PREVENTION OF PNEUMOCOCCAL PNEUMONIA BY IMMUNIZATION WITH SPECIFIC CAPSULAR POLYSACCHARIDES*

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Many studies on prophylactic immunization against pneumococcal pneumonia have been made, using a number of different antigenic preparations. Almost all investigators have concluded that immunization exerts a beneficial effect. In most of the studies, however, certain variables have clouded interpretation of the results. Among the variables, the following appear to be of greatest moment: differences in the composition of the immunized and control groups; uncertainty as to whether the specific pneumococcal types included in the immunizing preparation were the same as those currently causing pneumonia; failure to determine whether the observed decline in cases in the immunized group was due to a decrease in cases caused by the pneumococcal types included in the vaccine; inadequate control of the antigenicity of the preparations used.

The subject of antipneumococcal immunization has been reviewed recently by Heffron (1) and references will be made in the present paper to certain aspects only.

The studies of Lister and Ordman (2) among native laborers in South African mines between 1930 and 1934 suggest that immunization with a polyvalent pneumococcal vaccine reduces the incidence of pneumonia caused by the same pneumococcal types. Pneumococci of types I, II, III, V, VII, XII, and XIV were represented in the vaccine,

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1 cc. containing 1000 million organisms of each type, except for type II, of which 2000 million per cc. were included. In addition, the vaccine contained pneumococcus "type T" as well as varying numbers of *Streptococcus pyogenes*, *Streptococcus salivarius*, *H. influenzae*, *N. catarrhalis*, *Staphylococcus aureus hemolyticus*, and *K. friedländeri*. The total bacterial count of the vaccine was 11,000 million per cc. The organisms were suspended in saline, killed by heating to 60°C. for 1 hour, and preserved with 0.5 per cent phenol. Admnistration was in three doses of 1 cc. subcutaneously at intervals of 1 week. The best results were obtained in the prevention of pneumonia caused by pneumococcus type II. In the control group 27.9 per cent of the cases were caused by type II whereas in the vaccinated group this was reduced to 4.0 per cent. With the other types represented in the vaccine a beneficial effect was also observed, but in no instance was as good as with type II. In prior studies of Lister and Ordman, the prophylactic value of antipneumococcal immunization appeared to be less definite. The studies of immunization in South Africa carried out prior to 1931 have been reviewed critically by Orenstein (3), who concluded that in only one instance the results "appeared to justify the adoption of prophylactic vaccination."

Cecil and Austin (4) tested the effect of prophylactic vaccination against pneumococcal pneumonia at Camp Upton, New York, in 1918. Three or four subcutaneous injections of vaccine were given at weekly intervals to 12,519 men. The vaccine consisted of a saline suspension of heat-killed pneumococci types I, II, and III preserved with 0.3 tricresol. Each dose of 0.5 cc. contained 6000 to 9000 million organisms of each of types I and II and 4500 to 6000 million of type III pneumococcus. During a 10 week period following vaccination, no cases of pneumonia caused by types I, II, or III occurred among the immunized men, whereas in the control group of 19,400 men there were 26 cases of pneumonia caused by these types. The validity of the data is lessened by differences in the composition of the vaccinated and control groups, since the vaccinated group was made up entirely of seasoned troops, whereas in the control group approximately 25 per cent were new recruits.

Subsequent studies by Cecil and Vaughan (5) at Camp Wheeler, Georgia, were directed toward assessing the prophylactic effect of a lipovaccine of pneumococci given in a single subcutaneous dose of 1 cc. Each cubic centimeter contained 10,000 million pneumococci of each of types I, II, and III. Following vaccination, pneumonia rates were lower in the inoculated than in the control group, although the differences were not as great as in the experience of Cecil and Austin (4) at Camp Upton, New York. Rapid population changes and an epidemic of influenza at Camp Wheeler were felt to have influenced the results.

Following the injection of saline suspensions of pneumococci, Cecil and Austin (4) observed the development of small sterile abscesses in 1.2 per cent of inoculated subjects. With the lipovaccine the incidence of local reactions at the site of inoculation was only 0.04 per cent. General reactions severe enough to require hospitalization occurred in 0.2 per cent of subjects inoculated with saline suspensions of pneumococci in the study of Cecil and Austin (4) and in 0.7 per cent of the subjects inoculated with lipovaccine (5).

It has been demonstrated repeatedly that animals can be protected against infection by virulent pneumococci by means of antibodies directed against the

specific capsular polysaccharides. Antibodies to the somatic portion of the cell are of considerably less importance in this respect. Francis and Tillett (6) showed originally that the purified capsular polysaccharides of pneumococci are antigenic for man when injected intracutaneously in a single dose as small as 0.01 mg.

There are various reasons why the purified capsular polysaccharides should be advantageous immunizing agents for man as compared with whole bacterial vaccines. Not only should it be possible to avoid the local abscess formation associated not infrequently with whole pneumococcal vaccines, but in addition a stable, water-clear solution of known composition can be used. It becomes possible also to standardize the dose of antigen on a weight basis. It is of theoretical and practical importance for each of the various pneumococcal types that a single, purified antigen relatively free from heterogeneous somatic constituents can be used. Therefore, the injection of several purified capsular polysaccharides in a single does probably involves fewer antigens than when a whole bacterial vaccine of a single serological type is used, since the whole bacterial vaccine contains numerous unrelated protein and carbohydrate antigens in addition to the specific capsular polysaccharide.

Ekwurzel, Simmons, Dublin, and Felton (7) employed solutions of capsular polysaccharides for the immunization of man against pneumococcal pneumonia over a 4 year period between 1933 and 1937. Volunteers in Civilian Conservation Corps camps in New England and western United States were given a single subcutaneous injection of 1 mg. of each of the capsular polysaccharides of pneumococcus types I and II prepared by the calcium phosphate precipitation method of Felton, Kauffmann, and Stahl (8). These polysaccharides differed from those prepared in other laboratories in that their injection into man gave rise to antibodies affording heterologous protection. The specificity of the protective action of the antigens against pneumonia caused by pneumococcus types I and II was not tested satisfactorily because of incomplete typing of the organisms causing pneumonia. The results in the western camps, however, appeared to indicate "that the antigen may be effective in reducing the case incidence of pneumonia."

Suggestive evidence of prophylactic effectiveness of type I polysaccharide was reported by Smillie, Warnock, and White (9) from a study of an epidemic of type I pneumonia in a mental institution in Massachusetts. The preparation of polysaccharide used had properties similar to that described by Felton (10). The epidemic subsided shortly after the subcutaneous injection into most of the inmates of 2 mg. of polysaccharide. Unfortunately, in this study it was not feasible to inject alternate subjects only, so that adequate controls were not available. The prompt cessation of the epidemic following administration of the polysaccharide, however, indicates a specific protective action.

Siegel and Muckenfuss (11) studied in a New York State mental hospital the prophylactic effect of pneumococcal polysaccharides prepared by Felton. A single subcutaneous injection of a solution containing 0.3 mg. of type I, 0.2 mg. of type II, and 0.15 mg. of type III polysaccharide was given to one-third of the inmates. An

epidemic of type I pneumonia occurred in the institution subsequent to vaccination, but no evidence of a specific protective effect as concerned the disease was found. The cases of type II or type III pneumonia were too few to afford a test of the prophylactic effect against these types. The antigenicity of the type I preparation, however, must be considered as unsatisfactory, since specific antibodies, as measured by mouse protection tests, failed to appear in the serum of 11 out of 28 subjects within a period of 2 to 3 weeks following inoculation. Moreover, none of the patients who developed type I pneumonia showed a significant rise in titre of type I antibodies in their serum following inoculation. From the point of view of the antigenic response of the inoculated subjects, therefore, the study of Siegel and Muckenfuss was not a satisfactory test of antipneumococcal immunization in the prevention of pneumonia.

The present paper deals with the immunization of man with the polysaccharides of pneumococcus types I, II, V, and VII. It has been demonstrated that a single subcutaneous prophylactic injection of 0.03 to 0.06 mg. of each of these polysaccharides greatly diminished the incidence of pneumonia caused by pneumococci of the same types in immunized individuals. Moreover, evidence will be presented that the incidence of pneumonia caused by types I, II, V, and VII in the non-immunized controls who were thoroughly mingled with the immunized was also reduced.

Materials and Methods

Pneumococcal Capsular Polysaccharides.—The capsular polysaccharides of pneumococcus types I, II, and V were obtained from E. R. Squibb and Sons and were prepared by the phenol method (12) in which heat and strong acid or alkali are avoided. A single lot of each was used throughout the study. Two lots of type VII polysaccharide were employed. The first of these was prepared by Dr. Rachel Brown and the second by Dr. John W. Palmer.[1]

The solutions of polysaccharide were prepared by dissolving them in isotonic saline to which 0.5 per cent phenol was added as preservative. Sterilization was accomplished by filtration through Berkefeld candles. Filtration introduced an uncertainty since it was not known how much of the polysaccharides would be adsorbed by the filter. Adsorption was greater than had been anticipated, so that in the first lot, 1 cc. of solution, instead of containing 0.06 mg. of each polysaccharide as had been intended, contained only about one-half of the expected amounts. After 3755 men had been injected with a dose of 1 cc. (about 0.03 mg. of each polysaccharide), the dosage was increased to 1.5 cc. (0.045 mg. of each polysaccharide). This amount was given to 2193 men.

The second lot of polysaccharide solution, of which 1 cc. was injected, contained 0.06 mg. of type I and 0.05 mg. of type V polysaccharide per cc., with presumably similar quantities of type II and type VII polysaccharides. 2638 men were injected with this lot. The polysaccharide content of the solution used for immunization was determined by quantitative precipitin titrations with such analytical sera as were available (13). In the case of the type V and VII polysaccharides analytical sera were not available. Preliminary studies in student volunteers had indicated that amounts of the pneumococcal polysaccharides of each of types I, II, V, and VII between 0.03 and 0.06 mg. injected subcutaneously in a single injection evoke a definite antibody response, as measured by both the quantitative precipitin (13) and

[1] We are grateful to Dr. Rachel Brown of the New York State Department of Health and to Dr. John W. Palmer of E. R. Squibb and Sons for the gift of type VII polysaccharide.

mouse protection techniques. The results of these studies will form the subject of separate communications.

Determination of Antibody Response in Immunized Men.—Fifty specimens of serum obtained before immunization with polysaccharides and a similar number obtained 3 to 6 weeks later are being analyzed for their antibody content by the quantitative precipitin technique. In addition serial specimens of serum have been obtained from a group of 8 men at intervals of 3 days over a period of 2 weeks in order to determine when an antibody response can be first detected. These sera have been tested by the mouse protection technique.

Detection of Pneumococcal Carriers.—Prior to and during the immunization program a continuous carrier survey for pneumococci was carried out. The carrier survey included normal men in both the immunized and control groups, all hospital admissions for respiratory diseases, and a group of 100 surgical patients.

A dry sterile swab was passed firmly across both tonsillar fauci and then across the posterior pharynx. The swab was streaked immediately on the surface of a blood agar plate and then placed in broth containing 0.2 per cent glucose and rabbit blood (Avery tube). Following incubation at 37°C. for 4½ hours, 0.5 to 0.75 cc. of the Avery tube culture was inoculated intraperitoneally into a white mouse and a direct Neufeld typing carried out on the remainder of the culture. On the death of the mouse or upon sacrifice after 48 hours, a Neufeld typing was carried out on the peritoneal exudate. The heart blood was cultured in plain broth containing rabbit blood and on a rabbit blood agar plate. If no pneumococci were identified in the original Avery tube or mouse peritoneal exudate, an attempt was made to isolate pneumococci from cultures of the mouse heart blood or from the original plate which had been streaked with the throat swab. Commercially prepared antipneumococcal serum, types I to XXXIII, was used for typing by the Neufeld reaction. In addition, sera for 15 of the pneumococcal types above XXXIII were used as a routine.[2]

Experience showed that none of the above steps could be omitted in the identification of pneumococci from pharyngeal swabs without materially lowering the number of positives. It should be noted that carrier rates for pneumococci of approximately 60 per cent were obtained by these methods in a sampling of 3462 men over a period of 7 months during the fall and winter of 1944–45.

In addition to routine throat cultures, specimens of sputum were examined from all patients suspected of having pneumonia. For the identification of pneumococci in specimens of sputum, inoculation of mice was used as a routine during the winters of 1943–44 and 1944–45 in addition to direct Neufeld typing of the sputum.

The Background and Character of the Population

The experiment was carried out in an Army Air Force Technical School. There was much to recommend the choice of this population. First, there was the unusually high pneumococcal pneumonia rate which had prevailed in the School during the preceding two winters. From September 19, 1942, to July 1, 1944, more than 1500 cases of this disease occurred. For a number of weekly periods the rate exceeded 150 admissions per 1000 strength per annum, which may be considered a high epidemic level. The curves of the pneumonia rates for both years were similar in their seasonal distribution. Furthermore, much

[2] Sera for the 15 "higher" pneumococcal types were made available for this study through the courtesy of Miss Annabel Walter, Bureau of Laboratories, New York City Department of Health. We are also grateful to Miss Walter for the identification of a number of strains of the "higher" types of pneumococci.

information was available concerning the types of pneumococci responsible for these high disease rates. During both the 1942–43 and 1943–44 seasons, type II accounted, on the average, for about 34 per cent of the cases of pneumococcal pneumonia. Types I, V, and VII accounted for slightly more than 9 per cent each and types XII and IV followed with 7 and 5 per cent respectively. Since there had been no great change in the environment or the character of the troops, it was reasonable to expect that a high incidence of pneumonia would occur during the winter of 1944–45 and a good surmise could be made as to which pneumococcal types would be prominent.

Thorough epidemiological study of the respiratory disease experience of the preceding 2 years brought out another advantage in using the Technical School as an experimental population. It was found that for each of the diseases investigated, pneumococcal pneumonia, streptococcal sore throat, epidemic influenza, and common respiratory disease, the School reacted as a whole and not by its individual, component groups or units. The reason for this was clear. In contrast to troops of a tactical or administrative unit, the men in the School spent the bulk of their day in close, indoor contact with one another. Furthermore, the men comprising a given School class came from several different squadrons and from many different barracks. It is thus apparent that even should a focus of disease appear in a barrack, it would be spread rapidly to other barracks and other squadrons through the medium of schoolroom contact. All available data supported this assumption. It is clear that from the point of view of experimental epidemiology this uniform behavior of the School population was a valuable trait.

From yet another epidemiological aspect the population was highly satisfactory. It is known that many factors such as seasoning and environment exert a large effect on the incidence of respiratory disease. In the study of the Technical School several of these factors could be eliminated. The living conditions, duties, and even the recreations were identical for all the men in the School. Where factors could not be eliminated they were susceptible to controlled study. In order to insure this, a marginal punch card system was established by means of which the dates of arrival and departure, the basic epidemiological data, the immunization date, the respiratory disease experience, and, where pertinent, the pneumococcal carrier status were recorded for every man passing through the School. It was thus a simple procedure at any time to compare the characteristics of the immunized and control groups. Table I shows such a comparison made on the entire student body in late February, 1945, and includes data on length of service, age distribution, and previous history of pneumonia.

It is apparent from the data shown in Table I that there was no essential difference between the two groups in respect to length of service, age distribution, and previous history of pneumonia. Similar samplings were taken ir other periods of the study with practically identical results.

The average duration of stay in the School was 6 months which in a military population may be considered as long.

Finally, as discussed under Methods, facilities were available for extensive

TABLE I

Length of Service, Age Distribution, and Previous History of Pneumonia in Immunized and Non-Immunized Groups

Distribution according to length of service		
Length of service	Immunized subjects	Non-immunized subjects
yrs.	*per cent*	*per cent*
2/12	6.0	6.6
4/12	7.3	7.4
6/12	2.1	2.4
8/12	1.8	2.0
10/12	2.3	2.1
12/12	5.1	5.3
More than 1	75.4	74.2
Total	100.0	100.0
Distribution according to age		
Age	Immunized subjects	Non-immunized subjects
yrs.	*per cent*	*per cent*
18–20	30.1	30.8
21–23	25.6	24.6
24–26	22.8	24.4
27–29	12.8	11.9
30–32	5.9	5.2
More than 32	2.8	3.1
Total	100.0	100.0
Distribution according to previous history of pneumonia (all varieties)		
History of pneumonia	Immunized subjects	Non-immunized subjects
	per cent	*per cent*
Positive	19.3	16.9
Negative	80.7	83.1

and accurate typing of pneumococci both in hospital admissions and in the healthy population.

Immunization with Polysaccharides

Over a period of 5 days beginning September 20, 1944, the component squadrons of the Technical School were run through injection lines. A barrack was roped off longitudinally and as a squadron filed in, it was allowed to divide at

random. Men passing down one side of the barrack received the polysaccharide solution subcutaneously; those passing down the other side received an injection of 1 cc. of sterile isotonic saline containing 0.5 per cent phenol. At the end of the 5 days 3755 men had received the polysaccharides and 3975 had been injected with saline. After this time, new men arriving at the School received the polysaccharides or saline alternately. In all, 8586 men were injected with the polysaccharides and 8449 with saline. By this means the population of the School was kept almost equally divided into immunized and non-immunized fractions. In all the day by day activities of the troops the intermingling of immunized and non-immunized subjects was complete. Calculation of man-days of exposure gives approximately equal figures for immunized and non-immunized, 745,997 and 772,898 respectively.

TABLE II

Incidence of Pneumonia in Immunized and Non-Immunized Groups

Man-days exposure	745,997	772,898
Type of pneumonia	Immunized group	Non-immunized group
I	2	2
II	1	14
V	1	4
VII	0	6
Total of types I, II, V, and VII	4	26
IV	8	6
XII	21	25
Other types	27	28

Reactions to the injection were mild. A fair proportion of those injected with polysaccharide complained of sorness of the arm lasting 3 to 4 days. This did not interfere with their usual activities. Three men were admitted to hospital with systemic reactions attributed to the injection. All recovered promptly. The reactions to phenolized saline were negligible.

Effect of Immunization on the Development of Clinical Pneumonia

The effect of immunization on the development of clinical pneumonia is shown in Table II. During the 7 months period of observation following the beginning of the immunization program, 4 cases of pneumonia caused by types I, II, V, or VII occurred in the immunized group as opposed to 26 cases among the non-immunized subjects. This difference is highly significant. When the individual types are considered, it is apparent that the one to fourteen difference for type II is also highly significant and definitely establishes the

value of immunization with the polysaccharide of this type. The numbers of cases for types I, V, and VII were too small to permit definite statistical conclusions, although the zero to six difference in type VII would be due to chance only once in about four hundred times. Despite the inconclusiveness of the results for these three types, there is no reason for believing that polysaccharides of types I, V, and VII should differ in their immunizing capacity from the polysaccharide of type II. Furthermore, as will be shown in the discussion of the effect of the immunization program on the non-immune half of the popu-

TABLE III

Interval between Injection and the Development of Pneumonia in Immunized and Non-Immunized Subjects

Interval	No. of cases of pneumonia			
	Types I, II, V, VII in immunized subjects	Types I, II, V, VII in non-immunized subjects	All other types in immunized subjects	All other types in non-immunized subjects
wks.				
1	2	0	1	1
2	2	3	5	3
3	0	3	7	5
4	0	2	8	12
6	0	2	6	7
8	0	2	3	4
10	0	1	4	4
12	0	0	2	4
14	0	2	2	1
16	0	3	2	4
16+	0	8	16	14
Total.....	4	26	56	59

lation, the paucity of cases of types I, V, and VII pneumonia is in itself evidence of the effectiveness of the immunizing agents.

The distribution between the immunized and non-immunized groups of pneumonia due to types other than I, II, V, and VII adds validity to the differences noted in the distribution of the types against which immunization was practised. For type XII, which was the chief cause of bacterial pneumonia during the winter of 1944–45, 21 cases occurred in the immunized group and 25 cases among the controls. Type IV, which ranked second in importance, caused eight cases of pneumonia in the immunized and six in the non-immunized group. Pneumonia due to other types also was divided evenly, there being 27 and 28 cases respectively among the immunized and non-immunized subjects.

The four immunized men who developed pneumonia all did so within the first 2 weeks following the immunizing injection. In Table III and Fig. 1,

this distribution is contrasted with that of non-immunized men developing type I, II, V, or VII pneumonia and with immunized and non-immunized men who developed pneumonia due to other types. It is clear that the distribution of pneumonia in the three control groups as shown in the lower three curves of Fig. 1, was closely similar, and that the cases were scattered throughout the period in which the men were under observation. The concentration of control group cases in the first 6 weeks after injection is in keeping with the known behavior of pneumonia in the Technical School. Repeated observations had shown that more than half the cases of pneumonia developed during the first

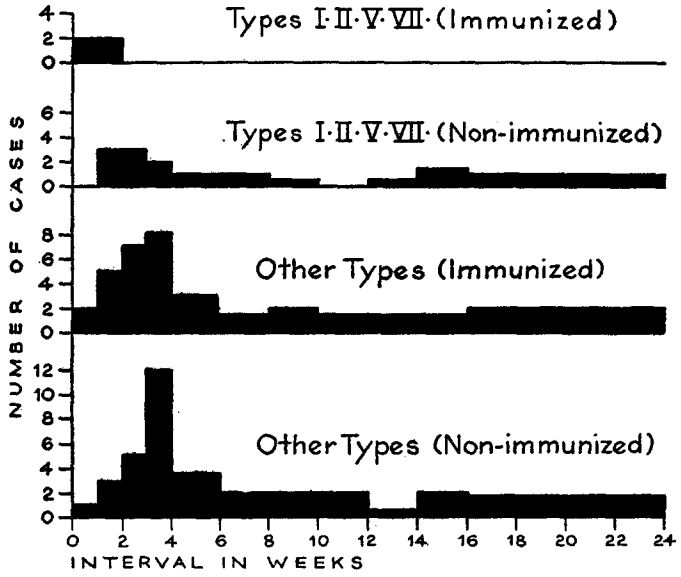

FIG. 1. Interval between injection and the development of pneumonia in immunized and non-immunized subjects.

6 weeks after arrival. In the majority of subjects in this study the date of arrival coincided with the date of injection.

The actual intervals between injection and admission to the hospital with pneumonia for the four immunized cases were 2, 6, 7, and 11 days respectively. It is believed that these cases may have occurred before specific immunity had developed to the full extent.

Time Required for Appearance of Circulating Antibodies Following Injection of Polysaccharides.—

Specimens of serum were obtained from eight men immediately preceding injection of the polysaccharides, at 3 day intervals thereafter for a period of 2 weeks, and a final specimen at 25 days. The presence of antibodies was determined by means of protection tests in mice

using 0.2 cc. of serum and 100 M.L.D. of pneumococci of the same serological types as were represented in the polysaccharide solution used for immunization. Serum was diluted to a volume of 0.5 cc. and mixed with an equal volume of broth containing the infecting dose of pneumococci immediately before injection. Three mice were used for each test. The animals were observed for 5 to 7 days following infection. The results of these tests are shown in Table IV.

From the data shown in Table IV it can be seen that immunity to pneumococci types I, II, V, and VII developed between 6 and 9 days after injection of the polysaccharides as estimated by the appearance in the serum of antibodies protective for mice. All subjects showed antibodies for all of the polysaccharides within a 9 day period following injection. Certain of them possessed antibodies to one or another pneumococcal type before injection. The incidence

TABLE IV

Number of Days Required for Appearance of Antibody in the Serum of Subjects Injected Subcutaneously with Pneumococcal Polysaccharides

Subject	Pneumococcal type			
	I	II	V	VII
1	9	9	9	9
2	0	0	0	0
3	9	9	9	0
4	9	9	6	6
5	6	6	9	6
6	6	6	9	0
7	9	0	0	0
8	0	9	9	0

0 indicates that antibody was present before injection of polysaccharides.

in the general population of immunity to pneumococcus types I, II, V and VII was not determined. It might be expected that the incidence of immunity in the subjects listed in Table IV would be higher than in the general population since these individuals were all members of the permanent party (medical detachment) and had been present on the post for a considerable time with more opportunity for exposure to these types.

Duration of Immunity.—An upper limit for the duration of immunity could not be determined in the present study. However, a large number of men were under observation for 6 months, which may be set, therefore, as a minimum for the duration of the immune effect. On the basis of previous studies in human volunteers (14) it seems probable that immunity as indicated by the presence of antibodies should endure for a period of at least 1 year, since antibody levels of $\frac{1}{8}$ to $\frac{1}{2}$ the original maximum level were still present after a period of 1 to 2 years in most subjects.

Effect of Immunization on the Pneumococcal Carrier Rates

Determination of pneumococcal carrier rates was made continuously throughout the course of the study. Pneumococci of various specific types were identified in 1212 instances out of 1785 cultures from immunized men (68.0 per cent) and in 1295 instances out of 1810 cultures from the non-immunized group (71.6 per cent). Pneumococci of more than one type were encountered in a considerable proportion of cultures. In Table V each of the types identified is scored as one regardless of how many other types were isolated from the same individual, so that this table shows the frequency with which the various types were encountered in the population and not the proportion of men who were

TABLE V

Distribution of Individual Types of Pneumococci between Immunized and Non-Immunized Groups

(Excluding Cases of Pneumococcal Pneumonia)

Pneumococcal type	Immunized men		Non-immunized men	
	Number	Per cent	Number	Per cent
I	6	0.3	12	0.7
II	14	0.8	30	1.7
V	1	0.1	5	0.3
VII	11	0.6	12	0.7
Total I, II, V, and VII	32	1.79	59	3.26
XII	127	7.1	139	7.6
All other types	1053	59.1	1099	60.8
Total of all types	1212	68.0	1295	71.6
Total cultures taken	1785		1810	

pneumococcal carriers. 57.7 per cent of the total population were found to be carrying pneumococci of one or more types.

Thirty-two men in the immunized group and fifty-nine in the non-immunized carried pneumococcus types I, II, V, or VII, or 1.79 and 3.26 per cent respectively. This is a significant difference ($\chi^2 = 9.0$). This difference becomes highly significant when the numbers of carriers in the two groups who developed clinical pneumonia are added to the figures for healthy carriers in Table V. Four men in the immunized group and twenty-six in the non-immunized developed pneumonia due to types I, II, V, or VII. These were case carriers. The total carrier incidence for these types in the immunized group, therefore, was 36 and in the non-immunized 85 ($\chi^2 = 27.0$). From these data it may be concluded that the carrier rate for pneumococci of types I, II, V, and VII

was significantly lowered in the immunized group, in all likelihood as a consequence of immunization.

It is probable that the carrier rates for types I, II, V, and VII were reduced not only in the immunized but in the non-immunized group as well. From Table V it can be seen that the carrier rate for pneumococcus type XII was 7.1 per cent in the immunized and 7.6 per cent in the non-immunized; that is, the rates were approximately equal. The high carrier rate for type XII takes on great significance when it is recalled that this type was responsible for more cases of pneumonia in the Technical School than any other single type during the 1944–45 season.

Previous studies such as those of Stillman (15), Smillie (16), Sydenstricker and Sutton (17), Strom (18), and Gilman and Anderson (19) have shown that during outbreaks of pneumococcal pneumonia in institutions or isolated communities, high carrier rates for the infecting types are encountered among the unaffected portion of the population. Carrier rates as high as 6 to 10 per cent were encountered in these studies. In the present study the same has been found true for type XII which caused epidemic disease and was present in 7.3 per cent of 3595 cultures examined.

While carrier rates for pneumococci of types I, II, V, and VII were not determined during the winter of 1942–43 and 1943–44 when these types were causing epidemic disease, it is probable that high carrier rates for these types were present during these epidemic seasons. It is suggested that immunizing one-half of the School population not only was effective in reducing the carrier rate for types I, II, V, and VII in the immunized group, as shown above, but also aided in preventing the development of high carrier rates in the non-immunized population. In the succeeding section of this paper, further evidence bearing on these points is discussed.

The Effect of Immunization on the Incidence of Pneumonia in the Non-Immunized Half of the Population

Of equal importance with the prevention of types I, II, V, and VII pneumonia in immunized subjects was the reduction of infection due to these types in the non-immune half of the population. As has been stated above, based on the experience of the two preceding winters it was expected that large numbers of cases of pneumonia caused by these types would occur during the period of observation. This expectation was not fulfilled. The reason for the discrepancy became clear when the behavior of type XII and type IV pneumonia was studied for the 3 years in question. Fig. 2 contrasts the admission rates in 1942–43, 1943–44, and 1944–45 for pneumonia due to types I, II, V, VII, XII, and IV. The curves are aligned so that the corresponding 4 week periods for the 3 years occupy the same position on the abscissa. The rates for the first winter begin with January, 1943, since typing of pneumococci before that time was not sufficiently complete. It is apparent that the curves for type XII

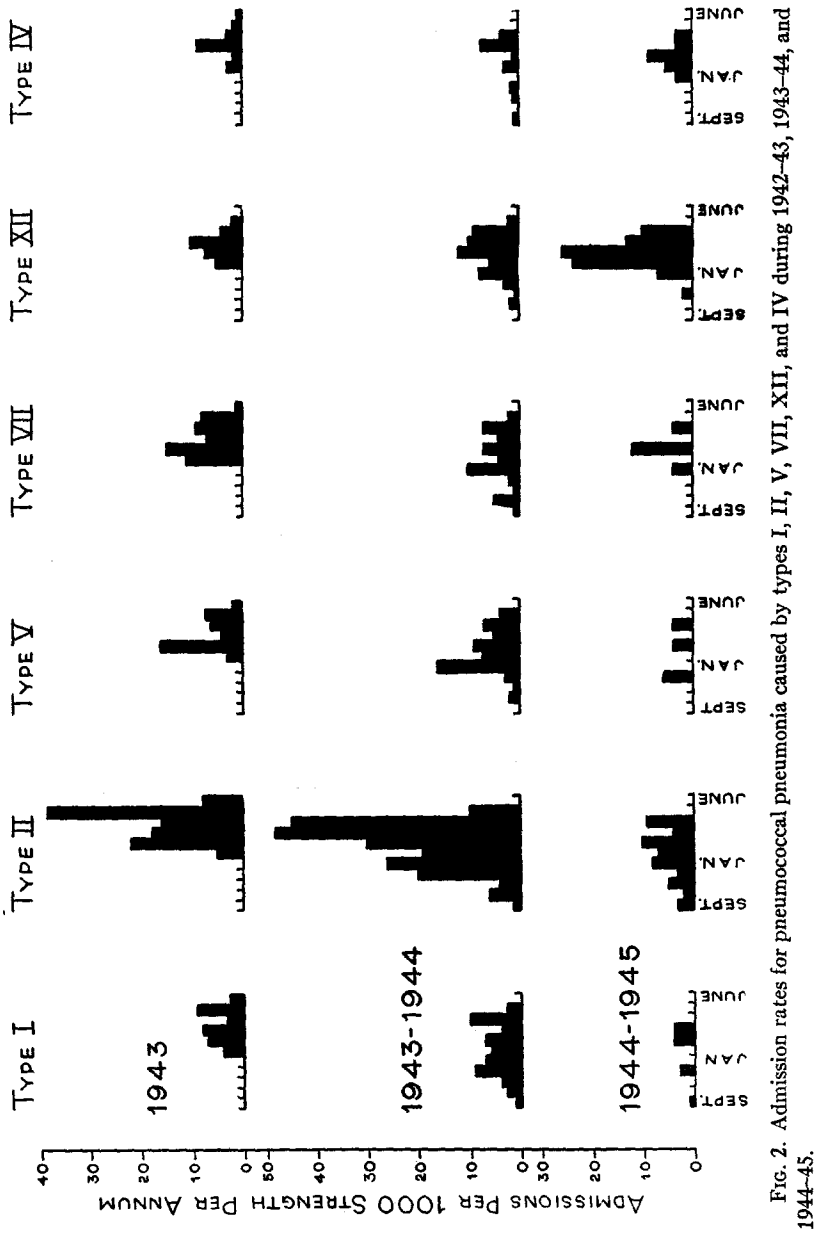

FIG. 2. Admission rates for pneumococcal pneumonia caused by types I, II, V, VII, XII, and IV during 1942–43, 1943–44, and 1944–45.

pneumonia are similar both in size and shape for the 3 years. The same is true for type IV although the small number of cases introduces some irregularity. It is also apparent that the 1943 and 1943–44 curves for types I, II, V, and VII respectively are closely similar. However, when the 1944–45 curves for types I, II, V, and VII in the non-immunized part of the population are

TABLE VI

Expected and Observed Incidence of Type I, II, V, and VII Pneumonia in Non-Immunized Group for 1944–45

Calculated from behavior of type XII					
Type	I	II	V	VII	No. of cases of type XII used for calculating expected incidence*
Expected from 1942–43 experience.	57	170	62	82	29
Expected from 1943–44 experience.	46	182	46	46	43
Observed 1944–45 (times 2).....	4	30	6	12	47
Calculated from behavior of type IV					
Type	I	II	V	VII	No. of cases of type IV used for calculating expected incidence*
Expected from 1942–43 experience.	25	77	26	36	20
Expected from 1943–44 experience.	49	182	47	46	13
Observed 1944–45 (times 2).....	4	30	6	12	14

	Type	No. of cases
Average expected for 1944–45	I	44
	II	153
	V	45
	VII	53

* These figures represent the actual numbers of cases of type XII and type IV pneumonia occurring for each of the 3 years in both immunized and non-immunized groups.

examined, it is evident that the rates fall far below the experience of the preceding 2 years. The consistent behavior of type XII and of type IV in each of the 3 years provides a means for calculating the amount of reduction in the incidence of pneumonia caused by types I, II, V, and VII among the non-immunes which was brought about by immunizing one-half the population.

In Table VI the expected incidence of type I, II, V, and VII pneumonia for the 1944–45 season is shown as calculated on four different bases. The values based on the 1943–44 experience were almost identical when either the type XII

or type IV incidence was used in the proportions. The values based on the 1942–43 experience showed a greater divergence, part of which may be due to the incomplete typing of pneumococci at that time. The observed incidence for 1944–45 was multiplied by two since the non-immunes represented only one-half the population. In Fig. 3 the expected and observed values are presented diagrammatically, the expected values being the average of values calculated on the four different bases. The observed incidence of pneumonia due to types I, II, V, and VII in the non-immunized men during 1944–45 was 17.6 per cent of the expected incidence.

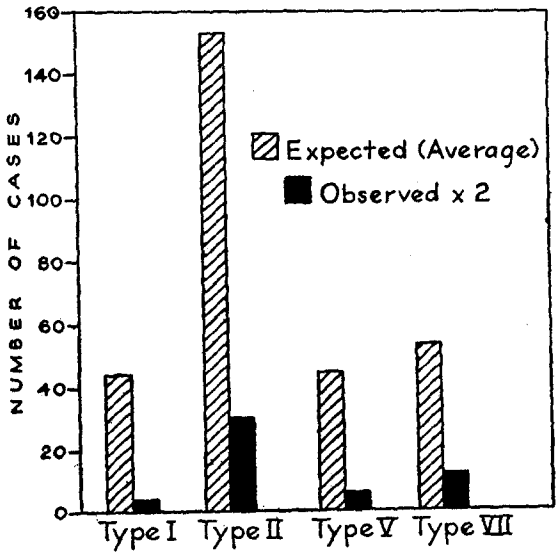

FIG. 3. Expected and observed incidence of pneumococcal pneumonia caused by types I, II, V, and VII for 1944–45. The observed incidence for 1944–45 has been multiplied by two since the non-immunes comprised only one-half the population.

The conclusion is inescapable that immunization of half the population greatly reduced the incidence of disease in the non-immune half. It explains why the number of cases available for testing the effect of the immunization was so far below the expected. Furthermore, the almost identical reduction in the incidence of pneumonia caused by each of the four types among the non-immunes, affords very strong evidence that immunization with the capsular polysaccharides of types I, V, and VII was just as effective as the more easily visualized protection given by the polysaccharide of type II. Since the actual number of cases of type I, V, and VII pneumonia was insufficient to give statistical proof of the value of these polysaccharides as immunizing agents, the evidence afforded by the comparisons with the preceding years is of very considerable importance.

Two possible explanations may be suggested to account for the protection afforded the non-immune half of the population. In the first place, elimination of type I, II, V, and VII pneumonia in half the population produced a corresponding reduction in case-contact carriers and thus lowered the carrier rate of the whole population. This assumes that the principal focus for dissemination was from subjects just developing pneumonia or recently recovered. In the preceding 2 years, when the pneumonia rates were very high, the rôle of cases in disseminating the organisms may have been important. For 1944–45, however, it is hard to believe that 43 cases of type XII pneumonia, occurring during the 4 months between January 6 and April 27, could account for a type XII carrier rate averaging 12.7 per cent over the same period.

A second possibility is that the immunization directly affected the carrier rate. It has already been shown that there was a highly significant difference between the I, II, V, and VII carrier rates of the immunized and non-immunized men. It is probable that the truth lies in a combination of the two explanations. With the thorough mixing of the immunized and non-immunized subjects in the population, the chances would be that in every other one of its transfers from man to man, the pneumococci would fall on infertile ground, either because the subject was prevented from developing pneumonia or because his ability to carry pneumococci was lessened by the immunization. The net result would be a lowering of the carrier rate for types I, II, V, and VII throughout the entire population.

The reduction of disease in the non-immune portion of a partially immunized population is not a new finding. Similar observations have been made following immunization against diphtheria. Likewise, Lister (20) concluded that in antipneumococcal immunization with bacterial vaccines, the unimmunized portion of the population derived benefit from contact with the immunized portion. He was unable to compute the magnitude of this benefit.

RÉSUMÉ AND DISCUSSION

In previous studies on the effect of antipneumococcal immunization in the prevention of pneumococcal pneumonia certain factors have made evaluation difficult. In the present study, it is believed that the important sources of error have been eliminated, and that as a result, the interpretation of the results can be made with more assurance than has been previously possible.

The population chosen, that of an Army Air Force Technical School, was large. During the two preceding winters, high epidemic rates for pneumococcal pneumonia had prevailed in the School. Information on the serological types of pneumococci identified from the 1500 cases of pneumonia occurring in the first 2 years was available. Types II, I, V, VII, XII, and IV, in that order, caused 75 per cent of the cases of disease, the rates for the individual types being approximately the same for each of the 2 years. The living conditions and duties of the population, which had been remarkably uniform during

the 1942–43 and 1943–44 seasons, were unchanged during the season of 1944–45. A reasonable prediction could thus be made that the incidence of pneumococcal pneumonia during the experimental period would be high and also what types of pneumococci would be involved. Accordingly, the solution used for immunization contained the specific capsular polysaccharides of pneumococcus types I, II, V, and VII. Type XII and type IV remained as controls.

The preparations of polysaccharides were of known antigenic potency as determined previously by inoculation of civilian volunteers.

Immunization by a single subcutaneous injection of the polysaccharides was carried out on alternate members of the population, thus insuring a thorough mixing of immunized and non-immunized subjects in all phases of their activities. To make certain that the two groups were epidemiologically identical a marginal punch card system was devised which made it possible at any time to compare the groups for such factors as length of military service, age, and previous history of pneumonia. At no time was any essential difference found. Computation of the man-days of exposure gave approximately equal values for the two groups. Finally, the members of the population could be observed for a reasonably long time, since the students remained at the School for 24 weeks.

Laboratory facilities were available for the typing of all cases of pneumonia. In addition, a continuous carrier survey for pneumococci was carried on throughout the period of observation, the total sample being 3757 pharyngeal cultures with an over-all pneumococcal carrier rate of 57.7 per cent.

The evidence given in the present paper demonstrates clearly that immunization of man with the specific capsular polysaccharides of pneumococcus types I, II, V, and VII is effective in preventing the development of pneumonia due to these types in the immunized subjects. Of equal interest is the observation that immunization of half the population against types I, II, V, and VII greatly reduced the incidence of pneumonia due to these types in the non-immunized subjects. This conclusion was based on the observed behavior of type XII and of type IV. For each of these types the rates of pneumonia were closely similar for the 1942–43, the 1943–44, and 1944–45 seasons. Furthermore, the ratios of the incidence of type XII pneumonia to the incidence of type I, II, V, and VII pneumonia, respectively, were quite similar for the 1942–43 and the 1943–44 seasons. The ratios of type IV pneumonia to the types I, II, V, and VII pneumonias were also close for the two seasons. From the actual incidence of type XII and type IV pneumonias in the 1944–45 season, it was thus possible to calculate on four different bases the expected incidences of type I, II, V, and VII pneumonias for the winter of 1944–45. The expected incidences obtained from the four separate calculations were close. The observed incidence of type I, II, V, and VII pneumonia in the non-immunized fraction of the population was but 17.6 per cent of the expected.

Earlier studies by other investigators have shown that when pneumococcal pneumonia is epidemic the carrier rates for the epidemic types are high. The same has been shown to be true for pneumococcus type XII during the present study. It is probable that the failure of the non-immunized portion of the population to develop high pneumonia rates was due to inhibition of the development of high carrier rates for types I, II, V, and VII as a consequence of its being thoroughly mixed with the immunized portion. In this regard, evidence has been presented that the carrier rates for these types in the immunized portion of the population were significantly lower than in the non-immunized. It should also be considered that the elimination of pneumonia cases in half the population should bring about a comparable elimination of case-contact carriers. For these reasons, it is suggested that the ability of the immunized subjects to carry and disseminate types I, II, V, and VII pneumococci was greatly reduced by specific immunity to these types and that this immune barrier, composed of half the population, greatly reduced the dissemination of these types throughout the whole population.

It is realized that a high carrier rate for pneumococci of certain serological types is only one of the factors responsible for epidemic outbreaks of pneumonia. For example, the association of pneumococcal pneumonia with non-bacterial respiratory disease has long been recognized. The carrier rate for pneumococci of certain types, however, is of sufficient importance in the production of high pneumonia rates that it is probable that any procedure which inhibits the development of a high carrier rate will also reduce the incidence of pneumonia.

The time required for the development of immunity following injection of the polysaccharides is believed to be in the neighborhood of 2 weeks. This is based on the observation that the only cases of pneumonia among the immunized men that were caused by types I, II, V, and VII developed during the first 2 weeks after immunization. In support of this conclusion, studies on the serum of immunized individuals showed that specific antibodies developed within this time but usually required 3 to 6 weeks to reach their maximum (14).

Because of the relatively low incidence of pneumococcal pneumonia in civilian populations, antipneumococcal immunization is unlikely to become a general procedure. In certain groups at greater risk, for example foundry workers, miners, and inmates of mental institutions, however, immunization would appear to be a desirable procedure. In military populations the greatest incidence of pneumonia occurs in new recruits, so that most benefit would be derived from immunization of this group. Although it cannot be predicted in advance with complete assurance, it is probable that the immunizing preparation should contain the capsular polysaccharides of pneumococcus types I, II, V, VII, XII, and possibly that of type IV also. Experience in two other military installations in which a moderately high incidence has prevailed during the past 3 years has shown that these types were responsible for most of the

cases of pneumococcal pneumonia. Epidemics recorded among civilian groups in the United States and Europe almost without exception have been caused by pneumococcus types I, II, and V. It is of considerable interest, however, that pneumococcus type XII, which has previously not been considered of epidemic significance in this country, has also been important in the etiology of pneumonia among the laborers in South African mines where epidemic levels have prevailed.

SUMMARY AND CONCLUSIONS

1. Immunization of man with 0.03 to 0.06 mg. of each of the capsular polysaccharides of pneumococcus types I, II, V, and VII, given in a single subcutaneous injection, has been shown to be effective in preventing pneumonia caused by these types but not that due to heterologous types.

2. Immunity appears within a period of 2 weeks following injection of the polysaccharides. Its duration was not determined, although 6 months can be set as a minimum.

3. Immunization of alternate subjects in the population reduced greatly the incidence of pneumonia in the non-immunized.

4. The carrier rate for pneumococcus types I, II, V, and VII was lowered significantly in the immunized group as compared with the controls. It is suggested that an over-all reduction in the incidence of carriers was responsible for the lowered rates for pneumococcal pneumonia in the non-immunized group.

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