STUDIES ON EXPERIMENTAL PNEUMONIA.

IV. Results of Prophylactic Vaccination against Pneumococcus Pneumonia in Monkeys.

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Prophylactic vaccination against pneumonia has been practised with apparent success on the miners in South Africa by Wright,[1] and more recently by Lister.[2] Within the past 2 years similar investigations have been undertaken in the United States Army Camps by Cecil and Austin[3] at Camp Upton, and by Cecil and Vaughan[4] at Camp Wheeler. The results obtained in these later experiments were so encouraging that the whole question of active immunity against pneumococcus seemed worthy of thorough study.

It has long been recognized that injection into animals of killed cultures of pneumococcus would protect them against lethal doses of living virulent pneumococci injected intravenously. Animals vaccinated in this way usually develop agglutinins and protective substances in their sera. Dochez[5] has shown that in man protective bodies are usually demonstrable in the serum of a patient immediately following an attack of pneumonia; and Cecil and Austin[3] found that the injection of killed pneumococci in man would, in some cases at least, stimulate the production of agglutinins and protective bodies.

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[1] Wright, A. E., *Lancet*, 1914, i, 87.

[2] Lister, F. S., An experimental study of prophylactic inoculation against pneumococcal infection in the rabbit and in man, Publications of the South African Institute for Medical Research, No. 8, Johannesburg, 1916; Prophylactic inoculation of man against pneumococcal infections, and more particularly against lobar pneumonia, Publications of the South African Institute for Medical Research, No. 10, Johannesburg, 1917.

[3] Cecil, R. L., and Austin, J. H., *J. Exp. Med.*, 1918, xxviii, 19.

[4] Cecil, R. L., and Vaughan, H. F., *J. Exp. Med.*, 1919, xxix, 457.

[5] Dochez, A. R., *J. Exp. Med.*, 1912, xvi, 665.

In spite of the fact that the injection of killed cultures of pneumococcus in rabbits, horses, and other animals will protect these animals against lethal doses of the living organism, this accomplishment is not equivalent to preventing the disease, pneumonia, itself. Indeed, very little is known concerning the whole subject of active immunity against pneumonia and comparatively little experimental work has been done along this line.

Wadsworth[6] immunized eleven rabbits against pneumococcus by injecting them with pneumococci which had been dissolved in rabbit bile. The eleven immunized rabbits and five controls were then injected intratracheally with 1 cc. of a virulent pneumococcus culture. Of the five control animals, three died in 48 hours without lung lesions; a fourth lived 4 days and a small patch of pneumonia was found at autopsy. The fifth was dying on the 5th day when it was killed. A small area of consolidation was found in this animal also. Of the eleven immunized animals, none died, but a few were seriously ill from 24 to 36 hours. All the immunized animals, when killed, showed more or less extensive pulmonary consolidation.

In the experiments referred to above, rather large doses of pneumococcus were used for infecting the animals, and it is probable that the controls were overwhelmed by the infection before there was an opportunity for pneumonia to develop. It is possible that if smaller doses had been employed for the intratracheal injections, Wadsworth would have produced pneumonia in the controls, and, on the other hand, the immunized animals would have escaped infection altogether.

The production experimentally of typical lobar pneumonia in monkeys affords an excellent method of testing the value of pneumococcus vaccine. It has been shown[7,8] that pneumococcus pneumonia in monkeys differs in no respect clinically or pathologically from pneumococcus pneumonia in man. An inflammation of the lungs can be produced in rabbits, dogs, and other laboratory animals by introducing virulent pneumococci or streptococci into the trachea, but animals injected in this manner do not run the typical course of lobar pneumonia as observed in man. The object of the present study has been to determine first the value of prophylactic pneumococcus vaccination in general, and secondly, the relative merits of the different types of pneumococcus vaccine that have been employed.

[6] Wadsworth, A., *Am. J. Med. Sc.*, 1904, cxxvii, 851.
[7] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.
[8] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 445.

Technique.

All the vaccine employed in this study was monovalent and was prepared from an old culture of Pneumococcus Type I which had been carried in the laboratory stock for several years. This organism appeared to have lost practically all its virulence. 1 cc. of a 24 hour broth culture had no effect whatever on a mouse when injected intraperitoneally. This was the same culture which was used at the Army Medical School in the preparation of pneumococcus vaccine for army camps.

The culture used for infecting the monkeys was a highly virulent Pneumococcus Type I, originally isolated from a case of lobar pneumonia. This organism killed a mouse in doses of 0.0000001 cc., and 0.00000001 cc. was usually lethal.

Method of Producing Experimental Pneumonia.—Experimental pneumonia was produced by introducing a small quantity of an 18 hour broth culture of pneumococcus (0.000001 to 1 cc.) with a Luer syringe directly into the trachea by the method previously described.[7] Symptoms of pneumonia developed 24 to 48 hours after injection. In testing for resistance to infection following pneumococcus vaccination, the animals were injected intratracheally, in most cases 2 to 4 weeks after vaccination.

Experiments with Pneumococcus Type I Lipovaccine.

The first vaccine to be experimented with was a Pneumococcus Type I lipovaccine which had been prepared at the Army Medical School according to the process described by Whitmore, Fennel, and Petersen.[9]

18 hour glucose broth cultures of Pneumococcus Type I were centrifuged in a Sharpless machine. The sediment was dried at 53°C. for 24 hours. This killed all the pneumococci. The dried sediment was then weighed and ground with steel balls for 24 hours.

[9] Whitmore, E. R., Fennel, E. A., and Petersen, W. F., *J. Am. Med. Assn.*, 1918, lxx, 427.

Finally, the dry powder was suspended in cottonseed oil containing 2 per cent lanolin and diluted to the desired strength.[10]

Dosage.—In order to make the results comparable with those in man only one injection of lipovaccine was administered to each monkey. As in man, the vaccine was injected subcutaneously, the abdominal wall being the site of inoculation.

Two series of monkeys were vaccinated; the first series received each the same dose that a man received of Pneumococcus Type I in the triple pneumococcus lipovaccine prepared by the Army Medical School; that is, 16 billion pneumococci, or 0.8 mg. of the dried bacteria. The second series received a dose proportional to their weight as compared with the weight of man. The average weight of a man is 70 kilos; the average weight of a Philippine monkey is 4 kilos. This series therefore received $\frac{1}{16}$ of man's dose, or 1 billion pneumococci (0.05 mg. of the dried bacteria). The lipovaccine was always diluted so that the monkey received 1 cc. of the oily suspension. In the first experiment a lipovaccine was used which was about 4 months old. In the remainder of the experiments, however, a freshly prepared vaccine was substituted.

Preliminary Test of Pneumococcus Lipovaccine.—The first attempt to test the efficacy of pneumococcus lipovaccine was carried out before the minimal infecting dose had been determined. As a result, the infecting dose which was employed in this experiment was 1 million times the size actually necessary to infect a normal monkey. The experiment is reported, however, as it illustrates certain differences between pneumonia in vaccinated and unvaccinated monkeys.

Experiment 1.—Three *Macacus syrichtus* monkeys were used in this experiment (Table I). Monkey 14 had received a large dose (16 billion) of Pneumococcus Type I lipovaccine, Monkey 17 a small dose (1 billion) of the same, while Monkey 27 served as a control. On Mar. 26, 1919, each of these three monkeys received 1 cc. of an 18 hour broth culture of Pneumococcus Type I intratracheally.

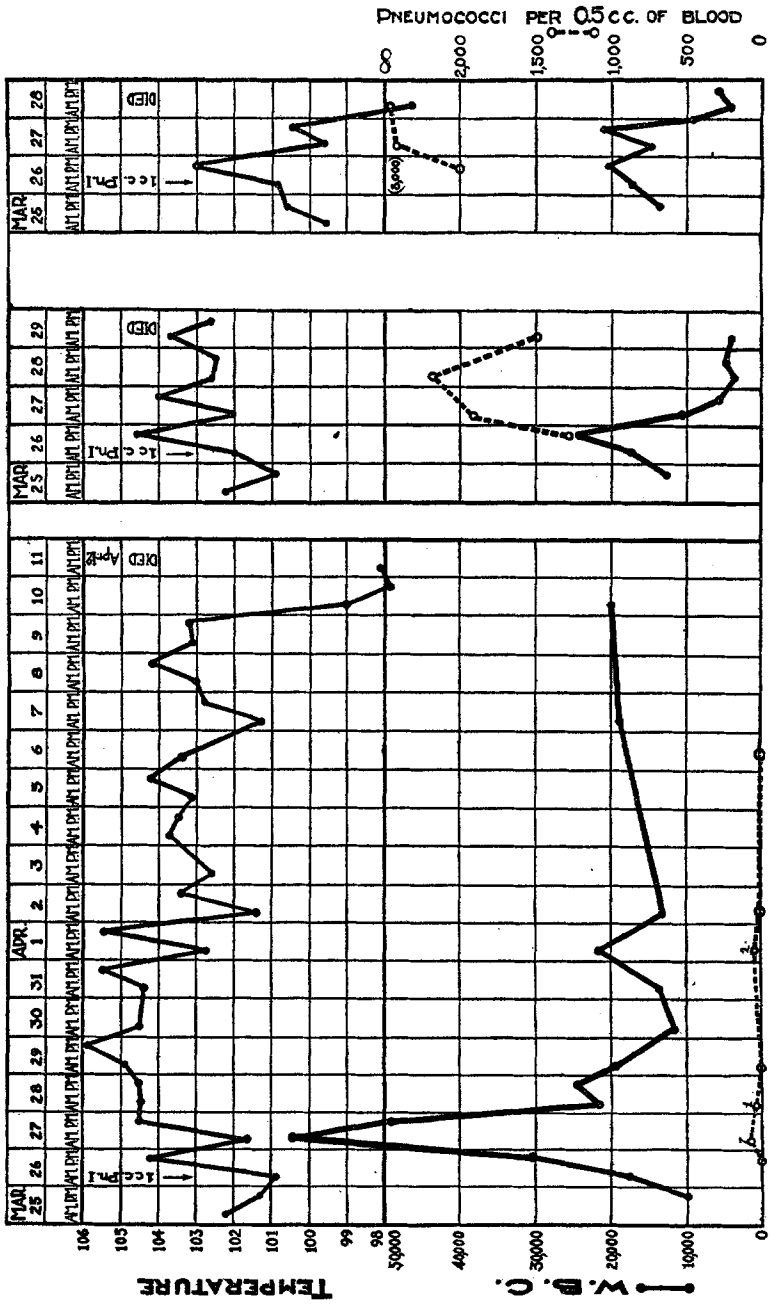
The results are shown in Table I and Text-fig. 1. All three monkeys died; the vaccinated monkeys, however, lived longer than the control. Monkey 14, which

[10] For full details for the method of preparing lipovaccine see articles by Whitmore, Fennel, and Petersen,[9] Whitmore and Fennel (Whitmore, E. R., and Fennel, E. A., *J. Am. Med. Assn.*, 1918, lxx, 1902), and Fennel (Fennel, E. A., *J. Am. Med. Assn.*, 1918, lxxi, 2115).

TABLE I.

Experimental Pneumonia Following Vaccination.

Monkey No.	Weight.	Mar. 11. Pn. I lipovaccine subcutaneously.	Mar. 25. Serum tests.		Mar. 26. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
			Agglutinins.	Protection.				Lung.	Heart's blood.
	gm.				*cc.*				
14	2,850	0.8 mg. (16 billion).	0	0	1	Died in 17 days.	Resolving lobar pneumonia; hypertrophy and dilatation of heart.	Sterile.	Pn. I
17	2,800	0.05 mg. (1 billion).	0	0	1	" " 4 "	Lobar pneumonia; red stage.	Pn. I	" I
27	2,620	0	0	0	1	" " 3 "	Acute bronchitis; acute suppurative pericarditis (Pn. I).	Sterile.	" I (bronchus, Pn. I).



TEXT-FIG. 1, *a*, *b*, and *c*. Pneumococcus Type I pneumonia in vaccinated monkeys following the intratracheal injection of a large dose (1 cc.) of Pneumococcus Type I culture. (*a*) Monkey 14; vaccinated on Mar. 11 with 0.8 mg. (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*b*) Monkey 17; vaccinated on Mar. 11 with 0.05 mg. (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*c*) Monkey 27; control.

received a large dose of vaccine, had apparently recovered on Apr. 10, but died suddenly on Apr. 12 from a greatly dilated heart. The leucocyte reactions were more marked in the vaccinated monkeys, though there was not a great deal of difference between Monkeys 17 and 27. Finally, the blood culture in Monkey 14, which received a large dose of vaccine, remained practically sterile, while in the other two monkeys the blood contained large numbers of pneumococci. The control monkey was overwhelmed by the huge infecting dose and died before frank pneumonia developed. He showed, in addition to hemorrhagic bronchitis, acute suppurative pericarditis.

In this experiment vaccination failed to protect either monkey against pneumonia, but the results in the case of one vaccinated monkey suggested that vaccination had modified to some extent the virulence of the infection.

Effect of Small Doses of Pneumococcus Lipovaccine.—If protection against pneumococcus infection in monkeys could be obtained by vaccination, it was desirable to find the minimum efficient dose; in other words, a dose that would be comparable with that used in man. In the following experiment the vaccinated monkeys had each received a dose of lipovaccine proportional to their weight as compared with the weight of a man; that is, 0.05 mg. of the dried bacteria, or 1 billion pneumococci.

Experiment 2.—Six *Macacus syrichtus* monkeys were used in this experiment. Three (Monkeys 64, 65, and 67) had received 1 billion each of Pneumococcus Type I lipovaccine; the other three (Monkeys 85, 86, and 87) were controls (Table II). May 6, 1919. All six monkeys were injected intratracheally with an 18 hour broth culture of Pneumococcus Type I. Monkeys 65 and 87 received 0.1 cc.; Nos. 67 and 85 received 0.001 cc.; and Nos. 64 and 86 received 0.000001 cc.

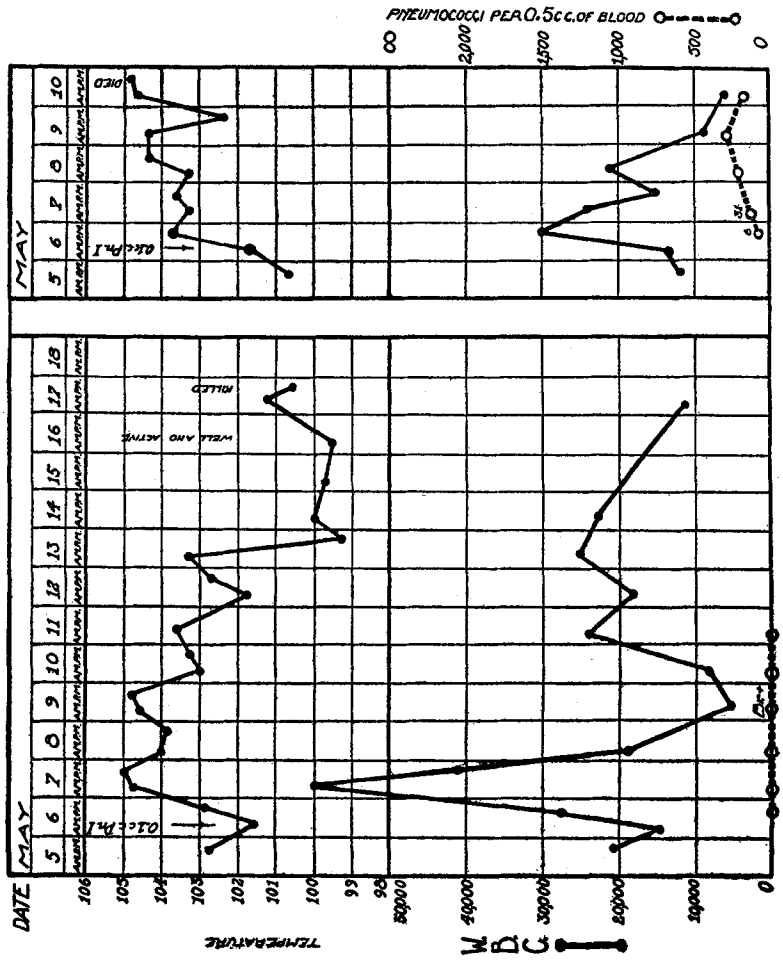
All six monkeys promptly developed symptoms of pneumonia. It will be observed, however, that while the three control monkeys died, two of the vaccinated monkeys recovered. The third vaccinated monkey (No. 67) had a crisis on the 9th day, but died suddenly on the 11th day of the disease. Autopsy revealed an old aortic endocarditis and insufficiency, with cardiac hypertrophy and dilatation. The size of the dose did not appear to exert a very pronounced influence on the course of the disease in either vaccinated or unvaccinated monkeys. Table II shows the protocols of these experiments, and Text-figs. 2, 3, and 4 exhibit the temperature, leucocyte, and blood culture curves.

TABLE II.

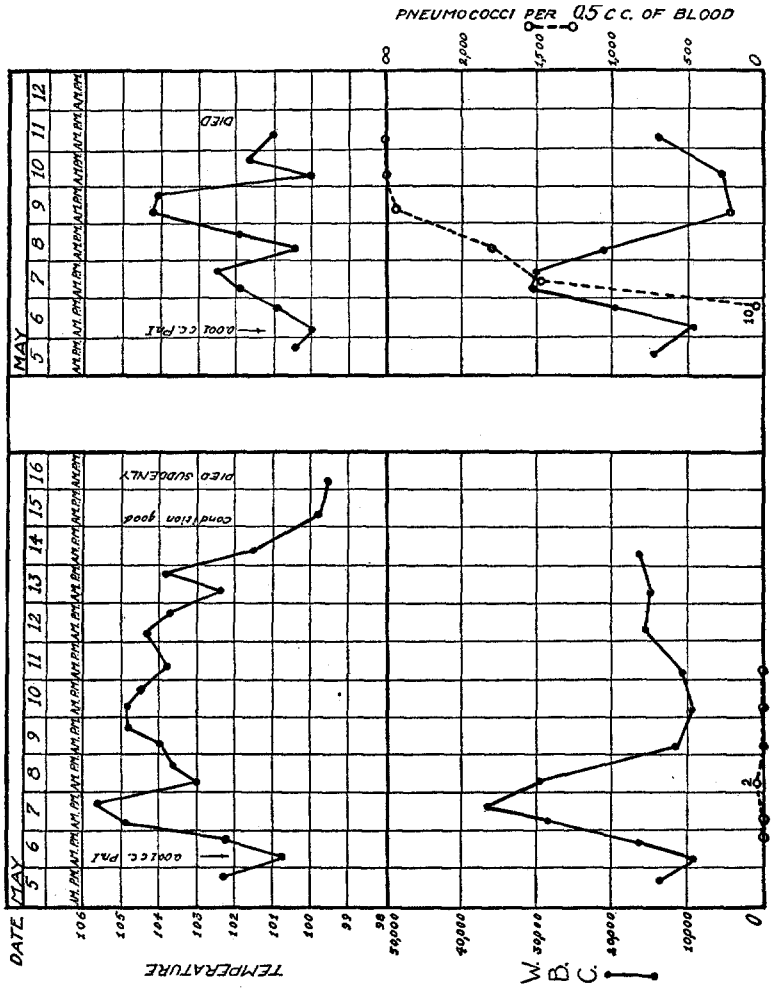
Results of Vaccination with a Small Dose of Pneumococcus Lipovaccine.

Monkey No.	Weight.	Apr. 21. Pn. I lipovaccine subcutaneously.	May 6. Serum tests.		May 6. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
			Agglutinins.	Protection.				Lung.	Heart's blood.
	gm.				*cc.*				
65	3,075	0.05 mg. (1 billion).	0	0	0.1	Clinical pneumonia. Recovery by crisis on 8th day. Killed on 12th day.	Resolving lobar pneumonia, R. L.*	No growth.	No growth.
67	2,629	0.05 mg. (1 billion).	0	0	0.001	Clinical pneumonia. Crisis on 9th day. Died on 11th day.	Resolving lobar pneumonia; chronic aortic endocarditis and insufficiency; cardiac hypertrophy and dilatation.	Pn. I	Pn. I
64	2,599	0.05 mg. (1 billion).	0	0	0.000001	Clinical pneumonia. Recovery by lysis on 26th day. Killed on 30th day.	Organizing lobar pneumonia, L. L.	" I (4 colonies).	No growth.
87	2,835	0			0.1	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, L. U., L. M., L. L.; red stage.	Pn. I	Pn. I
85	2,675	0			0.001	Clinical pneumonia. Died on 6th day.	Lobar pneumonia, L. M., L. L.	" I	" I
86	2,424	0			0.000001	Clinical pneumonia. Died on 14th day.	Lobar pneumonia, R. L., L. L.; gray stage.	" I	No growth.

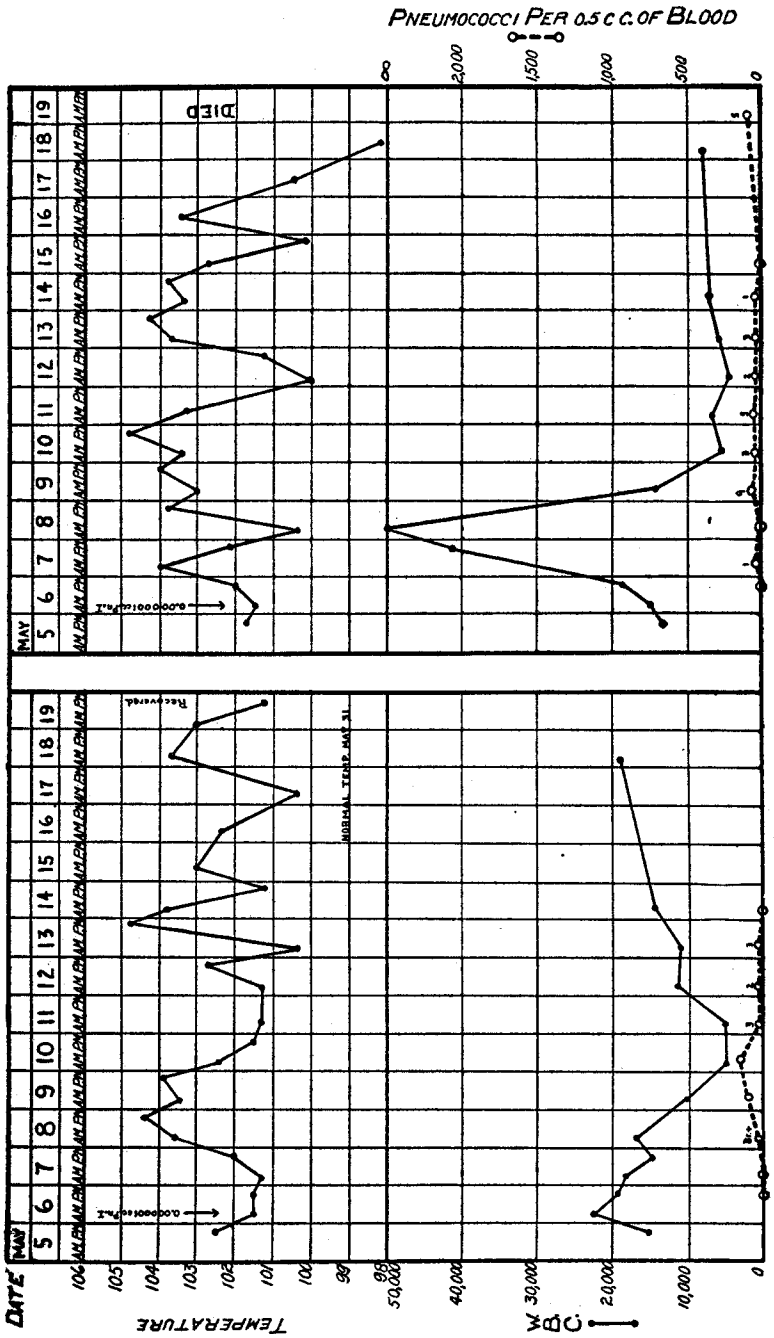
*L. U., L. M., L. L., etc., indicate lobes of the lung. The cardiac lobe is included as part of the right lower lobe.



TEXT-FIG. 2, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a small dose (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 65; vaccinated on Apr. 21. (*b*) Monkey 87; control.



TEXT-FIG. 3, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a small dose (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 67; vaccinated on Apr. 21. (*b*) Monkey 85; control.



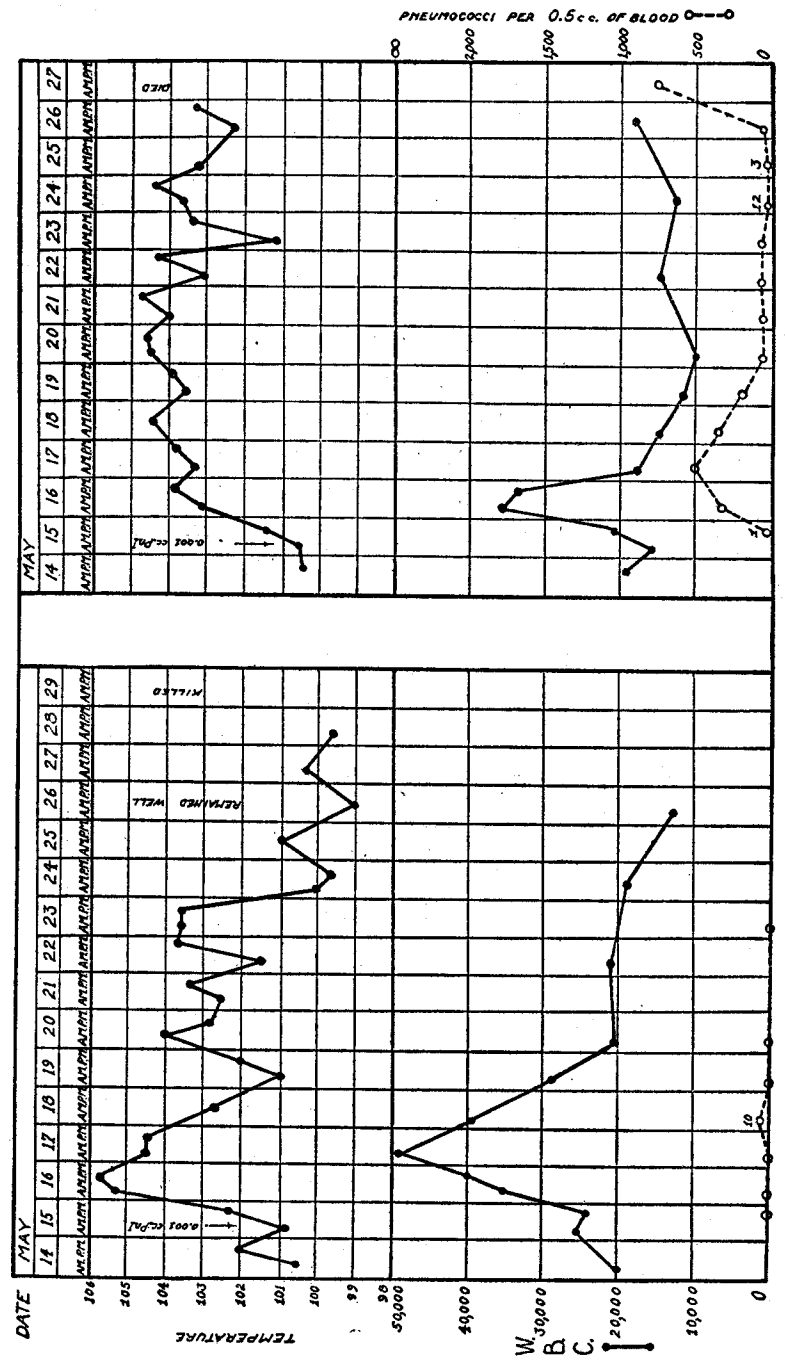
TEXT-FIG. 4, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a small dose (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 64; vaccinated on Apr. 21. (*b*) Monkey 86; control.

In these experiments, as in Experiment 1, it will be observed that the blood culture was sterile or weakly positive in vaccinated monkeys, while all the controls showed persistently positive blood cultures. These charts, however, demonstrate clearly that small doses of pneumococcus lipovaccine do not protect monkeys against pneumococcus pneumonia, even when the infecting dose is very small. It will also be observed that lipovaccine failed to stimulate agglutinins or protective substances in the monkeys' blood. Nevertheless, vaccination did appear to influence favorably the course of the disease.

Effect of Large Doses of Pneumococcus Lipovaccine.—In view of the failure of small doses of lipovaccine to protect against pneumonia, the next step was to determine the effect of large doses of pneumococcus lipovaccine. This experiment was carried out in the same manner as the one just described.

Experiment 3.—Six *Macacus syrichtus* monkeys were tested in this experiment (Table III, Text-figs. 5, 6, and 7). Monkeys 78, 80, and 81 had been vaccinated, each with 16 billion pneumococci. Monkeys 93, 95, and 96 were used for controls. All six monkeys were inoculated intratracheally with an 18 hour broth culture of Pneumococcus Type I. Monkeys 81 and 95 received 0.00001 cc., and Monkeys 80 and 96 received 0.000001 cc. on May 13, 1919. Monkeys 78 and 93 received 0.001 cc. on May 15. All six monkeys developed pneumonia. In this experiment two of the controls and two of the vaccinated monkeys recovered, while one in each series died. The death of the vaccinated monkey was unquestionably due to the complicating pericarditis which was discovered at autopsy.

As in Experiment 2, the vaccinated monkeys showed sterile or weakly positive blood cultures, with the exception of Monkey 80, in which the development of pericarditis probably contributed to the production of a fairly heavy blood infection. The character of the leucocyte reaction did not appear to be influenced by vaccination, nor was the disease appreciably shortened in the vaccinated group.



TEXT-FIG. 5, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 78; vaccinated on Apr. 29. (*b*) Monkey 93; control.

TABLE III.

Results of Vaccination with a Large Dose of Pneumococcus Lipovaccine.

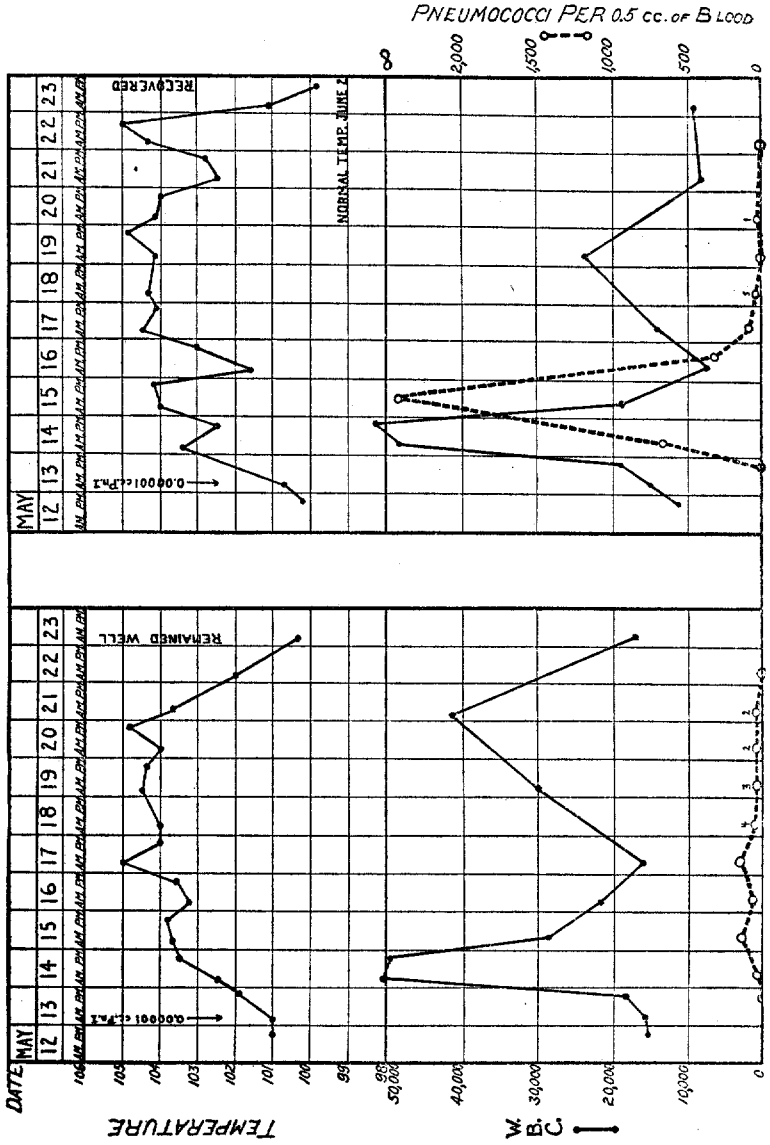
Monkey No.	Weight.	Apr. 29. Pn. I lipovaccine subcutaneously.	May 13. Serum tests.		May 13. Broth culture of Pn. I intratracheally.	May 15. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.		
			Agglutinins.	Protection.					Lung.	Heart's blood.	Pericardium.
	gm.				*cc.*	*cc.*					
78	5,322	0.8 mg. (16 billion).	0	0		0.001	Clinical pneumonia. Recovery by crisis on 10th day. Killed on 15th day.	Resolving lobar pneumonia, R. L., L. M.	No growth.	No growth.	
81	2,750	0.8 mg. (16 billion).	0	0	0.00001		Clinical pneumonia. Recovery by lysis on 10th day. Killed on 17th day.	Resolving lobar pneumonia, R. M., R. L.	" "	" "	
80	2,850	0.8 mg. (16 billion).	0	0	0.000001		Clinical pneumonia. Died on 12th day.	Lobar pneumonia, L. L., L. M.; acute pericarditis.	" I	Pn. I	Pn. I
93	5,110	0				0.001	Clinical pneumonia. Died on 13th day.	Lobar pneumonia; gray stage; entire right lung.	" I	" I	

95	4,110	0			0.00001		Clinical pneumonia. Crisis on 11th day. Relapse. Recovery on 21st day. Killed on 21st day.	Lobar pneumonia, L. L.; resolution and organization.	No growth.	No growth.
96	2,655	0			0.000001		Clinical pneumonia. Recovery by crisis on 8th day. Blood culture, Pn. I.			

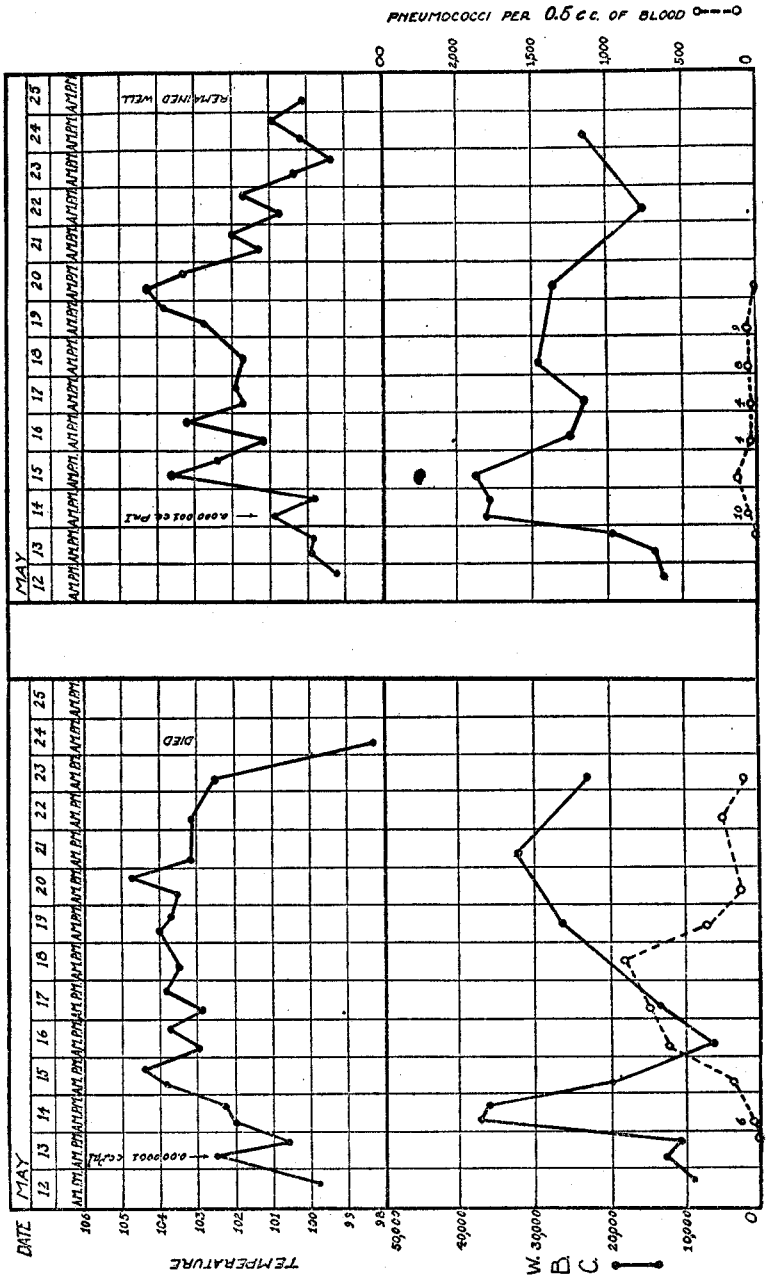
TABLE IV.

Spontaneous Pneumonia Following Vaccination.

Monkey No.	Weight.	Mar. 11. Pn. I lipovaccine subcutaneously.	Mar. 25. Serum tests with Pn. I.		Date of death.	Autopsy.	Autopsy cultures.		
			Agglutinins.	Protection.			Lung.	Heart's blood.	Pericardium.
	gm.				*1919*				
15	4,000	0.05 mg. (1 billion).	0	0	Apr. 9	Lobar pneumonia; stage of resolution.	Pn. IV	Sterile.	
13	5,055	0.05 mg. (1 billion).	0	0	" 25	Lobar pneumonia; gray stage; acute pericarditis.	Sterile.	Pn. IV	Pn. IV
21	2,815	0.05 mg. (1 billion).	0	0	" 29	Resolving lobar pneumonia; acute pericarditis.	"		Pn. (type undetermined).



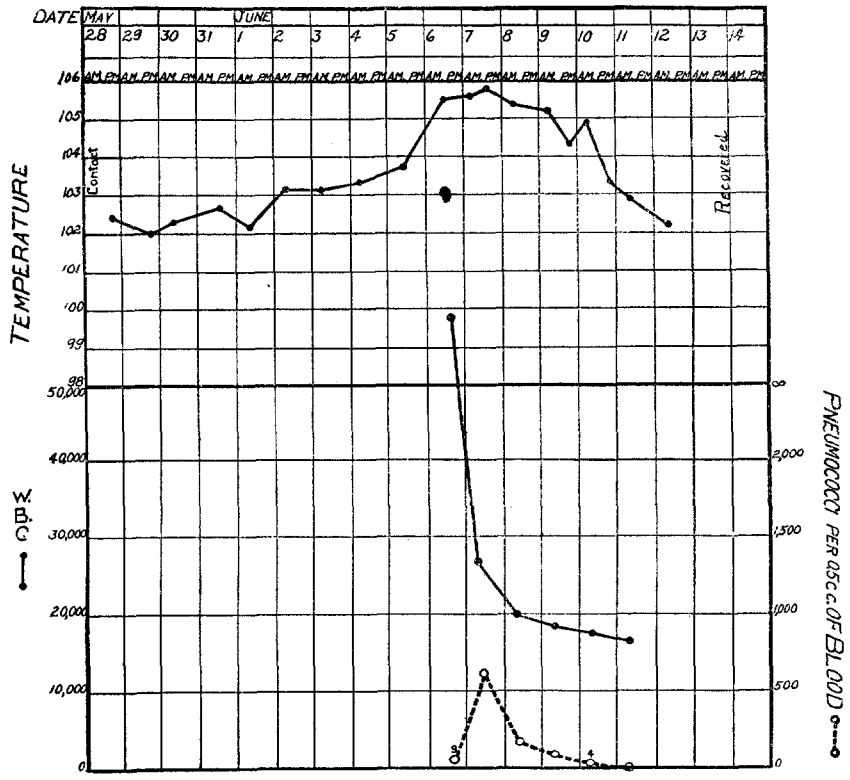
TEXT-FIG. 6, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 81; vaccinated on Apr. 29. (*b*) Monkey 95; control.



TEXT-FIG. 7, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 80; vaccinated on Apr. 29. (*b*) Monkey 96; control.

Contact Experiments.

From the preceding experiments it is evident that pneumococcus lipovaccine in the dosage employed failed to protect monkeys against experimental pneumonia. In order, however, to forestall the criticism which might be made that pneumonia had been produced by artificial means, it was decided to test the immunity of the vaccinated monkeys against spontaneous pneumonia by means of a contact test.



TEXT-FIG. 8. Spontaneous Pneumococcus Type I pneumonia developing in Monkey 16, vaccinated on Mar. 11 with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. Infection followed contact in the same cage with another case of Pneumococcus Type I pneumonia.

Experiment 4.—Three *Macacus syrichtus* monkeys which had previously been vaccinated with pneumococcus lipovaccine were placed in a cage with three normal healthy monkeys. Two monkeys in the active stage of Pneumococcus

Type I pneumonia were then put in the cage with the other six monkeys and the eight animals kept in intimate contact for 2 weeks. A few days after the experiment was started, Monkey 16, one of the vaccinated animals, became ill with pneumonia. The protocol follows:

Mar. 11, 1919. Monkey 16, *Macacus syrichtus*; weight 3,630 gm. Received 0.8 mg. (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. May 26. Monkey placed in the same cage with two other vaccinated monkeys, three normal monkeys and two monkeys suffering with Pneumococcus Type I pneumonia. June 6. Monkey appears sick; marked leucocytosis. Blood culture shows Pneumococcus Type I. June 7. Typical lobar pneumonia; blood culture shows 650 colonies of Pneumococcus Type I per 0.5 cc. of blood. June 11. Marked improvement. Monkey has run a typical course of lobar pneumonia.

This experiment shows that Monkey 16, in spite of having been vaccinated with a large dose of pneumococcus lipovaccine, was unable to resist infection with Pneumococcus Type I when exposed to pneumonia due to this type. It will be observed, however, in Text-fig. 8, that the disease ran a mild and fairly short course, which supports the observation previously made that vaccinated monkeys tolerate pneumonia more readily than unvaccinated animals. Strangely enough, another one of the vaccinated animals in the contact test developed pneumonia, but in this instance the infection proved to be with Pneumococcus Type IV.[11] None of the control monkeys became infected.

Spontaneous Pneumococcus Type IV Pneumonia in Monkeys Vaccinated against Pneumococcus Type I.—Some of the monkeys that had been inoculated with Pneumococcus Type I lipovaccine were put back into a large cage with a number of stock monkeys. An epidemic of Pneumococcus Type IV pneumonia broke out in this cage and a number of the vaccinated monkeys contracted the disease.

Experiment 5.—Three *Macacus syrichtus* monkeys (Nos. 13, 15, and 21) had received a small dose of Pneumococcus Type I lipovaccine (Table IV). Pneumonia developed spontaneously in all of them 4 to 7 weeks after vaccination, just at the time when presumably their immunity should have been at a high point. In Monkeys 13 and 15 Pneumococcus Type IV was recovered from the autopsy cultures, and in Monkey 21 a pneumococcus was seen in the pericardial fluid, but failed to grow in the culture.

[11] See Paper I.[7]

The protocols demonstrate the fact that monkeys vaccinated with Pneumococcus Type I lipovaccine possess no demonstrable cross-immunity against spontaneous Pneumococcus Type IV pneumonia.

Summary of Lipovaccine Experiments.—A review of the experiments so far reported brings out these facts:

1. Pneumococcus Type I lipovaccine, whether used in large or small doses has failed to protect monkeys against experimental and spontaneous Pneumococcus Type I pneumonia.

2. Vaccination, however, does appear to modify the course of a subsequent Type I pneumonia. The blood is not so heavily infected as in unvaccinated animals, in some cases remaining practically sterile throughout the entire course of the disease. Furthermore, the mortality rate is lower in the vaccinated monkeys and the disease seems to run a milder course.

3. No agglutinins or protective bodies were demonstrated in any of the monkeys inoculated with lipovaccine.

4. There is no evidence that Pneumococcus Type I lipovaccine confers any cross-immunity against other types of pneumococcus pneumonia.

Experiments with Pneumococcus Type I Saline Vaccine.

The failure of pneumococcus lipovaccine to protect monkeys against pneumonia prompted us to test the value of pneumococcus saline vaccine. Such experiments seemed all the more justified in view of the fact that the results of prophylactic vaccination against pneumonia at Camp Upton, where a saline vaccine had been used, were distinctly better than the results at Camp Wheeler where the lipovaccine had been employed.

The pneumococcus saline vaccine was prepared from the same avirulent strain of Pneumococcus Type I which had been used in the preparation of the Pneumococcus Type I lipovaccine. The saline vaccine was made as follows:

Pneumococci were cultivated for 18 hours in glucose broth and submitted to centrifugation. The bacterial sediment was then heated at 55°C. for 1 hour to kill the pneumococci. The vaccine was diluted with normal salt solution containing 0.25 per cent tricresol

and standardized by Wright's method. The saline vaccine used in the following experiments was prepared on May 1, and the experiments were started on May 6, 1919.

For the most part, the dosage and method of administration in the experiments with pneumococcus saline vaccine were the same as in the lipovaccine tests. In the following experiments each monkey received only one subcutaneous injection.

Results of Vaccination with Pneumococcus Type I Saline Vaccine.—In testing the saline vaccine the effect of the large and small dosage was determined in one experiment.

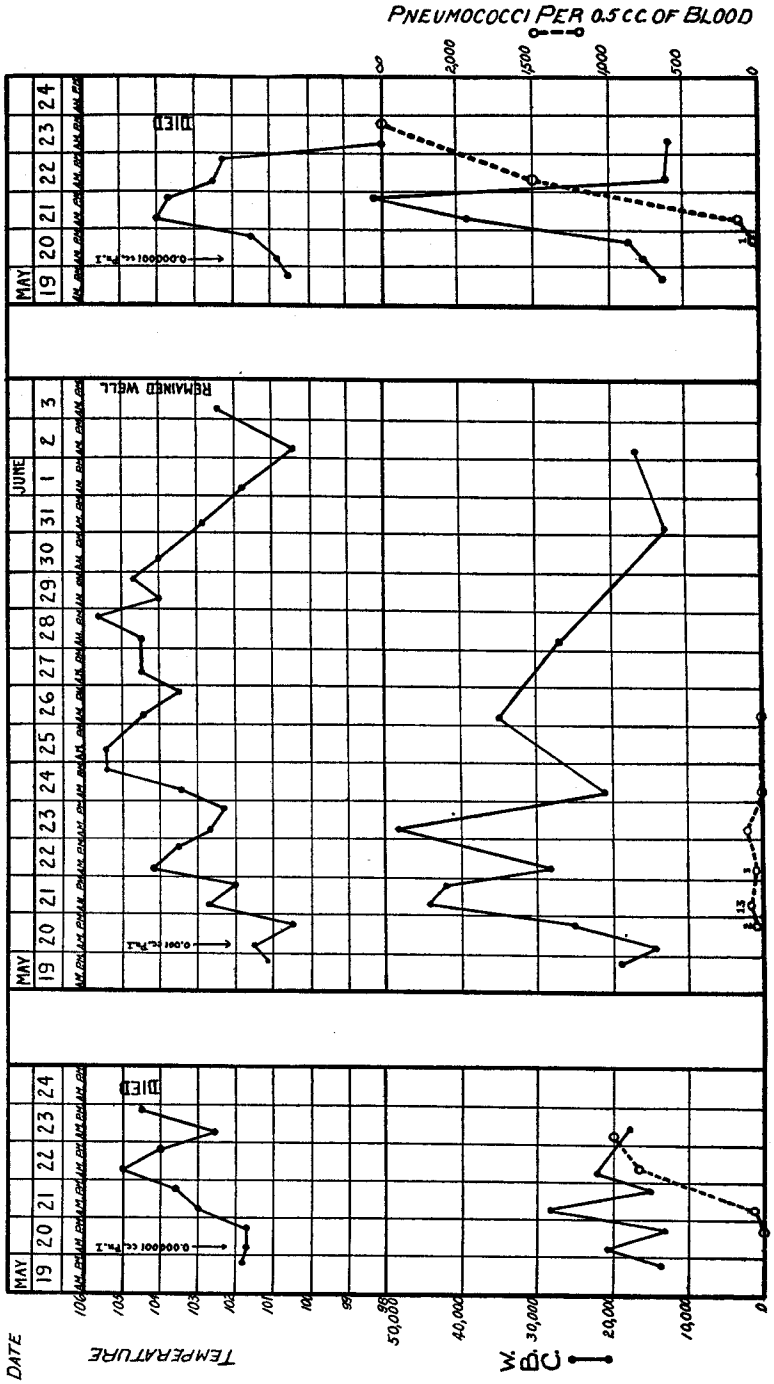
Experiment 6.—Four *Macacus syrichtus* monkeys were used in this experiment (Table V, Text-figs. 9 and 10). Monkeys 88 and 89 had each been vaccinated with 1 billion, Monkey 90 with 16 billion Pneumococcus Type I saline vaccine. Monkey 98 was the control. 2 weeks after these monkeys were vaccinated, their blood was tested for agglutinins and protective bodies. No agglutinins could be demonstrated, but all three monkeys showed the presence of protective bodies. In Monkeys 89 and 90 the protection was marked, in Monkey 88 slight. 2 weeks after vaccination the monkeys were injected intratracheally with an 18 hour broth culture of Pneumococcus Type I. Monkeys 88, 90, and 98 received each 0.000001 cc. of culture. Monkey 89 received 0.001 cc. of culture. The results are shown in Table V. While the four monkeys all developed pneumonia, the control monkey ran a rapid course and died on the 4th day. The vaccinated animals lived longer and two of them recovered (Monkeys 89 and 90). Monkey 88 died on the 5th day. The two cases that terminated fatally showed extensive lobar pneumonia at autopsy and Pneumococcus Type I was recovered from the lungs and heart's blood. One of the vaccinated monkeys that recovered was killed and at autopsy showed a resolving pneumonia, cultures from which were sterile. The temperature, leucocyte, and blood culture curves are shown in Text-figs. 9 and 10.

With pneumococcus saline vaccine as with lipovaccine, prophylactic inoculation failed to protect monkeys against pneumonia, but, as in the case of lipovaccine, inoculation seemed to modify favorably the course of the disease. The vaccinated monkey that died (Monkey 88) was the one which showed the smallest amount of protective substances in its blood. The two vaccinated monkeys which recovered showed only a moderate degree of bacteremia, whereas the two monkeys that died had heavy blood infections. The amount of pneumococcus culture used for infecting the monkeys appears to have

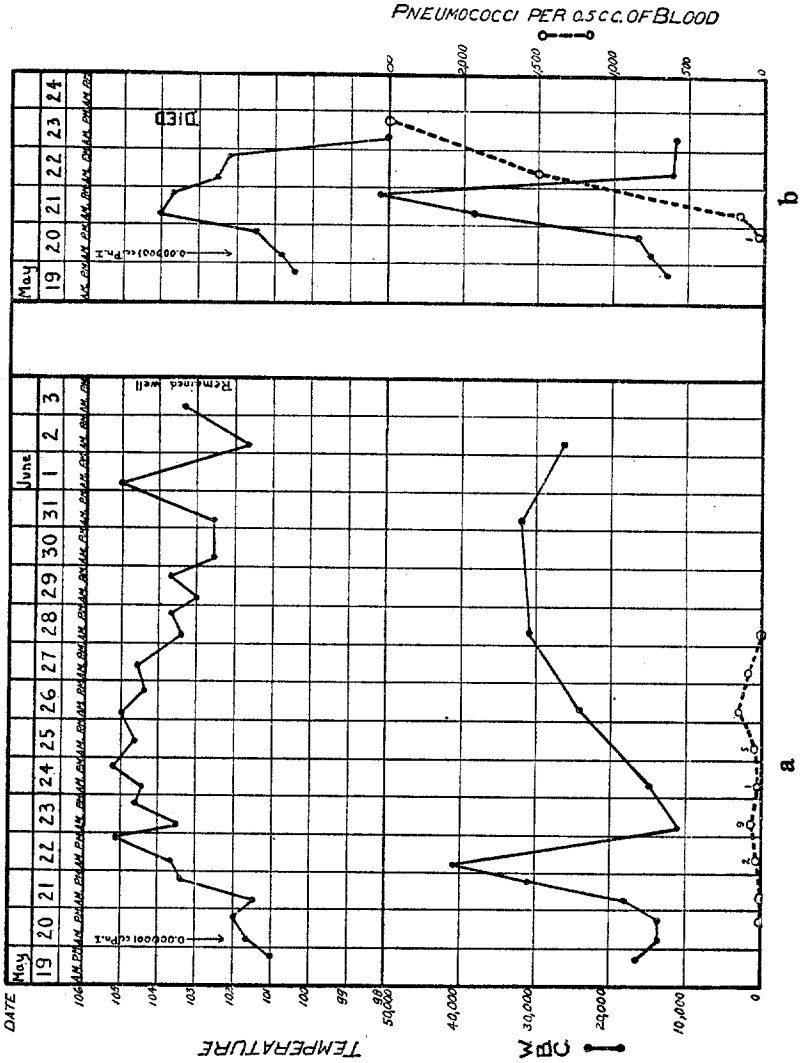
TABLE V.

Results of Vaccination with Pneumococcus Saline Vaccine.

Monkey No.	Weight.	May 6. Pn. I saline vaccine subcutaneously.	May 19. Serum tests.			May 20. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
			Agglutinins.	Protection.	Control.				Lung.	Heart's blood.
	gm.					*cc.*				
88	2,072	1 billion.	0	0.00001 cc. Died in 60 hrs. 0.000001 cc. Died in 60 hrs. 0.0000001 cc. Survived.	0.00001 cc. Died in 24 hrs. 0.000001 cc. Died in 48 hrs. 0.0000001 cc. Died in 48 hrs.	0.000001	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, entire left lung, R. L.; red stage.	Pn. I	Pn. I
89	2,462	1 "	0	0.00001 cc. Survived. 0.000001 cc. Survived. 0.0000001 cc. Survived.		0.001	Clinical pneumonia. Recovery by lysis on 12th day.	Resolving lobar pneumonia, R. L.	No growth.	No growth.
90	3,922	16 "	0	0.00001 cc. Survived. 0.000001 cc. Survived. 0.0000001 cc. Survived.		0.000001	Clinical pneumonia. Recovery by lysis on 11th day.			
98	4,100	0				0.000001	Clinical pneumonia. Died on 4th day.	Lobar pneumonia, R. U., R. L., L. U., L. L.; stage of engorgement.	Pn. I	Pn. I



TEXT-FIG. 9, *a*, *b*, and *c*. Pneumococcus Type I pneumonia in monkeys vaccinated with a small dose (1 billion) of Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 88; vaccinated on May 6. (*b*) Monkey 89; vaccinated on May 6. (*c*) Monkey 98; control.



TEXT-FIG. 10, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 90; vaccinated on May 6. (*b*) Monkey 98; control.

little influence, up to a certain point, upon the course of the disease. Monkey 88 received only 0.000001 cc. and died on the 5th day. Monkey 89 received 0.001 cc. (a thousand times as large a dose) and recovered from the infection after running a comparatively mild course.

Results of Vaccination with Three Injections of Pneumococcus Type I Saline Vaccine.—Although one injection of pneumococcus saline vaccine failed to protect monkeys against pneumococcus infection, the results were rather more encouraging than those obtained with the lipovaccine. In two of the vaccinated monkeys tested, a high degree of protection was demonstrable in the blood following the inoculation, and although these two monkeys both contracted pneumonia, the disease ran a mild course and both monkeys recovered. Therefore it seemed desirable to determine whether three injections of pneumococcus saline vaccine given at intervals of 1 week would not afford the necessary amount of protection.

Experiment 7.—This series of *Macacus syrichtus* monkeys was started on saline vaccine May 22, 1919, each monkey receiving weekly subcutaneous injections of 1 billion pneumococci until three inoculations had been given. 2 weeks after the third injection the blood from these monkeys was tested for agglutinins and protective bodies. No agglutinins or protective bodies could be demonstrated in any of the five monkeys tested.

June 20. Two of the vaccinated monkeys and a control monkey were injected intratracheally with 0.000001 cc. of an 18 hour broth culture of Pneumococcus Type I. Table VI and Text-fig. 11 show the results obtained. All three monkeys developed pneumonia, and in all three the disease was fatal. In one vaccinated monkey (Monkey 100) the disease presented features which are usually associated with a mild attack; namely, a moderate infection of the blood and a good secondary rise in the leucocytes, while in the other (Monkey 101) a septicemia equally as heavy as that of the control developed. At autopsy all three monkeys showed lobar pneumonia, and Pneumococcus Type I was recovered from the organs.

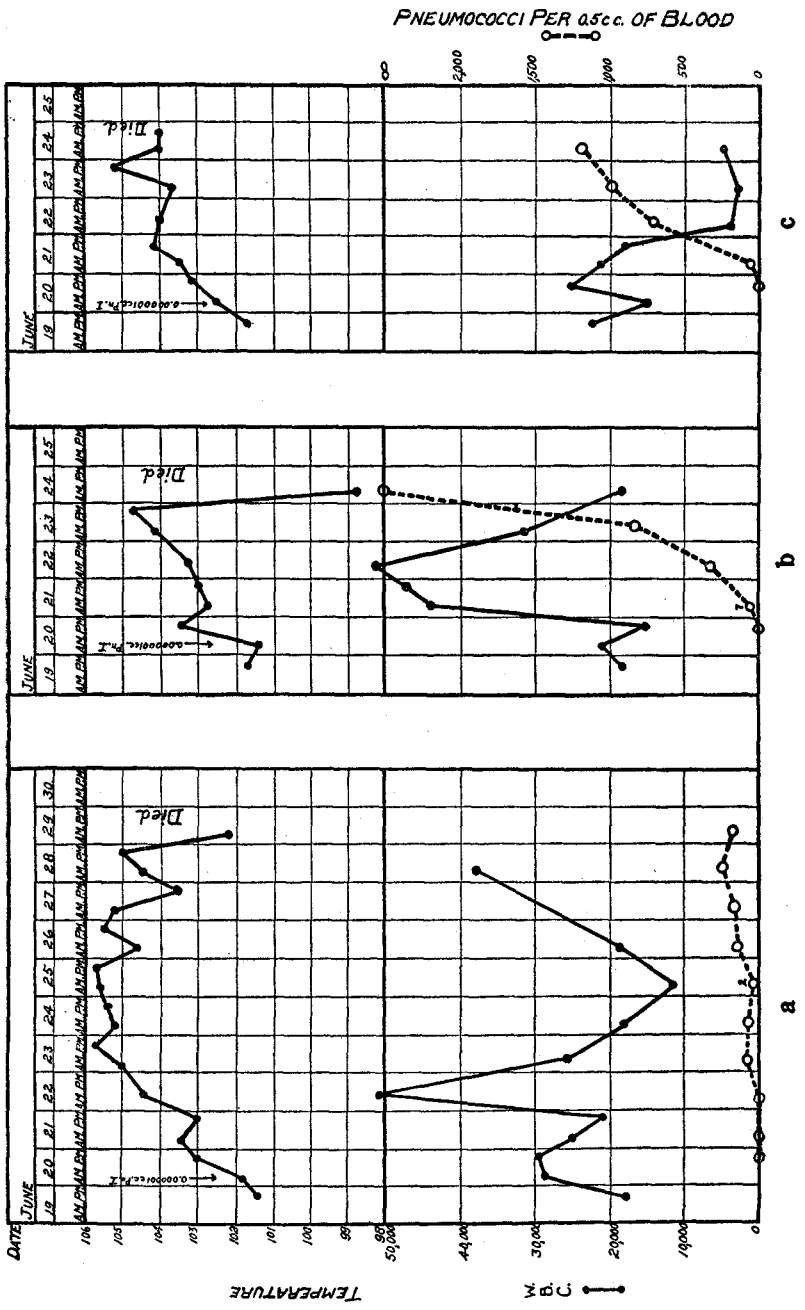
In this experiment three small doses of saline vaccine failed to give as much protection as had been obtained by a large single injection in the previous experiments.

Summary of Saline Vaccine Experiments.—The experiments which have been reported indicate that saline vaccine like lipovaccine, when injected subcutaneously in moderate doses, has failed to protect

TABLE VI.

Results of Vaccination with Three Injections of Pneumococcus Saline Vaccine.

Monkey No.	Weight.	Pn. I saline vaccine subcutaneously.			June 20. Serum tests.		June 20. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
		May 22.	May 29.	June 5.	Agglutinins.	Protection.				Lung.	Heart's blood.
	gm.						*cc.*				
100	2,392	1 billion.	1 billion.	1 billion.	0	0	0.000001	Clinical pneumonia. Died on 10th day.	Lobar pneumonia, L. U., L. M., L. L., R. L.; gray stage.	No growth.	Pn. I
101	2,532	1 "	1 "	1 "	0	0	0.000001	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, L. U., L. M., L. L., R. L.; stage of engorgement.	Pn. I	" I
114 (control).	3,000	0	0	0			0.000001	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, L. L., R. L.; stage of engorgement.	" I; *S. hæmolyticus.*	" I; *S. hæmolyticus.*



TEXT-FIG. 11, *a*, *b*, and *c*. Pneumococcus Type I pneumonia in monkeys vaccinated with three injections (1 billion each) of Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 100. (*b*) Monkey 101. Both these animals received vaccine on May 22, 29, and June 5. (*c*) Monkey 114; control.

monkeys against pneumococcus pneumonia. As for any relative superiority of one vaccine over the other, there appears to be little choice between the two. Saline vaccine, however, is more likely to stimulate the formation of protective bodies in the blood, and for this reason probably gives a somewhat better immunity. The individual variation in the natural resistance of monkeys to pneumococcus infection is a factor of primary importance and one which must always be considered. No definite decision can be reached as to the relative merits of pneumococcus lipovaccine and saline vaccine, except by testing a large series of monkeys with each type.

Effect of Intravenous Injection of Living Pneumococcus Type I Cultures in Monkeys Vaccinated with Pneumococcus Type I Vaccine.

In the preceding experiments it has been shown that pneumococcus vaccine does not protect monkeys against intratracheal infection with pneumococcus. It seemed desirable, therefore, for the sake of comparison, to determine whether these vaccinated monkeys would be protected against intravenous infection.

Experiment 8.—July 8, 1919. Two *Macacus syrichtus* monkeys that had been vaccinated 4 weeks previously with three injections (1 billion each) of Pneumococcus Type I saline vaccine and one control monkey were injected intravenously with 0.001 cc. of a broth culture of living virulent Pneumococcus Type I. This dose is often fatal for a normal monkey. The results are shown in Table VII and Text-fig. 12. The vaccinated monkeys showed few or no clinical symptoms following the injection. Monkey 105 remained perfectly well, with sterile blood cultures. Monkey 104, the other vaccinated monkey, had a mild febrile reaction and a temporary infection of the blood of 48 hours duration. The control (Monkey 116) was ill for 6 days with high fever and heavy septicemia.

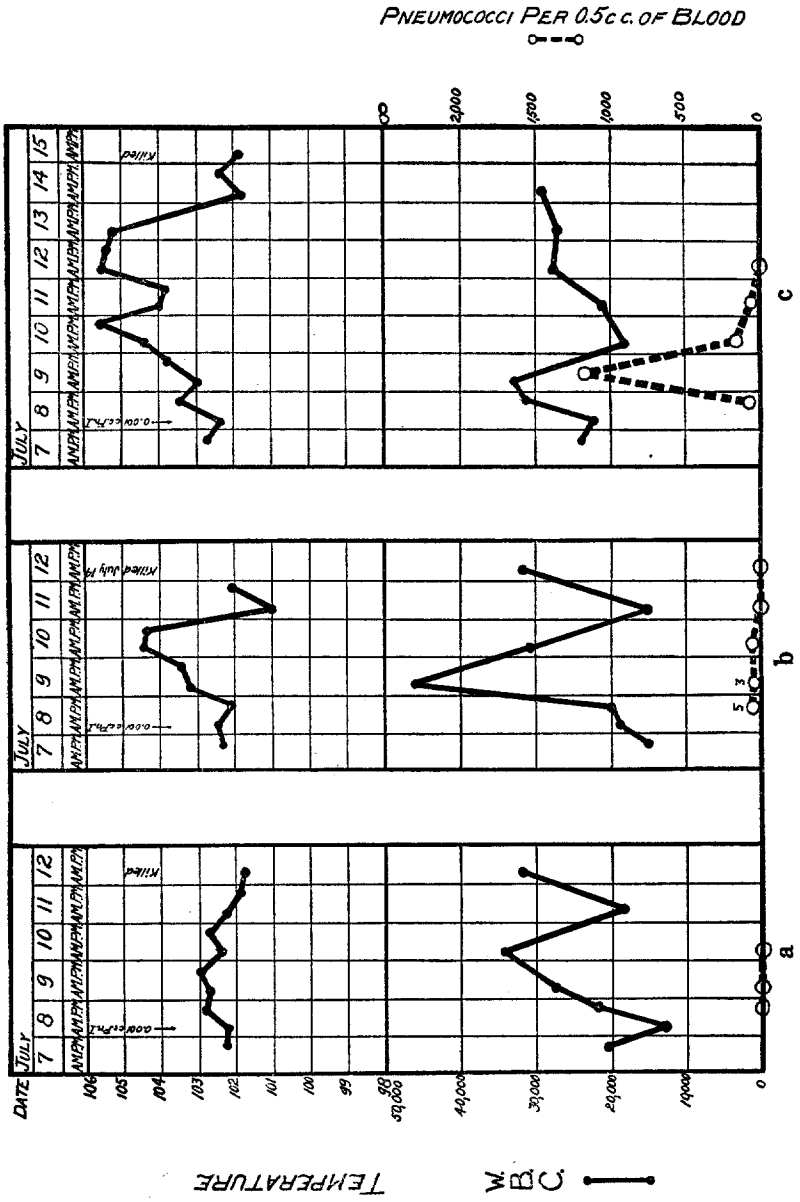
All three monkeys were killed just after their temperature had returned to normal, and all showed perfectly normal lungs.

This experiment has considerable significance in suggesting that distinction must be made between a humoral immunity against pneumococcus and a local immunity, possibly cellular, in the lungs. The same dose of culture which Monkeys 104 and 105 received intravenously with impunity would have produced a severe pneumonia if administered intratracheally. Although this phenomenon may at

TABLE VII.

Effect of Intravenous Injection of Virulent Pneumococcus Type I in Monkeys Previously Vaccinated with Pneumococcus Type I Saline Vaccine.

Monkey No.	Weight.	Pn. I saline vaccine subcutaneously.			June 21. Serum tests.		July 8. Broth culture of Pn. I intravenously.	Result.	Autopsy.	Autopsy cultures. Heart's blood.
		May 22.	May 29.	June 5.	Agglutinins.	Protection.				
	gm.						*cc.*			
105	1,375	1 billion.	1 billion.	1 billion.	0	0	0.001	No clinical symptoms; no septicemia. Killed on 5th day.	Normal lungs.	No growth.
104	1,735	1 "	1 "	1 "	0	0	0.001	Fever for 2 days; mild septicemia for 2 days; no symptoms of pneumonia. Killed on 7th day.	" "	" pneumococci.
116 (control).	1,935	0	0	0			0.001	Fever for 6 days; heavy septicemia; no symptoms of pneumonia. Killed on 8th day.	" "	No growth.



TEXT-FIG. 12, *a*, *b*, and *c*. Reactions to intravenous injection of living virulent Pneumococcus Type I in monkeys previously vaccinated with Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 105. (*b*) Monkey 104. Both these animals received vaccine on May 22, 29, and June 5. (*c*) Monkey 116; control.

first seem paradoxical, it is in reality not out of harmony with the results obtained in the preceding experiments in which it has been clearly shown that prophylactic vaccination prevented to a large extent the development of septicemia during the course of lobar pneumonia. It furthermore demonstrates that tests of the prophylactic value of vaccination in animals may lead to false conclusions if these depend upon the demonstration of immunity to intravenous infection rather than to the actual disease against which the vaccination is directed.

DISCUSSION.

In this study all efforts to protect monkeys against pneumonia by subcutaneous vaccination with killed cultures of pneumococcus have failed. With our present ignorance concerning the nature of bacterial antigen, any investigation of this nature must necessarily be for the most part empirical. In the experiments reported, two kinds of vaccine have been tried—the oily and the saline. But obviously, many other factors determine the character of a vaccine, each of which should be considered in determining the value of the vaccine for prophylactic inoculation. Among these factors are the following:

Virulence of the Organism.—It has generally been assumed that a virulent organism produces a more efficient vaccine than an avirulent strain. The virulent strain, however, causes a more severe reaction, and for this reason most vaccines in general use are prepared from avirulent cultures. The question of whether a vaccine prepared from a highly virulent pneumococcus will afford better protection than one prepared from an avirulent strain has not been attacked in the present study. It may, however, be a more important factor than is generally assumed and it is hoped that this question can be investigated at a later time.

Method of Cultivation.—When vaccines are prepared from cultures grown on solid media, all the products of bacterial metabolism are presumably included in the vaccine. When, however, the bacteria are grown in liquid media, the supernatant fluid is discarded, and with it the bacterial metabolites. Just how much antigenic value these products have is problematical. It is obvious, moreover, that in vaccines prepared from broth media, the longer the period of incu-

bation, the greater the autolysis and the greater the amount of bacterial products in solution in the broth. The influence of sugars, animal sera, etc., when added to the culture medium may be an important factor in the quality of a vaccine.

Method of Killing the Bacteria.—Many methods have been used for killing the bacteria in vaccines, but heat and germicides remain the two most frequently employed. In the case of the lipovaccine and saline vaccine used in the present study the pneumococci were killed by heat; but in the lipovaccine the bacteria were heated for 24 hours at 53°C., whereas in the preparation of the saline vaccine they were heated for only 1 hour at 55°C. Whether this difference in the duration of heating had any effect on the antigenic value of the vaccine, it is impossible to say.

Vehicle in Which the Bacteria Are Suspended.—Until quite recently all vaccines were prepared in either water or normal salt solution. The employment of vegetable oils as a vehicle for suspending bacteria introduces a new factor into the question of antigenic value. It is possible that in oily vaccines a capsule forms around the body of the microorganism and interferes not only with its absorption but also with the production of specific antibodies. In these experiments monkeys injected with pneumococcus lipovaccine failed to develop demonstrable agglutinins and protective bodies.

Age of the Vaccine.—The age of the vaccine is undoubtedly an important factor in its antigenic value and one that has not been thoroughly investigated. The lipovaccine used in the first of these experiments was 4 months old at the time the experiment was started. In the later experiments a fresh lipovaccine was used, prepared in the same way as the older one, and no difference in effect was observed in the two vaccines. With the saline vaccine, the first experiment, which was carried out immediately after the vaccine was prepared, showed protective bodies in the three monkeys tested. A month later the same vaccine was used for testing the effect of three repeated injections of saline vaccine and no protective bodies could be demonstrated in any of the five monkeys vaccinated. Whether this was a matter of individual variation in the monkeys or whether there occurred certain changes in the vaccine due to standing 1 month in the ice box, it is hard to say. The former hypothesis would appear more reasonable.

The results obtained in this study of prophylactic vaccination against pneumonia in monkeys have been disappointing; but it should be borne in mind that the test applied has been a particularly crucial one. Comparatively small doses of vaccine have been used in order to make the results comparable with vaccination in man. No doubt a satisfactory immunity could have been obtained if repeated injections of large doses of vaccine had been administered, and still better results might have been reached if, in the case of saline vaccine, the injections had been given intravenously. Such an accomplishment, however, was not the aim of the investigation.

Furthermore, the Type I pneumococcus which was employed in the intratracheal injections was an organism of extraordinary virulence. It practically never failed to produce the disease even in doses of 0.000001 cc. of broth culture, and in unvaccinated monkeys the result was usually fatal. One hundred millionth of a cc. was in most cases lethal for a mouse.

Finally, it must be emphasized that the monkey is highly susceptible to the pneumococcus. The prevalence of respiratory infections among these animals is well known; and that they succumb readily to the pneumococcus is evidenced by the fact that an epidemic of Pneumococcus Type IV pneumonia broke out among our stock monkeys and killed between 30 and 40 of them in less than 4 weeks. The disease ran through these animals, fresh from the Tropics, in very much the same manner that measles and pneumonia ravaged our southern recruits in 1917 and 1918. In either instance it was a case where an organism was suddenly brought in contact with a disease to which it had not been previously exposed. These monkeys when living in their natural environment probably rarely encountered the pneumococcus and had acquired no racial immunity to pneumococcus infections. Man, on the other hand, at least in North America, and particularly in urban communities, is constantly exposed to pneumococcus infections, and by reason of this exposure has probably gradually built up a fair degree of immunity against the microorganism. Clough[12] has recently shown that 19 per cent of normal men have demonstrable protective substances against pneu-

[12] Clough, P. W., *J. Am. Med. Assn.*, 1919, lxxiii, 785.

mococcus in their serum. Lack of previous exposure to pneumococcus is evidenced in monkeys by the difficulty which one has in producing protective substances in their blood.

A close analogy exists in this connection between pneumonia and typhoid fever. Metchnikoff and Besredka[13] in their study of experimental typhoid fever found that it was impossible to protect apes against the disease by means of killed cultures. Most of the typhoid vaccine, however, used in this country and elsewhere has been composed of killed bacilli and the results obtained with this vaccine are sufficient justification for its further use. To reason too closely, therefore, from monkey to man may lead to false conclusions. The bearing of this discussion on the question of prophylactic vaccination against pneumonia in man is obvious. The value of such vaccination will have to be finally determined by vaccinating large groups of men living under approximately the same conditions, and the results controlled by observations upon similar unvaccinated groups.

Pneumococcus vaccine probably stimulates in every case the production of a certain quantity of antibody, an amount, however, which in monkeys is not sufficient to protect them against pneumonia. Usually the antibody production in monkeys is not of sufficient degree to be demonstrable by any laboratory test. It is sufficient, however, to modify the course of the disease. The bacteremia is distinctly less marked. In twelve vaccinated monkeys the mortality rate was 41.6 per cent, while for seventeen unvaccinated monkeys the mortality rate was 76.4 per cent. Other evidence for this antibody production is furnished by the resistance which vaccinated monkeys offer to infection by the intravenous route.

In conclusion, it must be emphasized that immunity is a purely relative term. Almost any animal's "immunity," so called, can be overcome by a sufficiently large injection of virulent bacteria.

[13] Metchnikoff, E., and Besredka, A., *Ann. Inst. Pasteur*, 1911, xxv, 931; 1913, xxvii, 597.

CONCLUSIONS.

1. The subcutaneous inoculation of monkeys with Pneumococcus Type I vaccine in doses comparable with those employed in man does not protect them against subsequent attacks of Pneumococcus Type I pneumonia, either spontaneous or experimental. Furthermore, the occurrence of Pneumococcus Type IV pneumonia among monkeys that have been vaccinated with Pneumococcus Type I lipovaccine indicates that the vaccinated animals develop no cross-protection against other types of pneumonia.

2. Vaccination does, however, modify the course of the disease. Invasion of the blood stream by the pneumococcus in vaccinated animals is usually slight, and the proportion of recoveries is considerably higher for vaccinated than for unvaccinated monkeys.

3. Pneumococcus saline vaccine produces a greater amount of protective substance in the serum of the vaccinated animal than does pneumococcus lipovaccine and is probably, therefore, a better antigen. Both, however, fail to protect the animal against pneumococcus pneumonia.

4. Subcutaneous vaccination with pneumococcus vaccine gives definite protection against experimental pneumococcus septicemia. In other words, vaccination may induce a humoral immunity without protecting against intratracheal infection.

5. In view of the fact that monkeys are highly susceptible to pneumococcus infection, a strict analogy cannot be drawn between pneumococcus immunity in monkeys and pneumococcus immunity in man, since in the latter a considerable amount of resistance already exists, probably by reason of repeated exposure to pneumococcus infection.