RESULTS OF PROPHYLACTIC INOCULATION AGAINST PNEUMOCOCCUS IN 12,519 MEN.

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The success of prophylactic vaccination against typhoid fever as demonstrated by the results obtained in the United States Army naturally suggests the effort to combat other prevalent and serious diseases by the same method. Among such diseases pneumonia easily ranks first. During the past winter it has been responsible for probably 80 per cent of the deaths in the various training camps in this country, and though the streptococcus has been the causative agent in many of these cases the pneumococcus has also played a prominent part.

Animal experiments have shown that it is easy to produce active immunity to pneumococci by the injection of small doses of dead organisms even in animals as susceptible as the mouse and rabbit, and this immunity persists for a considerable time. It is theoretically possible, therefore, to immunize man to the fixed types of pneumococci by injection of dead cultures.

The only place to our knowledge where preventive inoculation against pneumonia has been hitherto attempted has been among the workers in the mines in South Africa, where the disease occurs with great frequency and causes the death of large numbers of native workmen. The first inoculations on a large scale were carried out by Sir Almroth Wright (1) in 1911 and 1912. However, in these experiments no attention was paid to differences in types of pneumococci and this fact renders the interpretation of the results of the inoculations extremely difficult. In 1913 Dochez and Gillespie (2) published a classification of pneumococci and Lister (3) independently reported shortly afterwards a similar classification

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of the pneumococci encountered in South Africa. Lister then undertook an experimental study of prophylactic inoculation against the various types of pneumococci in animals and man. He demonstrated that immunity can be produced in man against at least certain ones of these types either by subcutaneous or intravenous injection, more readily by the latter. He found that subcutaneous inoculation of 40 billion cocci of the strains he employed caused little if any toxic reaction in the guinea pig, rabbit, or man, and intravenous inoculation of 20 billion in the rabbit and 40 billion in man gave rise to but slight toxic reaction. On the basis of these experiments Lister undertook the prophylactic inoculation of large groups of miners against pneumococcus and has recently reported the results of his experiments. He at first advocated inoculation at 7 day intervals, each dose to consist of 6 billion cocci of each type against which immunity was desired. Subsequently he greatly reduced this dosage and gave three subcutaneous inoculations at 7 day intervals, each injection consisting of 2 billion of each type.

The workers in three different mines, the Crown, Premier Diamond, and De Beers Diamond, were inoculated with a vaccine composed of the three types of pneumococcus which were most prevalent in these mines. They were known as Types A, B, and C. Types B and C correspond to Types II and I, respectively, in Dochez and Gillespie's classification. Type A has not been encountered in America. In the De Beers Diamond Mine a fourth group was added, called Type H. In the De Beers experiment 1 billion of Type H was added to each injection, making a total dosage at each injection of 7 billion. The vaccinated miners were then observed over a period of 6 to 12 months and in all three mines a definite decrease in the incidence and mortality rate from pneumonia was observed. In the case of the Crown Mines, every case of pneumonia which occurred among the vaccinated individuals was studied bacteriologically and the type of pneumococcus determined. No cases of the types against which the men had been vaccinated (Types A, B, and C) developed during the 9 months of observation. Lister contends that this fact, namely the alteration of a relative group prevalence by means of specific group inoculation, is a more critical test of the efficacy of pneumonia prophylaxis than the simultaneous comparison of pneumonia rates in inoculated and uninoculated (control) groups when the comparison is based upon the erroneous assumption that all cases of disease due to the pneumococcus are bacteriologically indistinguishable. He emphasizes the probability that the protection of a considerable part of the community by inoculation lessens the number of carriers, and perhaps the virulence of the strains found in the community, and hence confers a definite benefit upon the uninoculated group which would affect the use of this group as controls in a statistical sense. Lister reported no unpleasant effects from the vaccine.

Bacteriological study of the first hundred cases of pneumonia occurring at Camp Upton showed that about 70 per cent were due to the pneumococcus and of these, about 50 per cent were caused by Type

I, II, or III. In consideration of this fact and of the results achieved by Lister in South Africa, it seemed desirable to employ prophylactic vaccination against the pneumococcus at Camp Upton. At the outset it was decided to incorporate only Types I, II, and III in the vaccine. Vaccination against Type IV was obviously not feasible on account of its many varieties. The preliminary problems to be determined were: First, how large a dose of such a vaccine could be administered without producing a severe reaction? Second, would the dose, injected three or four times subcutaneously, produce an efficient and demonstrable immunity?

Preliminary Experiments.

For the preparation of the vaccine preliminary experiments showed that agar media were unsatisfactory because of the small yield of organisms. Experiments in immunizing rabbits with pneumococci grown on a variety of media indicated that those obtained from 0.5 per cent glucose broth were as effective antigenically as those obtained from agar media or from plain broth, and as the yield from glucose broth is much more abundant this medium was adopted. To prevent autolysis, incubation was stopped after 12 to 14 hours. Centrifuging was carried out by the use of a continuous feed type of centrifuge (Sharpless laboratory centrifuge) which will take 8 to 12 liters of broth per hour and give a clearer supernatant fluid than is usually obtained from the bucket type of centrifuge. The cultures were killed before centrifuging by heating to 53°C. for $\frac{1}{2}$ hour. This conserves the antigenic effects of the culture. After centrifuging, the organisms were suspended in normal saline solution, shaken to secure even distribution, standardized by dilution and comparison of the opacity with known suspensions of pneumococci, and again heated at 55°C. for $\frac{1}{2}$ hour. Sterility of the vaccine was established by aerobic and anaerobic cultures and by subcutaneous inoculation into guinea pigs and intraperitoneal inoculation into mice. Tricresol was added to a concentration of 0.3 per cent as a preservative.

In order to determine the optimum dosage and interval of injection inoculations were given to forty-two adult volunteers in varying dosage and at different intervals, and the effect was gauged by means of tests made upon the serum taken 8 days after the last in-

jection. The character of the local and general reactions to the inoculation was also observed. The tests applied to the serum were the agglutination titer against the three types included in the vaccine and the protective power of the sera against these types injected into mice.

The agglutination titer was carried out with 24 hour plain broth cultures of the same strains used in preparing the vaccine. The serum dilutions used were 1:1, 1:3, 1:10, and occasionally 1:30. The tubes were incubated for 2 hours at 37°C. in a water bath, given a first reading, placed in the ice box over night, and then given the final reading. The results charted are based on the final reading.

For the protection experiments the method described by Dochez (4) was employed. A plain broth passage culture from the heart's blood of a mouse was used for each of the three strains employed in preparing the vaccine. Several dilutions of these three cultures were made with sterile broth so that 0.5 cc. of the dilutions would contain respectively 0.01, 0.001, 0.0001, 0.00001, and 0.000001 cc. of original culture. 2 parts of each serum were diluted with 3 parts of normal salt solution so that 0.5 cc. of the diluted serum contained 0.2 cc. of serum. With each serum mice were injected intraperitoneally with some or all of the following mixtures of diluted serum and diluted cultures.

Mouse 1, 0.5 cc. of diluted culture (equals 0.01 cc. of culture of Type I) + 0.5 cc. of diluted serum.

Mouse 2, 0.5 cc. of diluted culture (equals 0.001 cc. of culture of Type I) + 0.5 cc. of diluted serum.

Mouse 3, 0.5 cc. of diluted culture (equals 0.0001 cc. of culture of Type I) + 0.5 cc. of diluted serum.

Mouse 4, 0.5 cc. of diluted culture (equals 0.01 cc. of culture of Type II) + 0.5 cc. of diluted serum.

Mouse 5, 0.5 cc. of diluted culture (equals 0.001 cc. of culture of Type II) + 0.5 cc. of diluted serum.

Mouse 6, 0.5 cc. of diluted culture (equals 0.0001 cc. of culture of Type II) + 0.5 cc. of diluted serum.

Mouse 7, 0.5 cc. of diluted culture (equals 0.01 cc. of culture of Type III) + 0.5 cc. of diluted serum.

Mouse 8, 0.5 cc. of diluted culture (equals 0.001 cc. of culture of Type III) + 0.5 cc. of diluted serum.

Mouse 9, 0.5 cc. of diluted culture (equals 0.0001 cc. of culture of Type III) + 0.5 cc. of diluted serum.

As controls six mice were injected intraperitoneally as follows:

Mouse 1, 0.5 cc. of diluted culture (equals 0.00001 cc. of culture of Type I) + 0.5 cc. of normal saline solution.

Mouse 2, 0.5 cc. of diluted culture (equals 0.000001 cc. of culture of Type I) + 0.5 cc. of normal saline solution.

Mouse 3, 0.5 cc. of diluted culture (equals 0.00001 cc. of culture of Type II) + 0.5 cc. of normal saline solution.

Mouse 4, 0.5 cc. of diluted culture (equals 0.000001 cc. of culture of Type II) + 0.5 cc. of normal saline solution.

Mouse 5, 0.5 cc. of diluted culture (equals 0.00001 cc. of culture of Type III) + 0.5 cc. of normal saline solution.

Mouse 6, 0.5 cc. of diluted culture (equals 0.000001 cc. of culture of Type III) + 0.5 cc. of normal saline solution.

The time of injection of each mouse was noted and the number of hours to time of death recorded. When a mouse had survived over 140 hours it was recorded as "survived." Upon dying the mice were autopsied and films made from the peritoneal exudate and stained to determine the presence of Gram-positive diplococci. From the control mice, upon dying, cultures were made from the heart's blood and the type was verified by agglutination. The range of survival exhibited in the controls throughout the tests is shown in Table I.

TABLE I.

Mouse Controls for Protection Tests.

	Type I.		Type II.		Type III.	
	0.00001 cc.	0.000001 cc.	0.00001 cc.	0.000001 cc.	0.00001 cc.	0.000001 cc.
Minimum.............	28*	31	18	16	14	33
Maximum............	36	36	40	32	36	45

*The figures indicate the hours of survival of each mouse after injection.

All inoculations except in Individual 9 were given subcutaneously. The first one inoculated, Individual 9, received two subcutaneous inoculations of 16 billion cocci of each type at each inoculation, 4 days apart, followed 4 days later by one-tenth this dose intravenously. An extensive area of redness with some tenderness followed the subcutaneous inoculations, reaching the maximum in 48 hours but without incapacitating the subject; there was no accompanying general

reaction. The intravenous injection was followed 48 hours later by a slight malaise and a temperature of 100.4°F. of a few hours' duration.

All other inoculations were given subcutaneously as this was considered the only method feasible for use later on a large scale in the camp.

Individuals 1 to 8 (Table II) received a first dose subcutaneously of 8 billion cocci of each type. In four adults a moderate local reaction resulted. In two, however, the local reaction was severe, leading

TABLE II.

Individuals Receiving a Single Large Inoculation.

Individual No.	Total dosage in billions.			Reaction.		Tests on sera 7 days after injection.																	
						Agglutination.								Mouse protection.									
						Type I.			Type II.			Type III.		Type I.			Type II.			Type III.		
	Type I.	Type II.	Type III.	Local.	General.	1:1	1:10	1:30	1:1	1:10	1:30	1:1	1:10	0.01 cc.	0.001 cc.	0.0001 cc.	0.01 cc.	0.001 cc.	0.0001 cc.	0.01 cc.	0.001 cc.	0.0001 cc.
1	8	8	8	Sl.*	N.	++	–	–	++	–	–	–	–	S.	S.	S.	27	S.	S.	25	28	52
2	8	8	8	Mod.	"	+	–	–	++	–	–	–	–	30	103	"	21	"	"	25	37	S.
3	8	8	8	"	"	–	–	–	++	–	–	–	–	20	73	"	21	"	"	25	26	57
4	8	8	8	Sev.	Sl.	–	–	–	++	–	–	–	–	18	29	50	14	"	"	17	24	31
5	8	8	8	Mod.	Sev.	–	–	–	–	–	–	–	÷	30	S.	S.	21	80	"	19	27	S.
6	8	8	8	Sev.	"	–	–	–	–	–	–	–	–	17	"	41	10	54	"	12	27	51
7	8	8	8	Mod.	Sl.	–	–	–	+	–	–	–	–	17	29	50	12	80	"	10	51	S.
8	8	8	8	Sl.	Sev.	–	–	–	–	–	–	–	–	20	39	S.	22	44	"	24	29	43

* In the tables N. indicates none, Sl., slight, Mod., moderate, Sev., severe, and Inf., infiltration. The figures indicate the hours of survival of each mouse after injection. S. indicates survival over 140 hours.

to a reddening and swelling of the area from the shoulder almost to the wrist, persisting 3 days and rendering use of the arm during this time difficult. In three adults a chill with some nausea, marked malaise, and slight rise of temperature occurred in the first 24 hours. It was concluded that reactions of this type were too severe to be permissible in a large scale vaccination. A series of persons (Nos. 10 to 21, 25, and 27, Table III) was accordingly inoculated with four or five injections of much smaller doses at 3 to 7 day intervals (except

in two cases in which the interval between the first two doses was 2 days). Inasmuch as the suggestion had been made that Type III was especially prone to induce severe local reactions this type was omitted from one of the inoculations in Individuals 10 to 16 and 27. No difference could be observed between the local reaction in these cases when Type III was omitted. The dosage used in Individuals 12 to 16 was as follows:

Day.	No. of billions of Type I.	No. of billions of Type II.	No. of billions of Type III
1	1	1	1
4	2	2	2
7	4	4	4
13	6	6	

The dosage of each type in other individuals was as follows: Individual 17: first injection 1 billion; second injection 2 billion; third injection 3 billion; fourth injection 4 billion. Individuals 18 and 20: first injection 1 billion; second injection 2 billion; third injection 2 billion; fourth injection 3 billion. Individuals 19 and 21: first injection 1 billion; second, third, and fourth injections each 2 billion. Individual 25 received only half as much of Type III as of Types I and II at each injection. Of these fourteen adults, marked local reactions occurred after at least one of the inoculations in four instances but not severe enough to incapacitate the individual. In none of these instances was there any severe general reaction. These doses appeared to be such as could be satisfactorily employed on a large scale without unduly incommoding a command. Still smaller doses were employed in Individuals 32, 33, 36, 37, and 38. These were without any severe local reactions and with moderate or no general reaction.

The experimental work of Cole and Moore (5) showed that in rabbits an immunity could be more rapidly induced by small daily intravenous inoculations of antigen than by much larger intravenous inoculations at longer intervals. To test the applicability of this principle to subcutaneous injections in man, four adults (Nos. 39 to 42) were given daily subcutaneous injections of 1 billion cocci of each type for 7 days. These injections were associated with only the mildest local reactions and with no constitutional reaction. The

TABLE III.

Cases Receiving Multiple Doses of Varying Amounts.

Individual No.	No. of injections	Intervals	Total dosage in billions — Type I	Type II	Type III	Reaction — Local	General
9	3	days 4	34	34	34	Mod.	Sl.
10	5	4–2	12	12	6	Sev.	"
11	5	4–2	12	12	6		
12	4	6–3	13	13	7	Sl.	N.
13	4	5–3	13	13	7	Mod.	Sl.
14	4	4–3	13	13	7	Sl.	N.
15	4	5–3	13	13	7	Mod.	Sl.
16	4	6–3	13	13	7	Sl.	N.
17	4	5–3	10	10	10	"	"
18	4	4–3	8	8	8	Mod.	Mod.
19	4	4–3	7	7	7	Sev.	"
20	4	4–3	8	8	8	"	"
21	4	4–3	7	7	7	Mod.	Sl.

Agglutination (Before vaccination — Type I, II, III at 1:1, 1:3; 8 days after last injection — Type I, II, III at 1:1, 1:3, 1:10, 1:30) and Mouse protection (Before vaccination — Type I, II, III; 8 days after last injection — Type I, II, III at 0.01 cc., 0.001 cc., 0.0001 cc.) data as recorded in the table.

No.																													
22	3	7	6	6	5	Inf.																90	S.	S.	S.	S.	41
23	3	7	6	6	5	"																27	"	"	24	"	89
24	3	7	6	6	5	" N.																S.	"	"	17	"	64
25	3	7–4	7	7	3½	Inf. Sev.																21	"	"	36	"	23
26	3	7	6	6	5	Sl. to Mod.																	73	92	16	"	63
27	3	3	9	9	3	Sev.																16	24	33	17	37	28
28	3	7	6	6	5	Inf.																24	46	34	12	S.	S.
29	3	7	6	6	5	"																24	S.	38	18	"	"
30	3	7	6	6	5	"																65	"	S.	36	24	46
31	3	7	6	6	5	"																22	41	S.	12	103	31
32	4	7–4	4	4	4	Mod.	N.															14	S.	11	"	S.	33
33	4	7–4	4	4	4	"	"																				
34	2	7	3	3	3	Inf.																19	89	76	12	19	76
35	2	7	3	3	3	"																36	54	19	16	S.	40
36	3	7	2½	2½	2½	Mod.	N.															25	78	65	13	56
37	3	7	2½	2½	2½	"	Mod.															37	78	S.	13	S.
38	3	7	2½	2½	2½	Sl.	"															22	56	"	17	22
39	7	1	7	7	7	"	N.															11	18	39	29	40
40	7	1	7	7	7	"	"															13	S.	S.	20	28
41	7	1	7	7	7	"	"															15	21	43	14	35
42	7	1	7	7	7	"	"																		15

immune body response in the sera of these various groups is shown in Tables II and III.

From sixteen persons serum was obtained before commencing the vaccination. In none of these was there any agglutinin demonstrable against any of the three types and in only one instance (No. 9) were there any survivals in the mouse protection test, in this case against Type I. On the other hand, 8 days after the vaccination agglutinins to some extent were demonstrable in twenty-seven of the forty-two individuals studied and a definite degree of protective power as shown by the survival of at least some of the mice in all of the forty cases in which the mouse protection test was performed.

The degree of agglutinin production and of mouse protection varies so greatly in the individual cases in any one group that it becomes difficult to draw positive deductions as to the merits of the various methods of administration employed. This difficulty is indicated if we group the persons according to dosage and number of inoculations and tabulate the percentage of positive agglutinations at each titer and of mouse survivals for each dose of culture for these groups. This we have done in Table IV. From a study of this table the conclusion seems justified that the first group (Adults 10 to 21) which comprises those receiving the largest total dosage exhibits definitely the best response as judged by both agglutination and the mouse protection test. The second group (Adults 32, 33, 36, 37, and 38) received the smallest total dosage and gave definitely less satisfactory response. A comparison of the third group (Adults 1 to 8), the fourth group (Adults 18 to 21, 25, and 27), and the fifth group (Adults 39 to 42), all receiving practically the same total dosage but in the third group given in a single large dose, in the fourth in three or four moderate doses at 3 to 7 day intervals, and in the fifth in seven very small daily doses, shows no definite difference that can be detected in the response of the three groups. The conclusions that we draw from these experiments are that the immune response as measured by these tests will depend on the total dosage of vaccine and is little influenced by the number of doses into which this quantity is divided. The difference in toxic reaction, however, in these three groups was definite. The small daily doses gave hardly any reaction. The single very large dose gave rise to several severe local and constitutional reactions.

TABLE IV.

Summary by Percentages of Agglutination and Protection Tests in Sera.

Group.	Percentage of positive agglutinin reactions.						Percentage of mouse survivals in protection test.						
	Type I.			Type II.			Type I.			Type II.			Type III.
	1:1	1:3	1:10	1:1	1:3	1:10	0.01 cc.	0.001 cc.	0.0001 cc.	0.01 cc.	0.001 cc.	0.0001 cc.	0.0001 cc.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Group 1* (Individuals 10 to 21).....	67	75	33	58	58	17	12	54	82	14	60	82	18
" 2 (" 32, 33, and 36 to 38)......................	40	20	0	20	20	0	0	25	75	0	25	75	25
Group 3 (Individuals 1 to 8)........	25		0	62		0	12	37	62	0	50	100	37
" 4 (" 18 to 21, 25, and 27)......................	50	50	0	33	33	0	0	40	40	0	25	100	0
Group 5 (Individuals 39 to 42)......	0	25	25	25	25	0	0	50	50	0	67	75	0
" 6 (" 22 " 24, 26, 28 to 31, 34, and 35)..........	67	44	0	44	22	11	11	40	90	10	50	70	20

* Group 1 received a total dosage of from 7 to 13 billion cocci of each type. Group 2 received a total dosage of from 2½ to 4 billion cocci of each type. Groups 3, 4, and 5 received a total dosage of from 7 to 9 billion cocci of each type, Group 3 in a single large injection, Group 4 in three or four moderate injections at 3 to 7 day intervals, and Group 5 in seven small daily injections. Group 6 received a total dosage of 3 to 6 billion cocci of each type and developed infiltrations.

Vaccinations of Troops.

On the basis of these preliminary experiments we adopted for the larger scale vaccinations a moderate total dosage of 6 to 9 billion cocci of each type and administered this in four small doses at weekly intervals. By this method we expected to secure a definite immune response in the vaccinated individuals with a minimum of severe reactions and without undertaking a larger number of injections than would be within the capacity of the medical staff of the camp to carry out.

The detailed dosage decided upon was:

1st inoculation:	Pneumococcus Type I	1	billion
	" " II	1	"
	" " III	1	"
	Total........	3	"

2nd inoculation:	Pneumococcus Type I	2	billion
	" " II	2	"
	" " III	2	"
	Total........	6	"

3rd inoculation:	Pneumococcus Type I	3	billion
	" " II	3	"
	" " III	$1\frac{1}{2}$	"
	Total........	$7\frac{1}{2}$	"

4th inoculation: Same as 3rd inoculation.

The vaccine was made up in three different concentrations so that the dose in every case was 0.5 cc.

Altogether, 12,519 men were vaccinated, about 40 per cent of the mean strength of the command. A great majority of these received three or four inoculations. Some, however, had only one or two. The vaccine was given at 5 to 7 day intervals, except in one organization where unavoidable circumstances necessitated a lapse of 20 days between the first and second inoculations. In most cases the vaccine was administered in the arm.

Reactions.

The constitutional reactions to the pneumococcus inoculations were usually negligible. Out of the entire number vaccinated, only twenty-five men were sufficiently ill to remain in quarters or the hospital for a short time. This number would probably have been larger if the vaccination had been compulsory. As it was, those who were upset by the first or second inoculation were usually not given the third or fourth. The impression prevailed among the regimental surgeons that the reactions to pneumococcus vaccination were milder than those to typhoid vaccination. The troops, however, were in better physical condition when they received the pneumococcus vac-

cine than when the typhoid inoculations were given. In those who
reacted severely the symptoms simulated an attack of influenza.
The patient complained of general malaise, chilly sensations, fever,
and muscular pains. In addition, a certain number of those who
reacted severely had symptoms referrable to the upper respiratory
tract, such as coryza, sore throat, cough, and pain in the chest. A
number of those who were suffering from infections of the air pas-
sages at the time of inoculation, claimed that their symptoms were

TABLE V.

Incidence of Infiltrations and General Reactions among the Vaccinated Troops.

Organization.	No. vaccinated (approximate).	Reactions.	
		Infiltrations.	General reaction (quarters or hospital).
305th Infantry..........................	2,500	43	12
306th "	2,800	62	6
307th "	1,500	6	0
308th "	2,150	28	5
302nd Sanitary Train.....................	700	3	1
367th Infantry (colored).....·...........	1,500	1	0
304th Machine Gun Battalion..............	400	2	0
305th " " "	400	4	1
306th " " "	500	3	0
302nd Engineers..........................	35	0	0
Base Hospital............................	25	0	0
Total................................	12,510	152 (1 in 82)	25 (1 in 500)

more marked after receiving the injection. Constitutional reactions
to pneumococcus vaccination apparently develop more slowly than
with typhoid inoculation, sometimes not appearing until 24 hours
after the injection.

The local reaction to pneumococcus vaccination differs little, as a
rule, from that to typhoid vaccination. At the point of inoculation
an area of tenderness and induration develops, usually about 5 to 10
cm. in diameter. The area of induration is nearly always oblong
and extends down the arm below the point of inoculation. The
axillary glands are sometimes swollen and tender. The tenderness

and swelling at the point of inoculation rapidly decrease, however, and at the end of 3 or 4 days have usually disappeared.

An unexpected and somewhat troublesome complication arose in connection with the vaccination which at first gave us some concern; this was the development of a certain number of small infiltrations at the site of inoculation. They developed slowly, rarely coming to the stage of fluctuation before the 6th or 7th day after inoculation. In size they were usually 2 or 3 cm. in diameter, and in only one instance extended down deeper than the subcutaneous tissue. At first they were looked upon as the result of careless technique, but repeated cultures showed them to be invariably sterile. Some of them developed after the first inoculation, but a greater number followed the larger doses. In a few cases an infiltration developed with each inoculation given. This would have probably happened more frequently had the vaccination not been discontinued in those who developed the condition after the first or second dose. At first the regimental surgeons made an incision, but later it was found that the infiltrations would progress favorably if left alone. Altogether, 152 men developed the lesion (1 in every 82, Table V). They were fairly evenly distributed throughout the various organizations vaccinated, with the exception of the 1,500 vaccinated negroes, among whom only one developed. The infiltrations were tender and painful in the early stage of their development but later became cold and painless. They apparently developed from a hypersensitiveness to the pneumococcus or pneumotoxin. In the hope of discovering whether these individuals exhibited a more or less marked immune response in their sera, the sera of ten such persons were studied (Table III, Adults 22 to 24, 26, 28 to 31, 34, and 35). The percentile results are tabulated in Table IV as Group 6. Apparently the immune response in this group is analogous to that of the other individuals receiving the same total dose of vaccine. We would conclude, therefore, that there is no demonstrable difference in the degree of immune response by the tests we have used in individuals showing pronounced local reaction even to the extent of local infiltration described. Few of the men responding in this way had ever had pneumonia. With only one exception they did not occur among the colored troops who are especially susceptible to pneumonia. They

are not related in any way to the method of administration of the vaccine.

In order to test the relation of these severe reactions to the susceptibility to pneumotoxin, the following experiments were performed. Pneumotoxin was prepared by Cole's (6) method, which consists of growing pneumococci in plain broth; centrifuging and washing in normal saline solution; solution of the pneumococci in weak sodium taurocholate solution at 37°C.; dilution to one-tenth the bulk of the original broth culture. This pneumotoxin was diluted 1: 10 with normal saline solution and injected intradermally to the amount of 0.1 cc. in a series of healthy volunteers. Little resulting reaction was noted in most individuals. However, in those who had shown severe local reactions to the vaccination or had developed infiltrations, an extensive areola developed about the pneumotoxin injection, reaching the maximum after about 24 to 36 hours and often being associated with considerable tenderness. The reaction to the pneumotoxin occurred regardless of the type used in its preparation, exhibiting no specificity for type. These findings suggest that the severe local reactions to the vaccine are due to an unusual sensitiveness to a pneumotoxin which is common to all the types of pneumococci.

Table V shows the number of men vaccinated in each organization and the incidence of infiltrations and severe general reactions in each.

Bacteriological Examination of Sputa.

Particular attention was directed to the bacteriological examination of the sputum in the cases of pneumonia that developed during the period of observation. In seven instances this examination, through some unavoidable circumstance, was not completed. In all the others, however, the sputum was examined and the predominant organism determined.

The Avery blood broth method (7) was used in a great majority of cases and in addition, whenever it was possible, a mouse was inoculated, or a direct culture from sputum was made on a blood agar plate. The following figures indicate the number of times each method was used:

1. Both mouse and blood broth.................................... 47
2. " blood broth and direct culture........................... 23
3. Blood broth alone.. 108
4. Mouse alone... 5

Results of Vaccination.

The vaccination of the troops began on February 4, 1918. The Division was transferred from Camp Upton about April 15, 1918. The following figures are based on the period extending from February 4 to April 15, about 10 weeks. The number of troops vaccinated was 12,519. The number of unvaccinated was approximately 19,481. The latter figure varied, of course, from day to day as new men came and others departed. The vaccinated men were in stable organizations where the personnel underwent little change.

Before discussing the results of vaccination, it is of interest to note the incidence of pneumonia previous to the beginning of vaccination in the various organizations which later received the vaccine and to compare this with the incidence of pneumonia at the same period among the organizations which did not later receive the vaccine. Previous to February 4, the day on which the experiment began, there had been 91 cases of pneumonia among the troops at Camp Upton. Of these, 29 occurred among the organizations which were subsequently vaccinated, while 43 cases occurred among the units which were later to be used as a control; 19 occurred among casuals not included later in either group. It will be seen from this that the cases of pneumonia were quite evenly divided between the 40 per cent of the troops which were to be vaccinated and the 60 per cent which were to be used for controls. Of the 29 pneumonias occurring among the organizations which were subsequently vaccinated, 9 were due to pneumococci of Type I, II, or III and 11 to Pneumococcus Type IV. Of the 43 pneumonias occurring among the control group, 16 were due to pneumococci of Type I, II, or III and 17 to Pneumococcus Type IV.

Furthermore, about 30 per cent of all these pneumonias were streptococcus cases and these, too, were fairly equally divided between the two groups of organizations, 9 occurring among the men vaccinated later, and 10 among the control group.

Incidence of Pneumonia among the Vaccinated Troops from February 4 to April 15, 1918.—There has been but one case of pneumonia due to pneumococcus of Type I, II, or III among the vaccinated troops during this period. This case, due to Type I pneumococcus, developed 24 hours after the first inoculation and therefore before protection could have been developed. During this period sixteen cases of other types of pneumonia occurred among the vaccinated troops (Table VI). There were nine Type IV pneumococcus cases; three of these had only received one injection of vaccine. The remaining seven cases were streptococcus infections, six due to the hemolytic streptococcus, and one to *Streptococcus viridans*. None of the pneumococcus cases died. The Type I case received Type I serum and

TABLE VI.

Incidence of Pneumonia among the Vaccinated Troops, February 4 to April 15, 1918.

Average strength of command, Feb. 4 to Apr. 15, 1918............ 32,000
No. of troops vaccinated against pneumonia.................... 12,519 (40 per cent)
"   " unvaccinated men (average)........................... 19,481 (60 "   ")

Incidence of pneumonia among the vaccinated troops, Feb. 4 to Apr. 15:
Pneumococcus Type I (developed 24 hrs. after 1st injection)...................... 1
"   "   IV (3 cases receiving only 1 injection)....................... 9
Streptococcus hæmolyticus.. 6
"   *viridans*.. 1
                                                                            ——
    Total No. of pneumonias among the vaccinated troops...................... 17

made an uneventful recovery. Most of the Type IV cases ran a mild course, so mild in some cases that the diagnosis of pneumonia was made only by the aid of the x-ray. Two of the streptococcus cases died, both being of the hemolytic group.

Incidence of Pneumonia among the Unvaccinated Troops from February 4 to April 15, 1918.—The unvaccinated fraction of the camp has been divided into two groups (Table VII). First, the old troops whose physical condition and resistance to infection were presumably the same as that of the vaccinated men. They constituted, numerically, about 75 per cent of the controls. Second, the new troops who consisted of newly drafted men coming into camp between February 26 and April 15 and who by reason of their lack of training were prob-

ably more susceptible to infection than the seasoned troops. These comprised about 25 per cent of the controls. Among the old troops there were eighteen cases of pneumonia due to Pneumococcus Type I, II, or III, and seventeen cases due to Type IV, making a total of

TABLE VII.

Incidence of Pneumonia among the Unvaccinated Troops, February 4 to April 15, 1918.

Type of pneumonia.	No. of cases of pneumonia
Old troops (75 per cent of control).	
Pneumococcus Type I	8 ⎫
" " II	5 ⎬ 18 ⎫
" " III	5 ⎭ ⎬ 35
" " IV	17 17 ⎭
Streptococcus hæmolyticus	45 ⎫
" *viridans*	23 ⎭ 68
B. influenzæ	1
Type undetermined	6
Total	110
New drafted men, Feb. 26 to Apr. 15 (25 per cent of control).	
Pneumococcus Type I	2 ⎫
" " II	4 ⎬ 8 ⎫
" " III	2 ⎭ ⎬ 24
" " IV	16 16 ⎭
Streptococcus hæmolyticus	27 ⎫ 38
" *viridans*	11 ⎭
Type undetermined	1
Total	63

Total No. of Type I, II, and III pneumonias among the unvaccinated troops....... 26
"   "   "   " I, II, III, and IV "   "   "   "   "   59
"   "   " streptococcus pneumonias among the unvaccinated troops........... 106
"   "   " pneumonias among the unvaccinated troops....................... 173

thirty-five cases of pneumococcus pneumonia.   There were sixty-eight streptococcus cases, forty-five of the hemolytic type and twenty-three of the non-hemolyzing type.   There was one *Bacillus influenzæ* case and six cases in which the type was not determined.

Among the new men the proportion was about the same. There were eight cases due to Pneumococcus Type I, II, or III. There were sixteen cases of pneumonia due to Pneumococcus Type IV, making twenty-four cases of pneumococcus pneumonia. There were thirty-eight cases of streptococcus pneumonia, twenty-seven of the hemolytic type and eleven of the non-hemolyzing type. There was one case in which the organism was not determined.

TABLE VIII.

Mortality Rates.

Type of pneumonia.	Deaths.	
Among unvaccinated troops.		
		per cent
Pneumococcus.		
Types I, II, and III...	7	27
Type IV..	6	18
Streptococcus hæmolyticus...	26	36
" *viridans*...	4	12
Type undetermined...	5	
Total...	48	28
Annual pneumonia death rate per 1,000 for unvaccinated troops........	12.8	
Among vaccinated troops.		
Pneumococcus..	0	
Streptococcus hæmolyticus...	2	
" *viridans*...	0	
Total...	2	11.7
Annual pneumonia death rate per 1,000 for vaccinated troops..........	0.83	

A summary of the control shows that there were twenty-six cases of pneumococcus pneumonia of Type I, II, or III. There were thirty-three Pneumococcus Type IV cases, making a total of fifty-nine pneumococcus pneumonias. There was a total of 106 streptococcus pneumonias, 72 of which were of the hemolytic type and 34 of the *viridans*, or non-hemolyzing type. Altogether there were 173 pneumonias among the unvaccinated troops.

There were seven deaths among the Pneumococcus Type I, II, and III cases, or 27 per cent (Table VIII). There were six deaths among the Pneumococcus Type IV cases, or 18 per cent. Of the streptococcus cases, hemolytic type, twenty-six died, or 36 per cent. There were only four deaths among the *viridans* cases, the mortality being 12 per cent.

DISCUSSION.

The first and most important deduction to be made from these statistics is that pneumococcus pneumonia of Types I, II, and III has not occurred among the vaccinated troops, whereas twenty-six cases have occurred among the unvaccinated part of the camp. This is the best test of the value of the vaccination as a prophylactic measure. The one case of Type I pneumonia that developed 24 hours after the patient had received his first inoculation may properly be excluded from among the vaccinated cases, as the patient was probably already infected at the time he received the injection and could not have had time for the development of any appreciable immunity.

As Lister pointed out, the diminution or disappearance of certain types of pneumonia as the result of specific type inoculation is a more critical test of the efficacy of pneumonia prophylaxis than the mere simultaneous comparison of pneumonia rates in vaccinated and unvaccinated groups. It is true that the period of observation in this experiment has been short, but the immunity produced by the vaccine appears to have been adequate for this period of time. How much longer this immunity will last can only be determined by following these men and studying the cases of pneumonia that subsequently develop among them.

There have been only six cases of Pneumococcus Type IV pneumonia among the men who received two or more injections of the vaccine, while the control column shows thirty-three cases among the unvaccinated troops. The marked difference in these figures and the mild course which the Pneumococcus Type IV pneumonias ran in the vaccinated series might suggest that some cross-protection against Type IV pneumococcus has been afforded by the Type I, II, and III vaccine. This theory, however, is hardly admissible when we note that the same difference occurs in the incidence of streptococcus pneumonias

in the vaccinated and the unvaccinated troops. There were 106 cases of streptococcus pneumonia among the unvaccinated troops, whereas there were only seven streptococcus pneumonias among the vaccinated troops. Among the 3,500 colored troops, half the companies were vaccinated against pneumonia, the other half were not. There were twenty-eight cases of streptococcus pneumonia among the unvaccinated half and only two cases of streptococcus pneumonia among the vaccinated half, yet these men were living in the same part of the camp and closely associated on drill-grounds, and in recreation and amusement halls.

We have no explanation to offer for this difference. That an epidemic of streptococcus pneumonia occurred at about this time in the camp, there can be no question. Not only the bacteriological studies, but the clinical course, the frequency of empyema, the mortality rate, and the character of the autopsy findings confirm the bacteriological diagnosis. Why this epidemic should to a large extent have spared the vaccinated troops cannot be explained. The incidence of pneumonias, pneumococcic and streptococcic, was approximately equally distributed in the two groups of organizations previous to the beginning of the vaccination. While no explanation of this phenomenon can be offered, it presents no argument against the use of prophylactic vaccination against the pneumococcus, nor does it in the slightest degree weaken the importance of the fact that while twenty-six cases of Type I, II, or III pneumococcus pneumonia occurred among the unvaccinated, none occurred among the vaccinated troops. While it is still too early to draw final conclusions from this experiment, we feel that the results are sufficiently encouraging to justify further investigation along these lines.

SUMMARY.

1. From a study of the agglutinins and protective power of the serum of 42 persons vaccinated against the pneumococcus, Types I, II, and III, it is demonstrated that a definite immune response has been secured to Types I and II by the dose of vaccine employed. Little evidence of response to Type III can be demonstrated by these methods, but this is of less significance in that in animals it is rela-

tively difficult to secure antibodies against this strain in the serum, even though a considerable degree of active immunity may have been produced in the vaccinated animal.

2. The degree of response to the vaccination appears to be dependent upon the total dosage of each type of pneumococcus administered. While some response may be elicited by $2\frac{1}{2}$ billion cocci of each type, a much more constant and greater response follows 13 billion.

3. In subcutaneous administration the manner in which the total dosage is divided, whether given in a single large dose, in seven small daily doses, or in three to five moderate doses at 3 to 7 day intervals, seems to have little influence upon the degree of immune response, provided the total dosage is the same.

4. The local and general toxic reaction varies greatly in different individuals. The smaller the individual doses, the fewer are the severe reactions. This makes it desirable to divide the total dosage into as many inoculations as circumstances make practicable.

5. At Camp Upton 12,519 men have been vaccinated against Pneumococcus Types I, II, and III. Three or four doses were given at intervals of 5 to 7 days with a total dosage of 6 to 9 billion of Types I and II and $4\frac{1}{2}$ to 6 billion of Type III.

6. During the 10 weeks that have elapsed since the vaccination, no cases of pneumonia of these three types have occurred among the men who had received two or more injections of vaccine.

7. In a control of approximately 20,000 men there were twenty-six cases of Pneumococcus Types I, II, and III pneumonias during the same period.

8. The incidence of Pneumococcus Type IV pneumonia and streptococcus pneumonia was much less among the vaccinated troops than among the unvaccinated. No explanation has been advanced for this difference.

9. Small sterile infiltrations disappearing spontaneously occasionally follow the injection of large doses of pneumococcus vaccine and appear to be an expression of cutaneous hypersusceptibility.

10. The persons who develop these lesions exhibit local reactions to each dose of vaccine. They also give abnormally marked reactions to intradermal injections of pneumotoxin. They do not, however,

exhibit anything notable in the agglutinative or protective powers of their sera after vaccination. Whereas the immune response is characteristically specific for the type of pneumococcus, this reaction is not specific for any type. We have found no evidence that Type III is more prone to elicit these severe local reactions than are Types I and II.

11. Prophylactic vaccination against pneumococcus of Types I, II, and III is practical and apparently gives protection against pneumonia produced by these types. It remains to be determined how long this immunity persists.

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