FACTORS INVOLVED IN THE PRODUCTION OF IMMUNITY WITH PNEUMOCOCCUS VACCINE

II. Induction of Active Immunity during the Course of Lobar Pneumonia

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In a previous paper (1) the rate of development of immunity after injection of pneumococcus vaccine into normal animals was studied. In addition, the antigenic function of a vaccine made from the intact cell was compared with that derived from a watery extract of the cell free from formed elements. In each instance, a type-specific immunity was initiated. With the vaccine made from the intact cell, active immunity was demonstrated on the 3rd day after injection and, in the case of the filtrate, on the 4th day, in both instances increasing on the 5th and 6th days after administration of the antigen.

In the present study we administered pneumococcus vaccine to patients with lobar pneumonia to determine whether a similar state of active immunity could be induced during the course of the infection.

The literature concerned with the experimental production of pneumococcus immunity was reviewed in the first report. The attempts to induce active immunity in man during the course of lobar pneumonia may now be referred to.

Lister (2) injected pneumococcus vaccine intravenously into 40 South African natives suffering from lobar pneumonia, and concluded that the disease was favorably influenced. One patient who received an intravenous inoculation of 10 billion organisms the first dose, 20 billion the second dose and 30 billion the third dose, showed agglutinins in his serum 6 days subsequent to the first injection. Other investigators have employed subcutaneous injections of much smaller doses, such as 30 to 300 millions of diplococci, with reported lowering of the mortality rate of pneumonia as compared with control series. (Wright (3), Wynn (4),

Girdwood (5), Lambert (6), French (7), Sutton (8).) Lister was unable to demonstrate agglutinins in the serum of animals or patients when the dose of vaccine was limited to hundreds of millions of cocci. In only one investigation of subcutaneous immunization was a study made of the appearance of protective substance in the patient's serum after administration of Pneumococcus Types I, II and III (Lambert). The results in this instance showed that protective substance appeared only against the type of pneumococcus which corresponded to that present in the patient's sputum.*

However, it was shown by G. and F. Klemperer (9) that the serum of patients after the crisis possessed curative powers in pneumococcus infection in the rabbit, and Neufeld (10) demonstrated agglutinins in patients convalescing from pneumonia.

That protective substance appeared in the serum of patients at the time of the crisis for the type of organism which was present in the sputum was demonstrated by Dochez (11), Clough (12) and others subsequently.

It is evident that the appearance of protective substance for the homologous type of pneumococcus may be ascribed to the natural course of the disease in recovered patients, and not necessarily to the injection of vaccine. It seemed to us necessary to inject heterologous types (*i.e.*, types of pneumococci not present in the sputum of the patient) and test for the appearance of protective substances against these types in order to demonstrate that active immunity may be induced by injection of vaccine during the course of lobar pneumonia. Thus, if specific protective substances against Pneumococcus Type II appear after injection of Type II vaccine in a patient suffering with Type I pneumonia, the initiation of this immunity might then be ascribed to the introduction of the vaccine.

The appearance of protective substances against the type of pneumococcus found in the patient's sputum following the injection of an homologous vaccine could only be ascribed to the vaccine if heterologous immunity was previously demonstrated under similar circumstances.

Another difficulty encountered is that the serum of normal individuals occasionally possesses demonstrable immunity against one or more types of pneumococcus (Neufeld and Haendel (13), Clough (14)). In certain of our patients the blood before injection of vaccine

* As these tables are unpublished, it is due to the kindness of Dr. Lambert that I have been allowed to inspect them and quote the results.

contained protective substances against one or two types of pneumococcus. These cases were therefore excluded.

Additional confirmation of the fact that heterologous protective substance does not spontaneously occur during the time interval covered by our experiments is obtained by the complete absence of heterologous immunity in two patients who were treated with subcutaneous immunization and a much larger group (14 cases) treated by intradermal injection.

Methods

The antigens were prepared as described in the previous paper (1). A virulent Pneumococcus Type I (or Type II or III) culture was passed through a mouse in preparation of the vaccine. 0.2 cc. of the heart's blood obtained from the animal immediately after death was inoculated into a test tube containing 5 cc. of beef-infusion broth with 2.5 per cent of human serum. After 8 hours' incubation, this was used to inoculate 250 cc. of similarly prepared broth. Incubation was carried on for 8 hours. The culture was then centrifuged and the supernatant broth poured off. Care was taken not to loosen the sedimented bacteria at the bottom of the centrifuge tube which was now carefully rinsed with normal saline to wash off all broth adhering to it. Normal salt solution was added to dilute the bacteria to a concentration of 10 billion organisms to 1 cc. of water. Sterilization was accomplished by heating the suspension for 1 hour at 60°C. This constituted the serum vaccine. For preparation of the filtrate the serum vaccine was shaken by hand for 10 minutes and centrifuged. The supernatant fluid was passed through a Berkefeld filter, bottled and placed in the ice-box. If sterile on culture, tricresol was added to a concentration of 0.3 per cent.

The filtrate was injected intravenously into patients in doses of 1 to 7 cc. Blood was withdrawn before injection and daily on the 3rd to the 6th day thereafter. An estimation of protective substance was made in the usual manner by injection of graded doses of virulent culture with 0.2 cc. of serum intraperitoneally into mice. The vaccine described above was injected intravenously in doses of 1 to 5 billion organisms. Intradermal injection was made in doses of 1 to 3 billion organisms (0.1 to 0.3 cc. of vaccine).

The test for protective substance was performed with maximally virulent organisms, the culture being generally fatal in 10^{-8} or 10^{-9} cc. in 24 to 40 hours. Controls (usually 3 to 5 mice for each type) were tested in every experiment. The dilution of culture employed was generally 0.001 cc., 0.0001 cc. and 0.00001 cc. Survival (noted S in tables) thus indicated protection against 100,000, 10,000 and 1,000 M. L. D. (minimal lethal doses), respectively. In instances in which the culture killed in a dilution of 10^{-9}, survival of an injection of 0.001 cc. indicated that the patient's serum protected a mouse against 1,000,000 M.L.D.

The "Before," 3rd, 4th, 5th and 6th day bloods were tested by injecting the graded dilutions of culture plus 0.2 cc. of patient's serum into nine mice for each type: I, II and III. Thus, each experiment consists of the results in terms of survival (S) or death (number of hours after injection) on approximately 150 mice, including controls. Since the complete tabulation of all the experiments would occupy too large a space, the findings are condensed into tables which note the day on which protective substance appeared. This consisted of survival of one to three animals on one day followed by a larger number on succeeding days. A single survival that was not followed on other days by more survivals was reckoned as probably due to chance variation and not as evidence of passive immunity. Illustrative tables of individual experiments will be cited in each group.

RESULTS

Pneumococcus vaccine was administered in all to 37 patients, 29 of whom had pneumonia and 8 had miscellaneous diseases. The pneumococcus filtrate was injected intravenously in 7 patients, the vaccine of the whole organism was injected intravenously in 15 patients, intradermally in 13 patients and subcutaneously in 2 patients.

In the accompanying table (Table I), the results of the subcutaneous injection of 3 billion organisms of each of the three types, I, II and III, are shown. The patient was a man 36 years old, suffering from lobar pneumonia with Pneumococcus Type I in the sputum, a sterile blood culture and consolidation of the R. M. L. and R. L. L. He was treated on the 6th day of disease.

It is apparent from Table I that active immunity which was passively transmitted to mice began on the 3rd day and increased on the 4th, 5th and 6th days after injection in the case of Pneumococcus Type I, but was absent in Pneumococcus Types II and III. Since recovery in Type I pneumonia is followed by the spontaneous appearance of protective substance for that type, the presence of mouse survival 9 days after onset of illness for the homologous type of pneumococcus and the absence of mouse survival in the heterologous types indicate that the subcutaneous injection of pneumococcus vaccine was not effective in inducing active immunity as demonstrated by mouse protection during the 6 day period of the experiment. The second case was a woman of 33 years who had lobar pneumonia, Pneumococcus Type IV in the sputum, sterile blood culture and consolidation of the L.L.L. On the 3rd day of the disease she was given a subcutaneous injection of Pneumococcus II and III, 2.5 billion organisms of each type. No evidence of protective substance was apparent during the 6 day period after injection for Pneumococcus II or III.

The first case in the group treated by intravenous injection of vaccine (Case 1) is that of a man 49 years old who had chronic pulmonary tuberculosis, moderately advanced, and an acute serofibrinous pleurisy (which at first simulated pneumonia)

of 2 days' duration. He was given 2 billion organisms of each type, I, II and III, intravenously.

As seen in Table II, protective substance was present on 4th, 5th and 6th days after injection for Pneumococcus Types II and III, and absent for Pneumococcus

TABLE I

Appearance of Protective Substance in Patient's Serum after Subcutaneous Injection of Pneumococcus Vaccine

Case 36.

Day after vaccination	Survival after injection of Pneumococcus I culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus II culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus III culture plus 0.2 cc. patient's serum		
	0.001	0.0001	0.00001	0.001	0.0001	0.00001	0.001	0.0001	0.00001
Before	24	24	40	24	24	24	24	24	24
	24	24	72	24	24	24	24	24	24
	24	24	72	24	24	24	24	24	24
3rd	24	24	40	24	24	24	24	24	24
	24	24	72	24	24	40	24	24	24
	40	72	S	24	24	24	24	24	24
4th	24	S	S	24	24	24	24	24	24
	24	S	S	24	24	24	24	24	24
	24	S	S	24	24	24	24	24	24
5th	24	24	40	24	24	24	24	24	24
	24	24	S	24	24	24	24	24	24
	24	S	S	24	24	24	24	S	24
6th	24	S	24	24	24	24	24	24	24
	40	S	40	24	24	24	24	24	24
	S	S	S	24	72	40	24	24	24
	Controls			Controls			Controls		
	0.00001 } 40 hrs.			0.00001 } 24 hrs.			0.00000001 } 40 hrs.		
	0.000001			0.000001			0.000000001		
	0.0000001			0.0000001					
				0.00000001					
				0.000000001					

Diagnosis.—Lobar pneumonia, Pneumococcus Type I. Patient was given pneumococcus vaccine subcutaneously, 3 billion organisms of each type, I, II and III.

Type I. Since the patient was not suffering from pneumonia or pneumococcus infection, the appearance of mouse survival leads to the conclusion that an immunity against Pneumococcus Types II and III was produced 4 days after intravenous injection of the corresponding vaccine, although no response was elicited in the case of Type I.

The next case cited (Case 4) is that of a man 28 years old who developed a postoperative bronchopneumonia involving the R.L.L. and L.L.L., in whose sputum were *Streptococcus haemolyticus*, staphylococcus and Friedländer bacillus, but no pneumococci. He was given 4 billion pneumococci, Type II, intravenously on the 6th day of disease, and 3 days later protective substance appeared in his blood for

TABLE II

Appearance of Protective Substance in Patient's Serum after Intravenous Injection of Pneumococcus Vaccine

Case 1.

Day after vaccination	Survival after injection of Pneumococcus I culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus II culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus III culture plus 0.2 cc. patient's serum		
	0.001	0.0001	0.00001	0.001	0.0001	0.00001	0.001	0.0001	0.00001
Before	20	30	30	40	40	40	40	40	40
	30	30	30	40	40	40	40	40	40
	30	30	30	40	40	40	40	40	40
3rd	20	35	30	30	40	40	40	40	40
	30	32	30	30	40	40	40	40	40
	30	35	30	30			40	40	40
4th	35	40	40	30	40	40	40	60	40
	40	40	40	30	40	S	40	80	S
	40	40	40	30	S	S	60	S	S
5th	20	40	40	60	S	S	S	40	40
	20	40	40	60	S	S	S	S	60
	40	40	48	S	S	S	S	S	S
6th	20	48	48	120	S	S	60	40	S
	48	48	48	S	S	S	60	60	S
	48	48	48	S	S	S	40	60	S

Controls		Controls		Controls	
0.00001	40 hrs.	0.00001	40 hrs.	0.00001	40 hrs.
0.000001		0.000001			
0.0000001		0.0000001			

Diagnosis.—Acute serofibrinous pleurisy. Patient was given pneumococcus vaccine intravenously, 2 billion organisms of each type, I, II and III.

Type II (continuing on the 4th, 5th and 6th days) but none developed for Types I and III.

In this case of pneumonia without pneumococcus infection, immunity was developed specifically for the type of pneumococcus injected and not for the other two types during the febrile period of the disease. It began 3 days after intravenous injection of the vaccine.

In the case shown in Table IV (Case 6), the patient was a man 47 years old who

had lobar pneumonia, Pneumococcus Type IV in his sputum, negative blood culture and consolidation of the R.L.L. He was given 4 billion pneumococci of each Type II and III in two doses on the 5th day of disease.

It will be observed that protective substance appeared on the 4th day after injection for Type II pneumococcus, and increased markedly on the 5th and 6th

TABLE III

Appearance of Protective Substance in Patient's Serum after Intravenous Injection of Pneumococcus Vaccine

Case 4.

Day after vaccination	Survival after injection of Pneumococcus I culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus II culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus III culture plus 0.2 cc. patient's serum		
	0.001	0.0001	0.00001	0.001	0.0001	0.00001	0.001	0.0001	0.00001
Before	24	24	24	24	40	40	24	40	40
	40	40	40	40	40	40	24	40	40
	40	40	40	40	100	40	40	40	40
3rd	24	24	40	24	100	S	24	40	40
	40	40	40	40	S	S	24	40	40
	40	40	40	40	S	S	24	40	40
4th	24	20	24	24	40	40	24	24	24
	24	40	40	40	40	S	40	24	24
	24	40	40	40	40	S	40	40	40
5th	24	40	40	24	40	S	24	40	40
	40	40	40	40	S	S	40	40	40
	40	40	40	40	S	S	40	40	40
6th	24	40	40	24	40	40	24	40	40
	24	40	40	24	40	S	24	40	40
	24	40	40	40	S	S	40	40	40

Controls		Controls		Controls	
0.00001	40 hrs.	0.00001	40 hrs.	0.00001	40 hrs.
0.000001		0.000001		0.000001	
0.0000001		0.0000001		0.0000001	
0.00000001					

Diagnosis.—Post-operative bronchopneumonia. Sputum contained *Streptococcus haemolyticus*, staphylococcus and Friedländer bacillus. Patient received 4 billion Pneumococcus Type II intravenously.

day. (On the latter day, 0.2 cc. of his serum protected against 0.01 cc. of culture with a virulence of 10^{-9}, approximately 10,000,000 M.L.D.) In the case of Type III, protective substance appeared on the 6th day after injection and was not as great, (0.2 cc. serum protecting against 0.001 cc. of a culture with a virulence of

10^{-7} or 10,000 M.L.D.). In this instance, definite heterologous immunity was developed during the course of pneumococcus pneumonia.

The case presented in Table V (Case 12), is a man 40 years old who had lobar pneumonia, with Pneumococcus Type I in the sputum, negative blood culture

TABLE IV

Appearance of Protective Substance in Patient's Serum after Intravenous Injection of Pneumococcus Vaccine

Case 6.

Day after vaccination	Survival after injection of Pneumococcus II culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus III culture plus 0.2 cc. patient's serum		
	0.01	0.001	0.0001	0.01	0.001	0.0001
Before	24	24	24	24	24	40
	24	25	25	24	40	40
	24	25	40	40	50	40
3rd	24	24	24	24	24	25
	24	24	40	40	25	40
	24	25	40	40	25	40
4th	24	24	S	24	25	40
	24	24	S	24	40	40
	24	24	S	25	40	40
5th	24	24	24	24	24	24
	24	S	S	24	24	40
	24	S	S	40	24	40
6th	40	S	S	24	40	40
	S	S	S	24	S	S
	S	S	S	24	S	S

Controls		Controls	
0.0001	24 hrs.	0.0001	24 hrs.
0.00001	25 hrs.	0.00001	25 hrs.
0.000001	28 hrs.	0.000001	28 hrs.
0.0000001	30 hrs.	0.0000001	30 hrs.
0.00000001	35 hrs.		
0.000000001	40 hrs.		

Diagnosis.—Lobar pneumonia, Pneumococcus Type IV. Patient received 4 billion organisms of each Type II and III vaccine.

and consolidation of the R.M.L. He received 3 billion pneumococci intravenously of each Type I and II on the 3rd day of illness.

As seen in Table V, protective substance developed for the homologous Type I on the 3rd day after injection, and gradually increased on the 4th, 5th and 6th days; for the heterologous Type II on the 4th day, increasing on the 5th and 6th

day after injection. The blood before injection contained protective substance for Type III pneumococcus, with increase in degree on the 3rd to the 6th days after injection.

Tables I to V illustrate the various types of response to intravenous injection of pneumococcus vaccine. The results in this series

TABLE V

Appearance of Protective Substance in Patient's Serum after Intravenous Injection of Pneumococcus Vaccine

Case 12.

Day after vaccination	Survival after injection of Pneumococcus I culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus II culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus III culture plus 0.2 cc. patient's serum		
	0.001	0.0001	0.00001	0.001	0.0001	0.00001	0.001	0.0001	0.00001
Before	24	24	48	24	24	24	24	48	S
	24	24	48	24	24	24	48	48	S
	48	48	48	24	24	24	48	48	S
3rd	24	24	24	24	24	24	24	24	48
	24	72	S	24	24	24	24	S	48
	S	48	S	24	24	24	24	S	S
4th	24	24	S	24	24	48	24	72	48
	24	24	S	48	24	48	24	48	S
	48	48	S	48	24	S	24	S	S
5th	24	48	72	24	24	24	24	24	S
	24	S	48	24	48	S	48	48	S
	24	S	S	24	48	S	48	48	S
6th	24	S	S	24	24	72	24	S	S
	24	S	S	S	24	S	48	S	S
	24	S	S	S	72	S	S	S	S

Controls		Controls		Controls	
0.00001	24 hrs.	0.00001	48 hrs.	0.00001	48 hrs.
0.000001	48 hrs.	0.000001	48 hrs.	0.000001	72 hrs.
0.0000001	48 hrs.	0.0000001	48 hrs.		
0.00000001	48 hrs.	0.00000001	24 hrs.		
		0.000000001	48 hrs.		

Diagnosis.—Lobar pneumonia. Sputum contained Pneumococcus Type I. Patient was given an intravenous injection of pneumococcus vaccine, 3 billion organisms of each Type I and II.

have been divided into a group in which heterologous vaccine (*i.e.*, composed of organisms of a different type than that present in the sputum) was administered intravenously (Table VII), and a group in

which homologous vaccine (composed of pneumococci of the same type as that in the sputum) was given (Table IX).

TABLE VI

Appearance of Protective Substance after Intravenous Injection of Pneumococcus Vaccine: Heterologous Type Experiments

Case No.	Organism in patient's sputum	Type of pneumococcus vaccine administered	Day after injection on which protective substance appeared
10	Pneumococcus II	I	6
13	—	I	4
15	Hemolytic streptococci	I	6
1	Tubercle bacillus	I	Negative
2	—	I	Negative
5	Pneumococcus III	I	Negative
1	Tubercle bacillus	II	4
2	Pneumococcus IV	II	3
3	Pneumococcus IV	II	5
4	Friedländer bacillus	II	3
5	Pneumococcus III	II	6
6	Pneumococcus IV	II	4
7	Pneumococcus IV	II	4
8	Pneumococcus I	II	6
9	Pneumococcus I	II	3
11	Pneumococcus I	II	3
12	Pneumococcus I	II	4
13	—	II	3
14	Pneumococcus I	II	4
15	Hemolytic streptococci	II	5
1	Tubercle bacillus	III	4
6	Pneumococcus IV	III	6
7	Pneumococcus IV	III	6

Total experiments, 23.

Total cases showing protective substance 20, or 87 per cent.

Average day of onset of 3 cases injected with Type I vaccine, 5.3 days.

Average day of onset of 14 cases injected with Type II vaccine, 4.1 days.

Average day of onset of 3 cases injected with Type III vaccine, 5.3 days.

Combined average day of onset of 20 cases injected with Type I, II or III vaccine, 4.4 days.

In the accompanying table (Table VI), there were 23 instances in which a heterologous vaccine was given intravenously. Of these 20, or 87 per cent, developed protective substance for the organism in-

jected. The average day of onset of active immunity as demonstrated by passively transmitted protection to mice was 4.4 days after injection; 4.1 days for 14 cases of Pneumococcus Type II, and 5.3 days for 3 cases of Type I and 3 cases of Type III. Patients without pneumococcus infection were included in this group in order to add further evidence that heterologous immunity was due to the

TABLE VII

Appearance of Protective Substance after Intravenous Injection of Pneumococcus Filtrate: Heterologous Type Experiments

Case No.	Organism in patient's sputum	Type of pneumococcus vaccine administered	Day after injection on which protective substance appeared
16	Pneumococcus IV	II	6
17	Pneumococcus III	II	6
20	Pneumococcus IV	II	6
21	Pneumococcus IV	II	4
16	Pneumococcus IV	I	6
17	Pneumococcus III	I	6
18	Pneumococcus II	I	5
19	Pneumococcus II	I	6
20	Pneumococcus IV	I	Negative

Total experiments, 9.

Total cases showing protective substance, 8, or 89 per cent.

Average day of onset of protective substance of 4 cases injected with Type II vaccine, 5.5 days.

Average day of onset of protective substance of 4 cases injected with Type I vaccine, 5.7 days.

Combined average day of onset of protective substance of 8 cases injected with Types I or II vaccine, 5.6 days.

introduction of vaccine and not to the spontaneous appearance of pneumococcus protective substance.

The results of the intravenous injection of pneumococcus filtrate have been summarized in two tables, (1) the appearance of protective substance after administration of heterologous filtrate (Table VII), and (2) appearance of protective substance after administration of homologous filtrate (Table VIII).

In the former table, (Table VII), 9 instances of heterologous filtrate

injection are presented. In 8, or 89 per cent, protective substance appeared during the period under observation. The average day of onset of active immunity was 5.6 days after injection; 5.5 days for Type II filtrate and 5.7 for Type I filtrate.

It is of interest to note that the intravenous injection of vaccine resulted in the appearance of protective substance 1.2 days earlier than the filtrate, being approximately the same difference that was found in the animal experiments reported in the previous paper (1).

TABLE VIII

Appearance of Protective Substance after Intravenous Injection of Pneumococcus Vaccine and Filtrate: Homologous Type Experiments

Case No.	Organism in patient's sputum	Type of pneumococcus administered. Vaccine or filtrate	Day after injection on which protective substance appeared
10	Pneumococcus II	Vaccine II	5
12	Pneumococcus I	Vaccine I	3
14	Pneumococcus I	Vaccine I	3
18	Pneumococcus II	Filtrate II	3
19	Pneumococcus II	Filtrate II	6
22	Pneumococcus II	Filtrate II	4
22	Pneumococcus I	Filtrate I	4

Total cases showing protective substance, 7, or 100 per cent.

Average day of onset of 3 cases injected with vaccine, 3.7 days.

Average day of onset of 4 cases injected with filtrate, 4.2 days.

Combined average day of onset of 7 cases injected with vaccine or filtrate, 4.0 days.

In Table VIII, the findings after intravenous injection of homologous vaccine and filtrate are summarized. The average day of onset of passively transmitted immunity in the case of 3 vaccine cases was 3.7 days, in the 4 filtrate cases, 4.2 days. In both instances immunity appeared earlier than in the corresponding heterologous cases. That this might be due to the spontaneous development of protective substances and not to the vaccine has been previously emphasized.

Two illustrative cases of intravenous filtrate injection will be mentioned.

TABLE IX

Appearance of Protective Substance after Intravenous Injection of Antigen Filtrate

Case 17.

Day after injection of filtrate	Survival after injection of test culture					
	Pneumococcus Type I			Pneumococcus Type II		
	0.001	0.0001	0 00001	0.001	0.0001	0.00001
Before	20	20	25	20	20	20
	20	25	40	20	20	25
	40	40	90	20	20	25
1st	20	20	25	20	20	24
	20	20	40	20	20	20
	40	40	70	20	20	20
2nd	20	25	20	20	20	20
	20	40	40	20	20	20
	75	40	40	20	20	20
3rd	20	20	24	20	20	20
	20	20	40	20	20	20
	40	40	40	20	20	S
4th	20	20	40	20	20	20
	20	20	40	20	20	20
	20	25	40	20	20	20
5th	20	25	40	20	20	20
	20	24	40	20	20	24
	25	40	96	20	40	24
6th	30	24	S	20	40	40
	20	20	S	20	40	S
	20	96	S	24	S	S
	SS		S			
2 weeks	30	S	S		S	S
	30	S	S			S
	60	S	S		S	

Controls		Controls	
0.001	20 hrs.	0.001	20 hrs.
0.0001	20 hrs.	0.0001	20 hrs.
0.00001	25 hrs.	0.00001	35 hrs.
0.000001	40 hrs.	0.000001	40 hrs.
0.0000001	40 hrs.	0.0000001	40 hrs.
0.00000001	40 hrs.	0.00000001	40 hrs.

Diagnosis.—Lobar pneumonia. Pneumococcus Type III in sputum. Patient received 17 cc. of Pneumococcus I and II filtrate.

In the first case (Case 17), a man of 38 years had lobar pneumonia with Pneumococcus Type III in the sputum, sterile blood culture and involvement of the L.L.L. On the 2nd day of illness, 16 cc. of Pneumococcus Type I and II filtrate were injected intravenously in 5 doses in 24 hours.

TABLE X

Appearance of Protective Substance after Intravenous Injection of Antigenic Filtrate

Case 18.

Day after injection of filtrate	Survival after injection of test culture					
	Pneumococcus Type I			Pneumococcus Type II		
	0.001	0.0001	0.00001	0.001	0.0001	0.00001
Before	20	40	40	20	20	20
	20	40	40	20	20	20
	40	40S	40	20	20	30
3rd	20	20	20	20	20	20
	20	40	20	20	20	20
	20	40	40	20	S	20
4th	20	40	20	20	S	S
	20	40	40	40	S	S
	40	40	40	40	S	S
5th	20	40	40	20	S	S
	20	40	S	20	S	S
	40	90	S	40	S	S
6th	S	S	40	20	S	S
	S	S	S	20	S	S
	S	S	S	S	S	S
8th	40	S	40	S	S	S
	90	S	S	S	S	S
	S	S	S	S	S	S

Controls		Controls	
0.000001	40 hrs.	0.000001	40 hrs.
0.0000001		0.0000001	
0.00000001		0.00000001	
0.000000001			

Diagnosis.—Lobar pneumonia. Sputum contained Pneumococcus Type II. Patient received intravenously 17 cc. of Pneumococcus I and II filtrate in 5 doses.

As seen in Table IX, protective substances appeared on the 6th day (and were still present on the 14th day) after injection against Pneumococcus Types I and II. In this instance of immunity induced against two heterologous types of pneumococcus, the filtrate appeared clearly to be the cause of the appearance of protective substance.

The second case (Case 18), was a woman 28 years old who had lobar pneumonia, with Pneumococcus Type II in sputum, sterile blood culture and consolidation of the R.L.L. On the 5th day of disease, 17 cc. of Pneumococcus Type I and II filtrate were injected intravenously in 5 doses.

TABLE XI

Appearance of Protective Substance after Intradermal Injection of Pneumococcus Vaccine

Heterologous Cases

Case No.	Organism in patient's sputum	Type of pneumococcus administered	Day after injection on which protective substance appeared
23	Pneumococcus I	II	5
24	Pneumococcus I	II	6
29	Pneumococcus I	II	4
23	Pneumococcus I	III	3
29	Pneumococcus I	III	4
26	Pneumococcus IV	II	4
27	Pneumococcus IV	III	5
25	Pneumococcus IV	II	5
28	Tubercle bacillus	II	5
34	—	III	4
33	—	III	Negative
32	—	II	Negative
32	—	II	Negative
33	—	II	Negative
33	—	I	Negative
34	—	II	Negative
34	—	I	Negative
35	Pneumococcus III	II	Negative
24	Pneumococcus I	III	Negative
27	Pneumococcus IV	II	Negative
25	Pneumococcus IV	III	Negative
26	Pneumococcus IV	III	Negative
27	Pneumococcus IV	I	Negative
35	Pneumococcus III	I	Negative

10 of 24 cases, or 42 per cent, developed protective substance after intradermal vaccination. Average day of onset, 4.5 days after injection.

As noted in Table X, protective substances appeared on the 3rd day after injection in the case of the homologous Type II filtrate and 5 days after injection in the heterologous Type I filtrate.

The results of the intradermal injection of vaccine may now be summarized.

Of 24 instances in which heterologous pneumococcus vaccine was injected intradermally, 10, or 42 per cent, showed a positive response,

TABLE XII

Appearance of Protective Substance in Patient's Serum after Intradermal Injection of Pneumococcus Vaccine

Case 27.

Day after vaccination	Survival after injection of Pneumococcus I culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus II culture plus 0.2 cc. patient's serum			Survival after injection of Pneumococcus III culture plus 0.2 cc. patient's serum		
	0.001	0.0001	0.00001	0.001	0.0001	0.00001	0.001	0.0001	0.00001
Before	40	40	40	20	40	20	20	40	40
	40	40	40	40	40	40	40	40	40
		40	40		40	40		40	
3rd	20	40	40	20	20	20	40	20	40
	40	40	40	20	40	40	40	40	40
		40	40		40	40		40	40
4th	20	20	40	20	20	90	40	40	40
	20	40	40	20	20	40	40	40	40
		40	40		40	40		40	80
5th	40	40	40	20	40	20	40	40	40
	40	40	40	40	40	20	40	40	40
		40	40		40	40		S	40
6th	48	48	48	20	20	40	40	40	40
	45	48	48	20	40	60	40	S	40
		60	48		40	60	40	S	80

Controls		Controls		Controls	
0.00001	20 hrs.	0.000001	40 hrs.	0.00001	40 hrs.
0.000001	25 hrs.	0.0000001	40 hrs.	0.000001	40 hrs.
0.0000001	30 hrs.	0.00000001	40 hrs.	0.0000001	40 hrs.
0.00000001	40 hrs.	0.000000001	80 hrs.	0.00000001	40 hrs.
0.000000001	45 hrs.				

Diagnosis.—Lobar pneumonia. Sputum contained Pneumococcus Type IV. Patient received an intradermal injection of pneumococcus vaccine, 2 billions of organisms of each Type I, II and III.

and had an average day of onset of immunity of 4.5 days after injection. The degree of immunity as well as its regularity is much less marked than in intravenous injection. One illustrative case of this group will be cited:

The patient (Case 27) was a man 38 years old who had lobar pneumonia, with Pneumococcus Type IV in the sputum, negative blood cultures and consolidation of the R.L.L. He was given an intradermal injection of 2 billion pneumococci of each Type I, II and III on the 3rd day of disease.

As will be seen in Table XII, no immunity appeared for Pneumococcus Types I and II, but one survival was present for Type III 5 days after injection and two survivals 6 days after injection. A slight though definite instance of heterologous pneumococcus immunity seemed to result from intradermal vaccination.

DISCUSSION

Inasmuch as pneumonia is a self-limited disease ending with recovery or death, generally between the 7th and 12th days after onset, 3 to 5 days are frequently afforded in which an attempt can be made to produce active immunity before the natural termination of the disease takes place. The previous study of the rate of development of pneumococcus immunity in animals was suggested by Dochez (11) in order to determine whether active immunity to the pneumococcus could be developed in a sufficiently short space of time as to make the injection of vaccine a therapeutic measure in lobar pneumonia. In addition, the life of the pneumonia patient, in the author's experience (15), was at times prolonged by oxygen treatment, a circumstance that seemed to increase the possibilities of vaccine treatment in an individual case.

That a rational basis for applying vaccine therapy in lobar pneumonia is now present has been recently emphasized by Zinsser (16). He states that autoimmunization from the patient's own lesion may be inefficient for two reasons: (1) the fact that considerable autolysis of the pneumococcus takes place in the lung (17), and (2) that autolyzed pneumococci possess little antigenic value, and are especially deficient in the capacity to evoke type-specific antibodies (18). Since active immunity in animals within a 3 to 5 day period was produced in the study above referred to, and similar results were reported by Goodner (19) at the same time, a rational basis for attempting vaccine therapy in lobar pneumonia seemed justified.

It may also be noted in this connection that the site of the antibody-producing tissues is thought to be in the capillary and lymphatic endothelium and other reticulo-endothelial cells (Boone (20) and Manwaring (21)). The presence of large numbers of partially auto-

lyzed pneumococci in the lung which escape in relatively small numbers into the blood stream might therefore provide a less efficient stimulus for the production of protective antibodies than the introduction into the vascular system of large numbers of whole dead pneumococcus organisms.

The intravenous injection of pneumococcus vaccine in human beings was followed by the appearance of specific protective substances in their serum 4 to 5 days after administration. This was demonstrated in cases of miscellaneous disease without lung involvement, in pulmonary inflammation without pneumococcus infection and in lobar

TABLE XIII

Temperature Range on Day Protective Substance Appeared after Intravenous Injection of Pneumococcus Vaccine

Case No.	Temperature range
1	100.8–103.8°
4	100.0–103.0°
10	100.4–102.0°
12	101.0–103.0°
13	100.2–104.0°
3	98.2– 99.8°
6	98.2– 99.2°
7	98.8– 99.4°
8	98.6– 99.6°
9	99.6–104.0°
11	98.0– 99.2°
14	99.4–100.5°

pneumonia caused by the pneumococcus. By the injection of heterologous types of pneumococcus, it was possible to evoke specific protective substance for the corresponding types during the febrile course of pneumonia. In this discussion, emphasis has been placed on the development of specific protective substance during the course of acute pneumonia. Although all patients were injected in the presence of fever, in many instances the temperature had declined to normal or nearly so when antibodies appeared in the serum. It was possible, however, to demonstrate the production of specific protective substance during the febrile period. In two cases of intravenous filtrate injection and five cases of intravenous vaccine injection, antibodies

were present in the serum while the acute febrile stage of the pneumonia was in progress. A table (Table XIII) of the temperature range in the cases who were given intravenous vaccine shows the degree of fever on the day when protective substances first appeared. The first five developed antibodies while definite fever was still present. The remainder were in a nearly afebrile condition when protective antibodies were demonstrated. No case was considered as belonging to the febrile group if the temperature at any time went below 100°. It seems evident, therefore, that active immunity may be induced during the febrile course of pneumonia as a result of intravenous injection of pneumococcus vaccine.

The appearance of protective substance after injection of homologous vaccine appeared almost a day earlier than that which followed heterologous vaccine, but it is not possible to affirm that the injection of the vaccine in an individual instance was responsible for the appearance of homologous protective substance. Our experiments indicate, however, that immunity may generally be established 4 to 5 days after intravenous injection of pneumococcus vaccine. In cases of pneumonia that are seen early in the disease, the capacity to evoke type-specific protective substances in 4 or 5 days may make this procedure of therapeutic value.

Subcutaneous injection of vaccine in two cases of pneumonia and in ten normal donors* did not produce protective substance within this period. In Whitmore's (22) experiments with pneumococcus vaccine injected subcutaneously in normal human beings, protective substances did not appear in the serum until 8 days after injection. The same result was reported by Cecil and Austin (23). The results of Lambert in his own series (referred to in the early part of the paper) showed no heterologous protection. Although clinically a lowered mortality was present in the treated group of Lambert, the factor responsible for the favorable result does not appear in immunological studies so far reported.

It must be remembered, however, that the object of injection of vaccine is to produce active immunity, and that the measurement of protective substance in the serum of the patients records only trans-

* The findings on donors is part of a separate report on immune transfusion.

ferable or passive immunity. A state of active immunity against the pneumococcus may be produced without giving evidence of its existence by the presence of protective antibodies in the serum. This has been demonstrated by several workers, particularly by Cecil and Steffen (24), who were able to immunize monkeys against pneumonia by three subcutaneous injections of pneumococcus vaccine at weekly intervals. Numerous instances occurred in which the monkeys survived lethal doses of virulent cultures without possessing either serum agglutinins or protective substance. Active immunity, however, was not produced within the first 6 days after subcutaneous injection of vaccine. Immunization of rabbits with Type III pneumococcus may also be effective in producing active immunity against infection in the absence of type-specific protective antibodies (Tillett (25)).

The results of intradermal injection of vaccine are confusing. Although the majority of cases were negative, apparent instances of induction of active immunity did occur. The degree and regularity of response in an individual case was much less marked than in the case of intravenous injection of vaccine. The findings were sufficiently indefinite as to permit no conclusion as to the effect of the intradermal injection of vaccine in the production of pneumococcus immunity.

The clinical results have not been emphasized in this series, since the production of *heterologous* protective substance seemed necessary to demonstrate that an immunity may actually be initiated during the febrile period of pneumonia by the intravenous injection of a suitable pneumococcus antigen. There were 20 cases of pneumonia, 17 lobar pneumonia and 3 bronchopneumonia, who received an intravenous injection of pneumococcus vaccine or filtrate. Of these, 2 died. One was a patient who had a Pneumococcus Type IV in the sputum with a persistent Type IV pneumococcus bacteremia. She received an intravenous injection of Pneumococcus Type I and II filtrate. The other death occurred in a patient who had a lobar pneumonia due to Pneumococcus Type II with a blood stream infection with Pneumococcus Type II. The patient was injected with Pneumococcus Type I and II vaccine intravenously. Of 9 patients with pneumonia, 7 lobar and 2 bronchopneumonia, who received an intradermal injection of vaccine, 1 died. Two patients who received a subcutaneous injection of vaccine recovered. Eight patients with

miscellaneous disease were treated, of whom one subsequently died of causes unrelated to vaccine injection. Four cases were entirely excluded because they died before observations on the appearance of protective substance could be made. Their clinical course gave no indication of having been in any way affected by the introduction of vaccine.

TABLE XIV

Clinical Data on Pneumonia Patients Who Received an Intravenous Injection of Pneumococcus Antigen

Case No.	Diagnosis	Organism in sputum	Day of disease injected	Pneumococcus antigen employed	Outcome
5	Lobar pneumonia	Pneumococcus III	3	Vaccine I, II	S
6	Lobar pneumonia	Pneumococcus IV	5	Vaccine II, III	S
7	Lobar pneumonia	Pneumococcus IV	4	Vaccine II, III	S
8	Lobar pneumonia	Pneumococcus I	3	Vaccine I	S
9	Lobar pneumonia	Pneumococcus I	2	Vaccine II	S
10	Lobar pneumonia	Pneumococcus II	5	Vaccine I, II	D
11	Lobar pneumonia	Pneumococcus I	2	Vaccine II, III	S
12	Lobar pneumonia	Pneumococcus I	3	Vaccine I, II	S
13	Lobar pneumonia	—	7	Vaccine I, II	S
14	Lobar pneumonia	Pneumococcus I	6	Vaccine I, II	S
16	Lobar pneumonia	Pneumococcus IV	1	Filtrate I, II	S
17	Lobar pneumonia	Pneumococcus III	2	Filtrate I, II	S
18	Lobar pneumonia	Pneumococcus II	5	Filtrate I, II	S
19	Lobar pneumonia	Pneumococcus II	3	Filtrate I, II	S
20	Lobar pneumonia	Pneumococcus II	4	Filtrate I, II	S
21	Lobar pneumonia	Pneumococcus IV	1	Filtrate I, II	D
22	Lobar pneumonia	Pneumococcus I, II	4	Filtrate I, II	S
2	Bronchopneumonia	—	—	Vaccine I, II	S
3	Bronchopneumonia	Pneumococcus IV	3	Vaccine I, II	S
4	Bronchopneumonia	*Streptococcus haemolyticus*, Friedländer bacillus	6	Vaccine II	S

None of the patients who received an intravenous or intradermal injection of pneumococcus vaccine experienced any reaction There was no change in pulse, respiration or temperature. No consistent alteration of the clinical course of the disease was discerned. The pneumococcus filtrate was administered intravenously 28 times to 7

patients and in only one instance did a foreign protein reaction occur. In this case, a chill of moderate severity was followed by a defervescence of fever in 8 hours with a return of the temperature to its previous level 12 hours later.

The number of cases of pneumonia treated by the intravenous injection of homologous pneumococcus vaccine is too small to permit conclusions as to its therapeutic value at this time.

SUMMARY

1. Pneumococcus vaccine was administered to 29 patients with pneumonia to determine whether a state of immunity could be induced during the course of the disease. Twenty patients received an intravenous injection of pneumococcus vaccine or pneumococcus filtrate. Nine pneumonia patients received an intradermal injection of vaccine. Eight patients with miscellaneous disease received an intravenous or intradermal injection of pneumococcus vaccine.

2. Of 23 tests in which the serum of the patient was studied for the appearance of protective substance after intravenous injection of heterologous pneumococcus vaccine, 20 or 87 per cent showed a positive response within 6 days after the administration of the antigen. The average day of onset was 4.4 days after injection.

3. Of 9 tests of the same character following the intravenous injection of pneumococcus filtrate, 8 or 89 per cent showed a positive response. The average day of onset of protective substance was 5.6 days after injection.

4. The appearance of specific protective substance following heterologous injection of pneumococcus vaccine appeared to be due to the introduction of the vaccine and not to the natural course of the disease, as was shown by negative control experiments.

5. Of 24 tests with intradermal injection of vaccine, 10 or 42 per cent developed slight protective substance of irregular degree 4.5 days after injection.

6. No immediate reactions were observed following the intravenous or intradermal injection of pneumococcus vaccine. One chill occurred after injection of pneumococcus filtrate. Of 20 cases with intravenous injection of pneumococcus vaccine or filtrate, 2 died of their disease, one a case in which homologous vaccine was used and one in which heterologous vaccine was administered.

7. Conclusions concerning the therapeutic value of the introduction of pneumococcus vaccine in pneumonia must await further investigation. These studies demonstrate that specific protective substances generally appear 4 to 5 days after intravenous injection of pneumococcus vaccine during the course of lobar or bronchopneumonia.

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