SEROLOGICAL REACTIONS IN PNEUMONIA WITH A NON-PROTEIN SOMATIC FRACTION OF PNEUMOCOCCUS*

By WILLIAM S. TILLETT, M.D., and THOMAS FRANCIS, Jr., M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

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It has been shown (1) that pneumococci contain two constituents which are chemically and antigenically distinct. One of these, the type-specific component, is a complex polysaccharide, predominantly present in the capsule of the organism; the other, a substance common to the pneumococcus species, is the so-called nucleoprotein, contained for the most part in the body of the cell. That these two chemically distinct fractions are responsible for the production of two qualitatively different antibodies has been demonstrated (1, 2).

The present report is based upon observations made with a third fraction derived from pneumococci and chemically distinct from both type-specific capsular polysaccharide and non-type-specific somatic nucleoprotein. For purposes of reference this substance is designated Fraction C. The chemical nature of Fraction C and the method of purification together with certain experimental observations are presented in a separate communication (3). In this report it is sufficient to state that Fraction C is a non-protein material of somatic origin and appears to be a carbohydrate common to the pneumococcus species. Although final proof of its exact nature rests upon chemical analysis, nevertheless convincing evidence of the separate identity of Fraction C is brought out by the serological reactions to be described.

Material and Methods

Preparation of Fraction C.—The material employed in the serological tests was derived from a degraded, non-type-specific R strain of Pneumococcus. A strain of this character was employed in order to minimize the presence of type-specific carbohydrate. Fraction C was obtained in the following manner: The organisms

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contained in several liters of full-grown broth culture of an R strain were centrifuged and resuspended in normal salt solution in 60 to 100-fold concentration. The bacteria were then frozen and thawed several times until dissolution had been effected. 0.3 cc. to 0.5 cc. of normal acetic acid was added and the solution boiled for 8 to 10 minutes. The heavy coagulum thus formed was removed by centrifugation. Acidulation and boiling were repeated to insure removal of all acid and heat precipitable material. The final water-clear supernatant fluid, neutralized with normal NaOH, contained Fraction C. Material prepared according to this simple procedure was comparable in reactivity and specificity to more highly purified preparations. Consequently, further steps in purification were not, as a rule, carried out. Some lots, however, were treated by repeated precipitation with 4 to 5 volumes of 95 per cent alcohol.

In the charts the reaction is represented as occurring in dilutions of C substance as high as 1 to 640,000. This figure is only approximate since a quantitative estimation was not made on all lots of material. However, accurate measurement was made with one sample; using it as a standard some of the sera were re-tested and found to react with a 1 to 1,000,000 dilution. Consequently the approximate figures given are believed to be conservative estimates.

The chemical fractionation of numerous bacteria by procedures similar in many respects to that just described, has been reported by others. Pick (4) derived from typhoid bacilli an apparently non-protein substance which reacted especially in immune serum. Zinsser (5) and Zinsser and Parker (6) isolated so-called residue antigens from tubercle bacilli, pneumococci, staphylococci, influenza bacilli, and typhoid bacilli. Laidlaw and Dudley (7) obtained a carbohydrate from tubercle bacilli. Furth and Landsteiner (8) obtained from typhoid and paratyphoid B cultures three fractions, one of which appeared to be non-protein. Day (9) derived from staphylococci an "antigenic specific substance." Lancefield (10) separated a somatic carbohydrate from hemolytic streptococci which she designated Fraction C and which is comparable in many respects to the pneumococcus Fraction C.

Pneumococcus Type-Specific Polysaccharides.—The preparations employed were made according to the method described by Heidelberger and Avery (11).

Pneumococcus Nucleoprotein.—The material was made by precipitation with acetic acid in the cold as described by Avery and Morgan (12).

Precipitin Tests.—0.2 cc. test serum was diluted to 0.5 cc. with physiological salt solution; to this was added an equal volume of varying dilutions of precipitinogens. Final readings were made after the mixtures had been kept in water bath at 37° for 2 hours and over night in the ice box.

Agglutination Tests.—0.5 cc. of varying dilutions of serum was added to 0.5 cc. of heat-killed pneumococci suspended in physiological salt solution. The tubes were incubated at 37° for 2 hours and allowed to stand overnight in the ice box before final reading was made.

Sera obtained at frequent intervals from patients acutely ill with pneumonia or convalescent from the disease have been mixed with varying dilutions of Fraction C and the presence or absence of precipitation noted. Charts 1 and 2 illustrate results which have been repeatedly obtained.

Chart 1 gives the results of observations in a case of Type II pneumococcus pneumonia. Serum obtained on admission to the hospital in the third day of disease reacted with high dilutions of Fraction C.

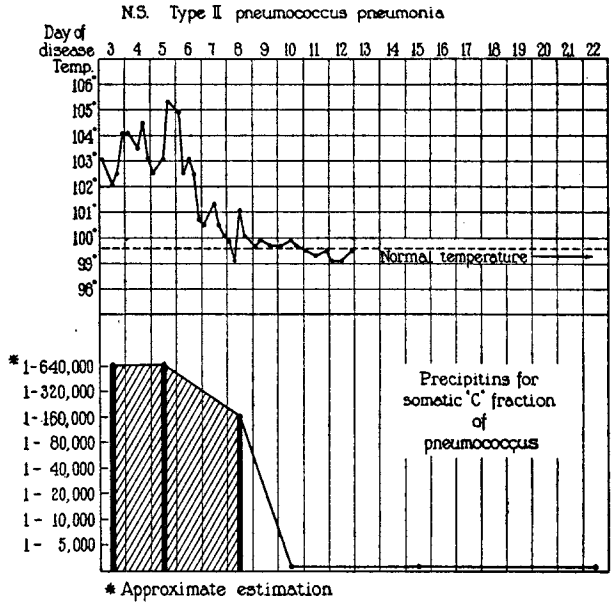

CHART 1

This reactive capacity dropped slightly on the day of crisis and completely disappeared in the next 2 days.

Chart 2 presents a similar course of events obtained with serum derived from a patient suffering from Group IV pneumococcus pneumonia. The phenomenon exhibited in Charts 1 and 2 has been observed in each of 50 patients ill with pneumococcus pneumonia. In each instance it has been found that serum obtained during the acute stage possesses a high titre of precipitins for Fraction C. A day or two after recovery this precipitating power is no longer detectable. The occurrence of the phenomenon is unrelated to the type of Pneu-

mococcus causing infection. The fact that serum obtained from patients on admission possessed, in every instance, anti-C precipitins is evidence of the early appearance of the reactivity. The serum obtained from one patient as early as 18 hours after the initial chill reacted with high dilutions of C substance. Individuals who succumbed to the disease maintained C precipitins until death. The age of patients, whose sera have been tested, ranged from 7 years to

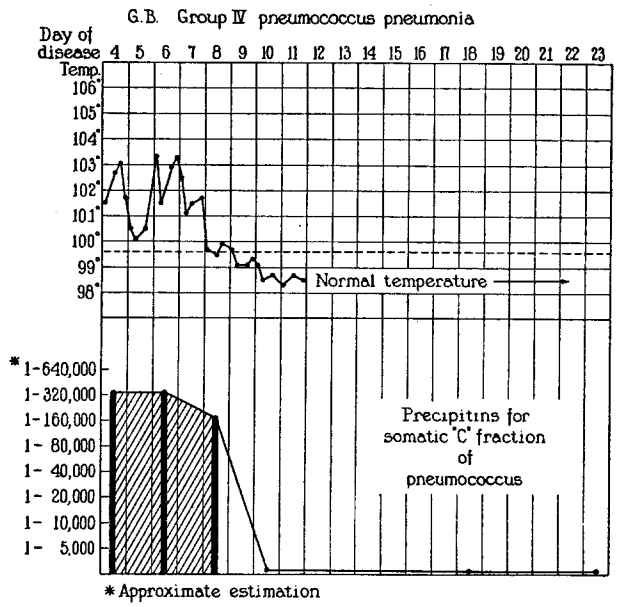

CHART 2

65 years; the phenomenon was present in the youngest and oldest. Sera from normal individuals have not been found to possess demonstrable precipitins for Fraction C.

The observations on the serological reactivity of pneumococcus Fraction C presented in this report are limited to precipitin tests made with sera from patients. However, it may be mentioned that antipneumococcus sera derived from animals, in some instances, precipitate Fraction C. This is particularly demonstrable with the antipneumococcus horse sera[1] used for typing of pneumococci. The

[1] The antipneumococcus horse sera were obtained through the courtesy of Dr. A. B. Wadsworth from the New York State Board of Health Laboratories, Albany, New York.

available lots of antisera of Types I, II and III all precipitate Fraction C, Type III serum apparently reacting in highest titre. Of sera derived from 24 rabbits immunized with either Types I, II, III, or R pneumococci, only 3 possessed demonstrable anti-C precipitins; each of these animals had received heat-killed

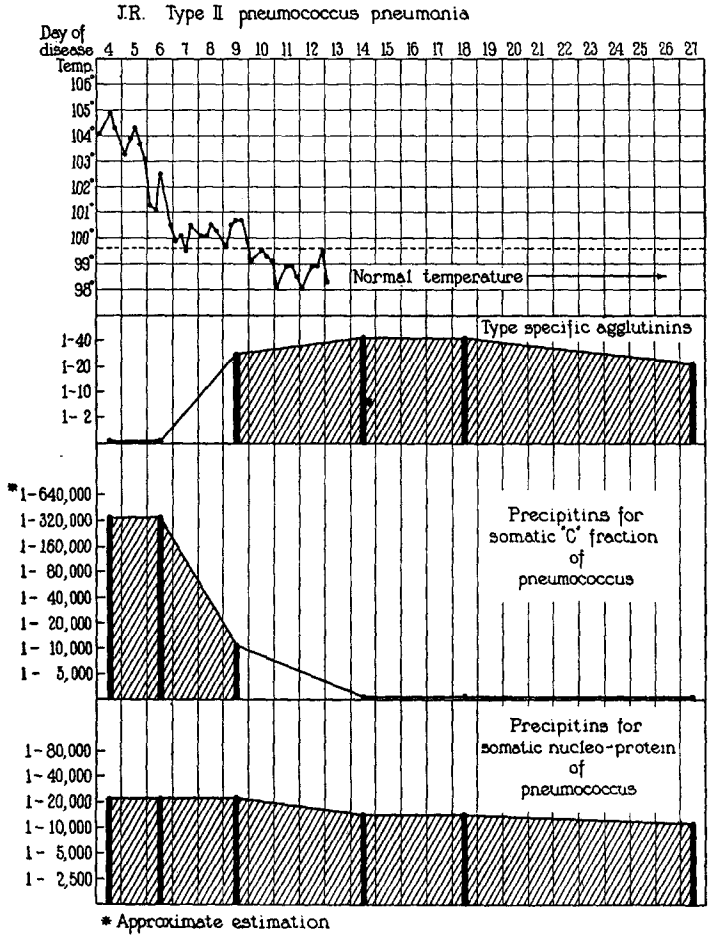

CHART 3

cultures of Type III Pneumococcus. At the present time the conditions which favor the production experimentally of anti-C antibodies are not understood.

For purposes of comparison, sera derived from patients during the course of pneumonia were tested for the presence of type-specific

antibodies and for pneumococcus protein antibodies as well as for C precipitins. The results when charted in relation to the course of pneumonia, present three different curves of antibody titre. Observations of this character are presented in Chart 3.

From Chart 3 it will be seen that type-specific antibodies are absent during the acute illness and are first demonstrable at the time of crisis, whereas precipitins for C substance are present in high titre during the febrile stage and are no longer detectable a few days after

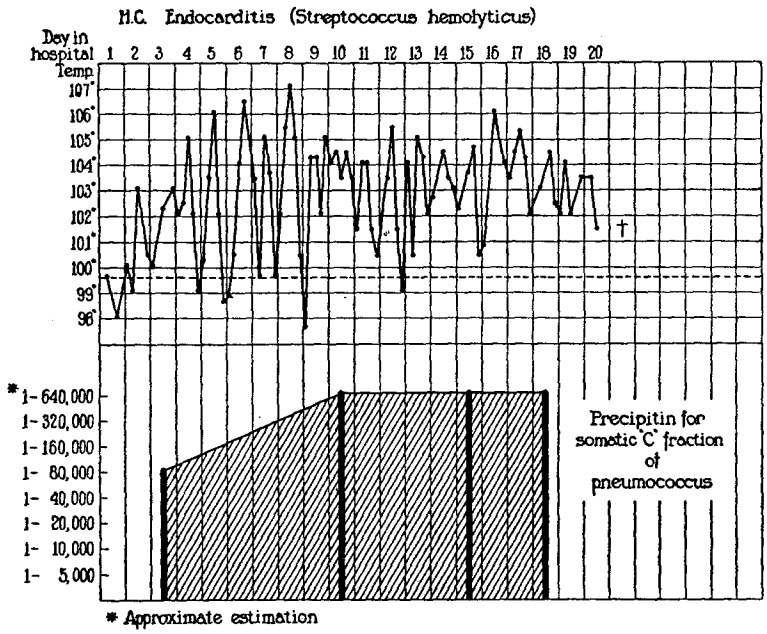

CHART 4

the critical fall in temperature. Antiprotein antibodies do not vary markedly during the course of illness, although there seems to be a slight reduction in titre as convalescence progresses. Observations similar to those given in Chart 3 have been made on twenty cases of pneumonia. It is an interesting fact, therefore, that with three chemically distinct fractions derived from the same bacterial cell, serological reactions involving three qualitatively different antibodies may be demonstrated during the progress of pneumococcus pneumonia.

Up to this point the report has been limited to the results obtained with the sera of patients suffering from pneumococcus infection. A limited number of individuals acutely ill with other febrile diseases have been available for comparative tests. Four cases of hemolytic streptococcus infection ending fatally were observed from admission to the hospital until death. Three of these had streptococcus pneumonia. In each instance their sera precipitated pneumococcus Fraction C equally as well as did sera from cases of pneumococcus pneumonia. The fourth patient had malignant endocarditis; hemolytic

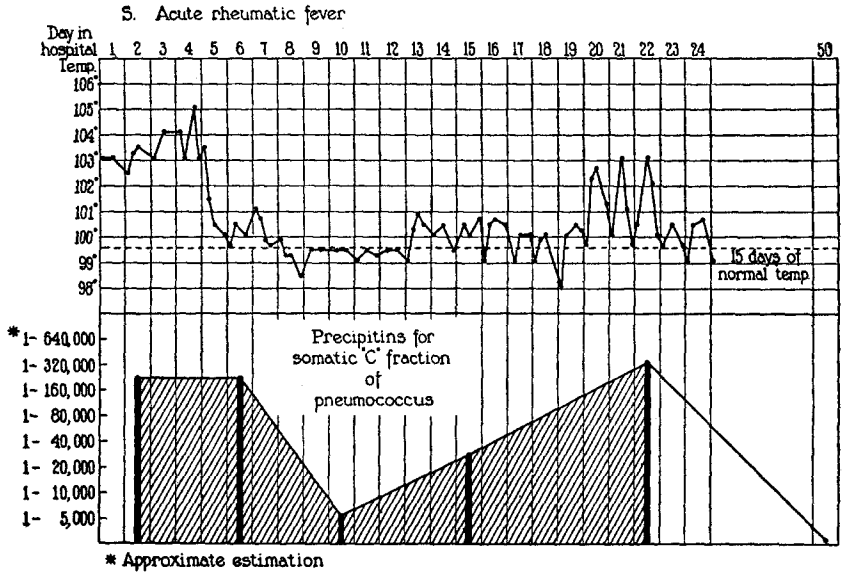

CHART 5

streptococci in large numbers were repeatedly demonstrated in blood cultures. The result of serological tests made with pneumococcus C substance in this case is presented in Chart 4.

As can be seen from Chart 4 the patient's serum maintained throughout the illness the capacity to react in high titre with C substance of Pneumococcus.

Fifteen cases of acute rheumatic fever have been followed by similar serological tests through stages of active disease and remission. Chart 5 records the results obtained in one of this group.

As demonstrated by Chart 5 the course of the temperature and the capacity of the patient's serum to precipitate pneumococcus Fraction C closely parallel each other. The same relations were found to hold in the other fourteen cases of rheumatic fever. All of the patients at the time of elevated temperature exhibited symptoms and signs of active disease.

TABLE I

Reactions with Fraction C in Febrile Diseases Other than Pneumococcus Pneumonia

Patient	Disease	Temperature	*Dilutions of Fraction C		
			1-10,000	1-80,000	1-120,000
Das.	Malaria	104°	−	−	−
Mek.	Lung abscess	102.5°	+	++	+++
Fol.	Cirrhosis of liver	103°	−	−	−
Mit.	Typhoid fever?	104°	−	−	−
Rid.	Pericarditis, rheumatic origin	103°	+	++	++
Reg.	Unexplained anaemia	104°	−	−	−
Hag.	Tuberculosis of lungs	102.5°	±	−	−
Mer.	Unexplained fever—Tbc.?	103.5°	−	−	−
Hin.	Osteomyelitis (staphylococcus)	105°	+++	+++	++
McD.	Measles	102.8°	−	−	−
Pe.	Chicken pox	103°	−	−	−
Mac.	Endocarditis (*Streptococcus viridans*)	104°	+	+	±

* Approximate estimation.

In addition to the hemolytic streptococcus infections and cases of acute rheumatic fever just mentioned, single tests were made on sera obtained in a miscellaneous group of diseases. The results are listed in Table I.

From Table I it can be seen that patients, whose sera precipitated pneumococcus Fraction C, were in each instance suffering from disease associated with Gram positive cocci. The number of cases so far available is too few to attach undue significance to this point. However, the results suggest that etiological agent and serological reactivity with Fraction C may have some causal relationship.

DISCUSSION

The observations recorded in this communication have been made with a constituent of pneumococcus cells which is chemically distinct from both the type-specific capsular carbohydrate and the somatic nucleoprotein. Although pneumococcus Fraction C in all probability is a nitrogenous sugar, it differs from the soluble specific substance in that it possesses no type-specificity and is contained, as a common species constituent, within the body of the cells. Pneumococcus Fraction C appears to be analogous to the somatic carbohydrate isolated from hemolytic streptococci by Lancefield (10).

By testing sera obtained from cases of lobar pneumonia during the progress of the disease two interesting observations were made. The first of these is the early appearance of precipitins for C substance and the second is the complete disappearance of the phenomenon 1 to 3 days after crisis. Sera obtained from patients on the day of admission to the hospital have in every instance reacted with Fraction C. This was true even of cases admitted in the first 24 hours of disease. Since the serum of no normal individual has exhibited this reaction, the appearance of C precipitins is associated closely with the onset of pneumonia. Although an explanation of the phenomenon is not yet available, a possible interpretation is suggested by the so-called "anamnestic" reaction. Cole (13) found that in an animal which had previously been immunized and in which the antibodies in the blood had disappeared a subsequent injection of the original antigen caused antibodies to reappear more rapidly than did the primary injections. Bieling (14) employing two antigens (*B. dysenteriae* and *B. typhosus*), found that the reinjection of one brought back heterologous as well as homologous antibodies. In the course of similar experiments McKenzie and Frühbauer (15) using four antigens of remote biological origin were able to cause heterologous antibodies promptly to reappear by the injection of one antigen. Applying the principles involved in the "anamnestic" reaction to the observations recorded in this paper, it is possible that the occurrence of pneumococcus C precipitins early in pneumonia may be determined by a previous experience of the individual with some suitable bacterial antigen.

In addition to the observations with Fraction C, the investigation

was extended to include a more complete study of the occurrence during pneumonia of antibodies reactive with pneumococcus constituents. In these latter experiments, therefore, tests were made for antibodies reactive with: 1. Type-specific capsular polysaccharide; 2. Non-type-specific somatic polysaccharide; 3. Non-type-specific somatic nucleoprotein. By charting the result of these serological reactions with reference to the course of the disease, the occurrence of each of the qualitatively different antibodies was shown to follow a different course. It is an interesting fact, therefore, that during a *single* infection, the body responds in such a manner that antibodies reactive with different components of the causative organism become demonstrable at different stages of the disease. The results serve to illustrate the complexity of the immunological mechanism which presides over the response to bacterial infection. The observations recorded in this paper also demonstrate that the reactivity of a patient's serum with pneumococcus C substance is not specific for pneumococcus infection. Sera from a limited number of febrile diseases due to other causes have been tested. In this small group, precipitation of C fraction occurred most definitely in those instances where Gram positive cocci were proven to be or were suspected of being the etiological agent. If the implications contained in these few cases are substantiated by a larger number of tests the results suggest some, as yet undetermined, broad relationship existing among certain bacterial infections.

SUMMARY

1. Sera from individuals acutely ill with lobar pneumonia possess the capacity to precipitate in high titre a non-protein somatic fraction derived from pneumococci (Fraction C). Following crisis the reaction is no longer demonstrable.

2. Sera obtained from cases of pneumococcus pneumonia during illness and convalescence have been tested for antibodies specifically reactive with three chemically distinct constituents of Pneumococcus. The results, when correlated with the course of disease, demonstrate differences in the occurrence of each qualitatively distinct antibody.

3. The precipitation of pneumococcus Fraction C is not limited to the sera of individuals ill with pneumococcus infection. But in the

few other cases available for comparative tests, definite reactions have been obtained only in streptococcus and staphylococcus infections and in acute rheumatic fever.

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