodies were detectable.

The 21 patients who served as controls were comparable to the test group with regard to age incidence, distribution of types of infecting Pneumococcus, severity of the infection and period of observation.

The patients in whom heterologous antibodies were observed, received injections of 0.01 mg. of the type-specific capsular polysaccharides derived from the Pneumococcus of Types I, II and III. The average number of injections before the appearance of antibodies for the corresponding type of Pneumococcus was three. In 3 cases the antibodies were detected after a single injection. The largest total amount which was followed by heterologous antibody production was 0.05 mg. whereas in other cases heterologous antibodies did not develop even after the injection of as much as 0.08 mg. of the polysaccharides. These irregularities demonstrate the fact that the production of antibodies is not solely dependent upon the quantity of polysaccharides injected. The average time between the last injection and the appearance of the antibodies was 6 to 10 days but in one case they were first detected 20 days after the last injection. The exact data regarding the individual patients in whom antibodies for heterologous types of Pneumococcus developed are presented in graphic form in composite charts. These charts show in relation to the day of disease, the time of appearance of type-specific agglutinins and mouse protective antibodies for homologous and heterologous types of Pneumococcus, the time, number and results of skin tests with the 3 type-specific polysaccharides and the period during which the observations were continued.

DISCUSSION

The primary interest of the present work centers in a group of 18 cases of lobar pneumonia 10 of whom, in convalescence, developed

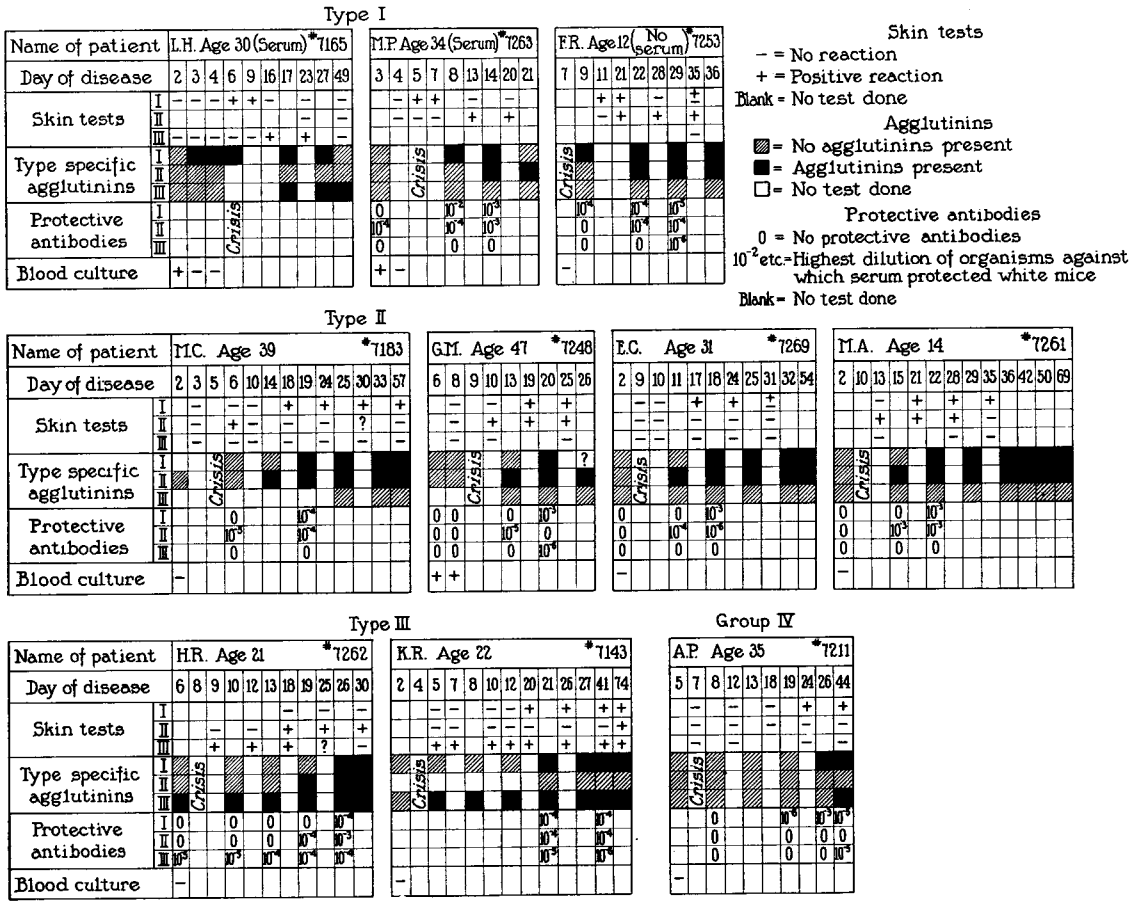

CHART 1. The development of heterologous antibodies in patients receiving intradermal injections of type-specific polysaccharides.

circulating antibodies for at least one type of Pneumococcus heterologous to that etiologically related to the disease. All of the patients in whom the phenomenon was observed had received repeated intradermal injections of 0.01 mg. of specific capsular polysaccharide from each of Types I, II and III Pneumococcus. Antibodies for the homologous type of Pneumococcus appeared at about the time of crisis. Antibodies for heterologous types, on the other hand, appeared usually in the second week of convalescence after 1 to 5 injections of the respective polysaccharides.

A control group of patients, 21 in number, received no skin tests. Their sera, in the great majority of instances, contained antibodies for the homologous type of Pneumococcus. In 3 of these cases antibodies for a heterologous type were present but the time of appearance differed from that of the antibodies observed in the first group in that they were detectable in low concentration not only in convalescence but during the acute illness as well. Nor was any increase in concentration observed during convalescence. Antibodies to the heterologous types which were encountered in these 3 cases, apparently preexisted and their appearance is presumably unrelated to any incident occurring in the course of the present infection. The validity of this supposition is borne out by the fact that type-specific antibodies may be found in the serum of certain normal individuals, who give no history of lobar pneumonia. But, in no case were heterologous antibodies first detected in convalescence unless the patient had previously received intradermal injections of the type-specific polysaccharides.

Many investigators have noted the presence of specific antibodies for the homologous strain or type of Pneumococcus in the blood of pneumonia patients at the time of crisis. Bacteriotropins (5, 6, 7), agglutinins (5, 7, 8, 9, 10, 11, 12) and protective antibodies (13, 14, 15, 6, 12) have all been demonstrated but so far as can be discovered, in no instance has the development of antibodies for a heterologous type been described. Chickering (11) in 1914 followed the course of agglutinin production to all types throughout convalescence in 40 cases but found only antibodies for the homologous type. Clough (7) in 1919, using the serum of patients convalescent from pneumonia due to known types of Pneumococcus, obtained similar results with phagocytic and agglutination reactions. In the sera of 25 normal

individuals, however, Clough (16) found protective antibodies for Type I in 4 of 18 tests, for Type II in 8 of 18 tests; and of 19 tests for Type III antibodies, 8 were positive. Ward (20) tested the capacity of the defibrinated blood of normal individuals to inhibit the growth of pneumococci of Types I, II and III. In each instance a variable degree of pneumococcidal action was observed against one or more types of the organism. Furthermore, this property was present in the blood of patients early in the course of pneumonia even against the type of Pneumococcus producing the infection. Robertson and Cornwell (21), employing serum-leucocyte mixtures from normal human beings, found that the blood of all persons tested by this method possessed pneumococcidal properties for at least one type of Pneumococcus and in the majority of instances for two or more types.

In evaluating the factors involved in the production of antibodies for types of Pneumococcus heterologous to that causing the infection several possible mechanisms may be considered.

It is possible that antibodies for other types were present before the onset of disease and were masked during the acute infection only to return with convalescence. Or that, present in minimal amounts before the disease, the infection served to cause an outpouring of antibodies in detectable concentrations,—the anamnestic reaction first observed by Cole (17).

Interconvertibility of types as described by Griffith (18) and later studied by Dawson (19) offers a possible explanation. One might assume that with the development of type-specific antibodies to the homologous organism a change of type occurred and, as a result, antibodies to the secondary type appeared.

The evidence presented in this report implicates the polysaccharides employed in intracutaneous tests and suggests that they were antigenic. On the basis of this assumption, the conditions under which the investigation was carried out may have favored antibody production. No previous data are available regarding the antigenicity in humans of the purified type-specific polysaccharides of the Pneumococcus. On the basis of animal experiments in this laboratory these substances, in the pure state, are considered non-antigenic. It is possible, however, that in the process of recovery from infection, a highly reactive state exists in the human organism which responds to stimuli otherwise

ineffective. In the removal of the inflammatory material from its focus in the lungs, as takes place in pneumonic resolution, the absorbed material may act as an adjunct to the polysaccharides (the "Schleppe" of the German investigators) forming a combination possessing antigenic properties. The polysaccharides which, under ordinary conditions, are haptens, may then acquire antigenic properties and determine the specificity of the antibodies produced. It is of course also possible that the particular preparations used in this study, although they were prepared with great care, still contained minute amounts of combined polysaccharides.

At present it is impossible to offer any final statement as to the exact mechanism by which antibodies for the heterologous types are produced. Further work is being carried on with normal individuals and patients in an attempt more completely to elucidate the factors which play a rôle in the development of these heterologous antibodies.

SUMMARY

The majority of patients convalescent from pneumonia due to Types I, II and III Pneumococcus develop at the time of recovery circulating antibodies for the homologous type of organisms. At the same time an immediate wheal and erythema reaction followed the intradermal injection of the homologous type-specific polysaccharide in 100 per cent of Type I patients, 58.8 per cent of Type II patients, and 44 per cent of Type III patients.

In a group of 18 patients repeatedly tested with the type-specific polysaccharides, 10 developed in the second or third week of convalescence circulating antibodies for one or more heterologous types. In none of 21 control patients was this phenomenon observed.

It is suggested that the development of circulating antibodies for heterologous types of Pneumococcus was associated with the previous intradermal injections of the type-specific polysaccharides.

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