STUDIES ON EXPERIMENTAL PNEUMONIA.

V. Active Immunity against Experimental Pneumococcus Pneumonia in Monkeys Following Vaccination with Living Cultures of Pneumococcus.

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In a preceding paper[1] it was shown that the subcutaneous injection of killed pneumococci in doses comparable to those employed in man will not protect monkeys against pneumococcus pneumonia of homologous or heterologous type. The aim of the present study has been to determine the prophylactic value of a vaccine composed of living pneumococci.

The conviction, based on considerable experimental evidence, has long been held by immunologists that a living virus stimulates a more effective resistance to infection than a killed virus.

Metchnikoff and Besredka,[2] in their studies on typhoid vaccination in the chimpanzee, found that very little protection was conferred by vaccination with dead typhoid bacilli, whereas animals vaccinated with small amounts of living cultures were efficiently protected. Haffkine's[3] method of vaccination against cholera consisted in the injection of 0.2 to 0.05 cc. of living culture of the cholera vibrio, first an attenuated culture being used and 5 days later a more virulent one. As early as 1893 Haffkine and his coworkers vaccinated 40,000 people in India by this method and obtained, on the whole, encouraging results. Kolle and Otto,[4] after experiments upon monkeys and other animals, concluded that dead plague bacilli were much inferior to attenuated living cultures for prophylactic vaccination.

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[1] Cecil, R. L., and Blake, F. G., *J. Exp. Med.*, 1920, xxxi, 519.

[2] Metchnikoff, E., and Besredka, A., *Ann. Inst. Pasteur*, 1911, xxv, 193; 1913, xxvii, 597.

[3] Haffkine, W. M., *Bull. Inst. Pasteur*, 1906, iv, 825.

[4] Kolle, W., and Otto, R., *Deutsch. med. Woch.*, 1903, xxix, 493; *Z. Hyg. u. Infectionskrankh.*, 1903, xlv, 507.

Strong[5] made a thorough study of the various methods of plague vaccination and concluded that the most efficient method was immunization with attenuated living cultures. He showed that when carefully done this method can be employed in human beings.

The investigations referred to above indicate that for the diseases of bacillary origin at least, living vaccines are in many ways preferable to dead cultures.

The experiments included in this study may be divided into two groups: (1) vaccination with a living virulent culture of Pneumococcus Type I, and (2) vaccination with a living avirulent culture of Pneumococcus Type I.

EXPERIMENTAL.

Two species of monkeys were used in this investigation, *Cebus capucinus* and *Macacus syrichtus*.

Method of Vaccination.—The inoculations were administered subcutaneously in the abdominal wall. In the experiments with the virulent culture the dose of vaccine was in all instances 0.001 cc. of an 18 hour broth culture diluted to 1 cc. with normal salt solution. This amount of culture contained approximately 400,000 pneumococci. In vaccinating with the avirulent strain a dose of 1 to 2 cc. of undiluted broth culture was employed.

Reactions.—The local reaction to living pneumococcus vaccine was very mild in the monkeys which were studied, consisting of merely a slight induration, free from redness and tenderness. The constitutional reaction depended, on the one hand, upon the dose and virulence of the culture, and, on the other hand, upon the individual resistance of the monkey. The latter is a factor of considerable variation. As the experiments will show, the constitutional reactions varied widely in severity, from no reaction whatever to a fatal pneumococcus septicemia.

The resistance of the vaccinated monkeys to experimental pneumonia was tested 2 or 3 weeks after vaccination by the intratracheal injection of virulent pneumococci, as described in Paper I.[6]

[5] Strong, R. P., *J. Med. Research*, 1908, xiii, 325.

[6] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

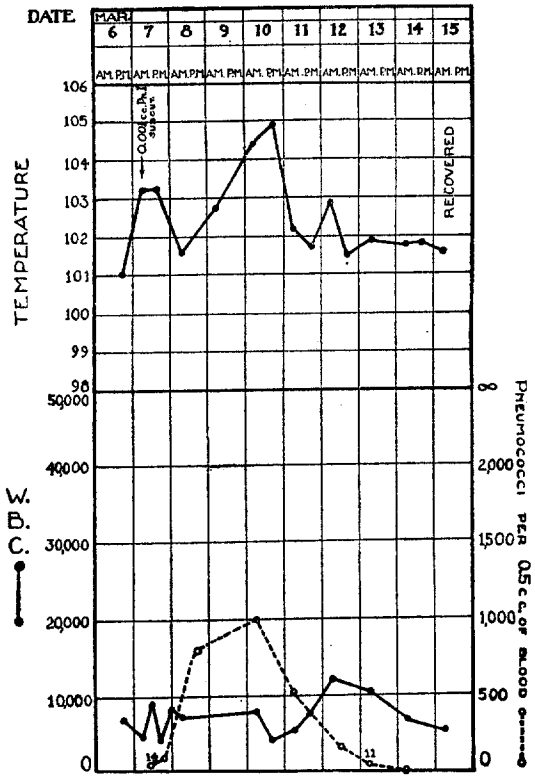
Vaccination with a Living Virulent Culture of Pneumococcus.

The strain of pneumococcus used as a vaccine in this group of experiments was a highly virulent Pneumococcus Type I which killed a mouse regularly in doses of 0.0000001 cc. of broth culture. The same strain was used for the intratracheal injections, and when injected into the trachea of monkeys in doses of 0.000001 cc. of broth culture would produce in practically every instance a classic lobar pneumonia.

Reaction of Monkeys to Subcutaneous Injection of Living Virulent Pneumococci.—The first monkey vaccinated with a living virulent pneumococcus was studied in considerable detail. The protocol therefore will be reported in full.

Experiment 1.—Monkey 7. *Cebus capucinus*, female; weight, 1,430 gm. Mar. 7, 1919, 10.30 a.m. Well and active. Subcutaneous injection of 0.001 cc. of 18 hour broth culture of Pneumococcus Type I. 1.30 p.m. Well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 14 colonies of Pneumococcus Type I. 4.30 p.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 84 colonies of Pneumococcus Type I. 7.30 p.m. Appears well and active. Mar. 8, 10.30 a.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 800 colonies (approximately) of Pneumococcus Type I. Mar. 9. Appears well and active. Mar. 10, 9 a.m. Monkey quiet at times, restless at times; evidently becoming sick. 10.30 a.m. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 1,000 colonies (approximately) of Pneumococcus Type I. 4 p.m. Appears sick; offers little resistance to handling; respirations not increased. Blood culture: one loop on surface of blood agar plate, 18 colonies of Pneumococcus Type I. Mar. 11, 10.45 a.m. Appears better; more active. Blood culture: 0.1 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar, 510 colonies of Pneumococcus Type I. 4.30 p.m. X-ray of chest. There is no evidence of consolidation in the chest. Mar. 12, 10 a.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 151 colonies of Pneumococcus Type I. Mar. 13, 10.30 a.m. Appears well and active. Blood culture: 0.5 cc. in broth, Pneumococcus Type I; 0.5 cc. in agar plate, 11 colonies of Pneumococcus Type I. Mar. 14, 11 a.m. Well and active. Blood culture: 0.5 cc. in broth, no growth; 0.5 cc. in agar plate, no growth. Mar. 15. Well and active. Mar. 27. Well and active. Monkey bled (10 cc.). Serum tests with Pneumococcus Type I: Agglutinins, 0. Protection, 0.0001 cc., survived; 0.00001 cc., survived; 0.000001 cc., survived 4½ days. Control, 0.00001 cc., died in 48 hours; 0.000001 cc., died in 48 hours.

Text-fig. 1 shows the temperature, leucocyte, and blood culture curves following vaccination. There was a sharp rise of temperature in response to the injection, but the leucocytes were unaffected. The most striking feature of the chart is the blood culture curve. The pneumococci invaded the blood stream within 3 hours after vaccination,



TEXT-FIG. 1. Monkey 7. Reaction following subcutaneous inoculation of 0.001 cc. of broth culture of living virulent Pneumococcus Type I.

and by the end of 24 hours there were nearly 1,000 pneumococci per 0.5 cc. of blood. In spite of this septicemia, however, the monkey did not appear seriously ill at any time. The blood cleared up rapidly, the temperature dropped, and 5 days after vaccination the monkey appeared well and active. It is surprising that a heavy septicemia of this kind could have been associated with such mild clinical symptoms.

It is also noteworthy that 20 days after vaccination the serum of this monkey protected mice against 1,000 times the minimal lethal dose of Pneumococcus Type I.

Experiment 2.—Apr. 15, 1919. Three *Macacus syrichtus* monkeys (Nos. 46, 47, and 48) were inoculated with a living virulent Pneumococcus Type I, each receiving the same dose that Monkey 7 received—0.001 cc. of broth culture subcutaneously. Condensed protocols are shown in Table I and Text-fig. 2.

TABLE I.

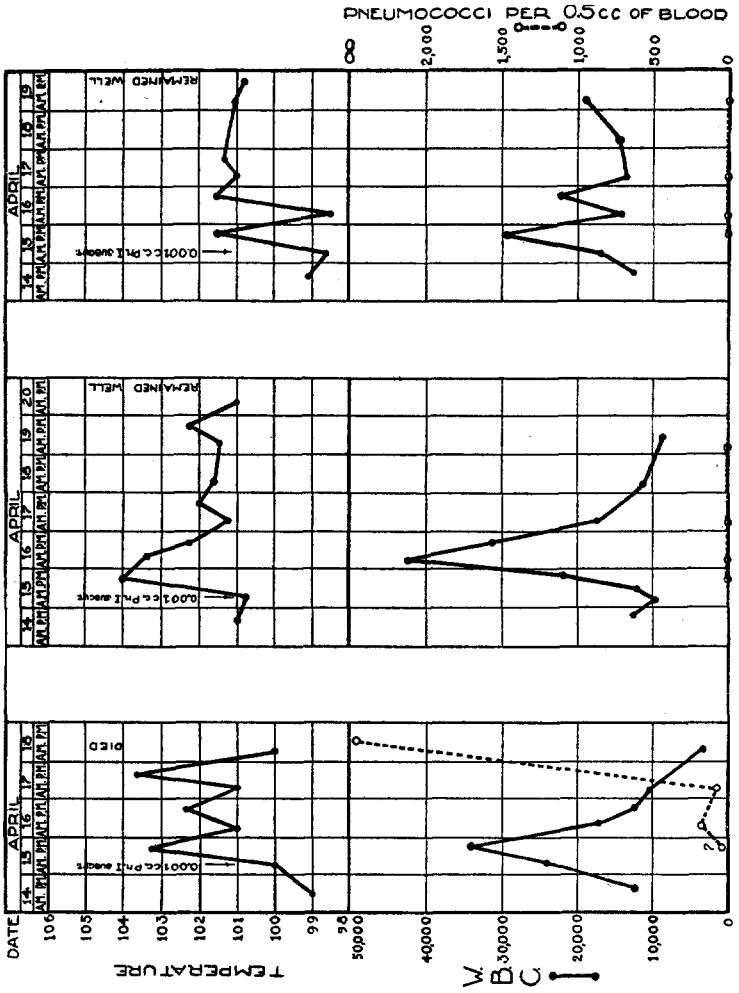
Effect of Subcutaneous Injection of Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Apr. 15. Broth culture of Pn. I subcutaneously.	Apr. 29. Serum tests with Pn. I.			Result.	Autopsy.	Autopsy cultures. Heart's blood.
			Agglutinins.	Protection.	Control.			
	gm.	*cc.*						
46	2,550	0.001				D. 4 days.	Lungs normal.	Pn. I
47	2,710	0.001	1:1++	0.00001 cc. S.* 0.000001 cc. S. 0.0000001 cc. S.	0.00001 cc. D. 24 hrs. 0.000001 cc. D. 36 hrs. 0.0000001 cc. D. 36 hrs.	Remained well.		
48	3,590	0.001	0	0		Remained well.		

* S. indicates survived; D., died.

It will be observed that each of these three monkeys reacted differently to the vaccine. The smallest one (Monkey 46) showed a severe constitutional reaction with heavy septicemia, and died on the 4th day. Monkey 47 reacted with a sharp but temporary rise in temperature and leucocytes, but the blood remained sterile and the monkey at no time appeared ill. The largest monkey (No. 48) showed no reaction of any kind except a slight leucocytosis.

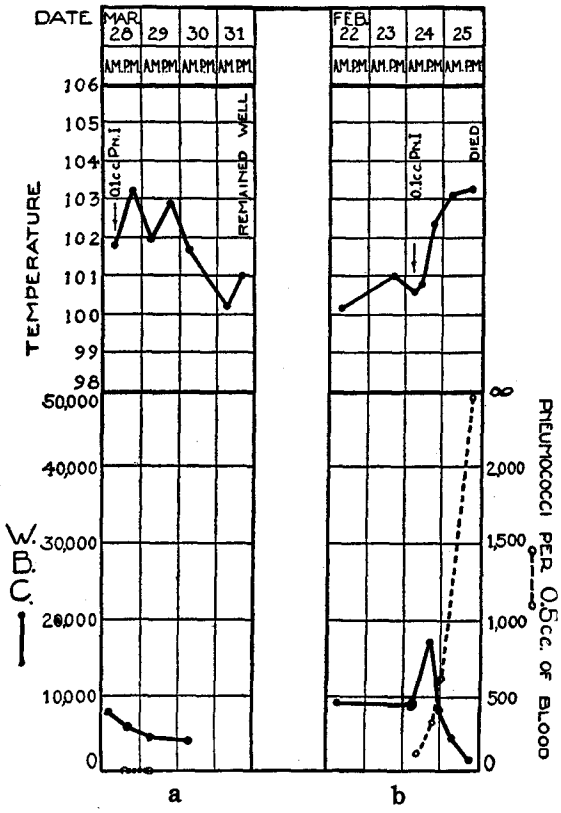
2 weeks after vaccination the two surviving monkeys were bled and their sera tested for agglutinins and protective bodies against Pneumococcus Type I. Monkey 47 showed both agglutinins and protective substances. Its serum protected mice against 100 times the minimal lethal dose of Pneumococcus Type I. Monkey 48, on the other hand,



TEXT-FIG. 2, *a*, *b*, and *c*. Reactions following subcutaneous inoculation of living virulent Pneumococcus Type I. (*a*) Monkey 46, (*b*) Monkey 47, (*c*) Monkey 48. Each of these animals received 0.001 cc. of living culture subcutaneously.

exhibited neither agglutinins nor protective bodies. These reactions indicate what a large factor individual variation may be in experiments of this kind.

Active Immunity Following Subcutaneous Injection of Living Virulent Pneumococci.—The immediate effect of subcutaneous injections of



TEXT-FIG. 3, *a* and *b*. Active immunity against Pneumococcus Type I following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of living culture subcutaneously. (*b*) Monkey 3; control.

living virulent pneumococci having been studied, the next step was to determine the degree of immunity conferred by this method of vaccination.

The object of the two following experiments was to test the vaccinated monkeys for active immunity against Pneumococcus Type I.

TABLE II.

Active Immunity Following Subcutaneous Injection of a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Mar. 7. Broth culture of Pn. I subcutaneously.	Mar. 11. X-ray.	Mar. 27. Serum tests with Pn. I.			Mar. 28. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
				Agglutinins.	Protection.	Control.				Lung.	Heart's blood.
	gm.	*cc.*					*cc.*				
7	1,430	0.001	Negative.	0	0.0001 cc. S. 0.00001 cc. S. 0.000001 cc. S. 4½ days.	0.00001 cc. D. 48 hrs. 0.000001 cc. D. 48 hrs.	0.1	Remained well.			
3	1,595	0	" (Feb. 19).				0.1 (Feb. 24).	D. 2 days.	Lobar pneumonia; red stage.	Pn. I	Pn. I

The identical strain of Pneumococcus Type I was used for the intratracheal injections that had been used for the subcutaneous vaccinations.

Experiment 3.—Mar. 28, 1917. Monkey 7, 21 days after vaccination with living virulent Pneumococcus Type I, was injected intratracheally with 0.1 cc. of an 18 hour Pneumococcus Type I broth culture. No monkey of this species was available as an actual control at the time. The record of Monkey 3, however, is introduced to indicate what had been the invariable result when comparable doses of Pneumococcus Type I were injected intratracheally in normal unvaccinated *capucinus* monkeys. The temperature, leucocyte, and blood culture curves are charted in Text-fig. 3. The results are shown in Table II.

The vaccinated monkey (No. 7), though it received 100,000 times the minimal infecting dose, remained perfectly well, while Monkey 3 died with incipient lobar pneumonia and an overwhelming pneumococcus septicemia on the day following injection. Even the leucocyte count in Monkey 7 remained unchanged.

In other words, a very small quantity of living virulent pneumococcus culture (0.001 cc.) injected subcutaneously was sufficient to confer on the monkey a high degree of active immunity against the homologous strain.

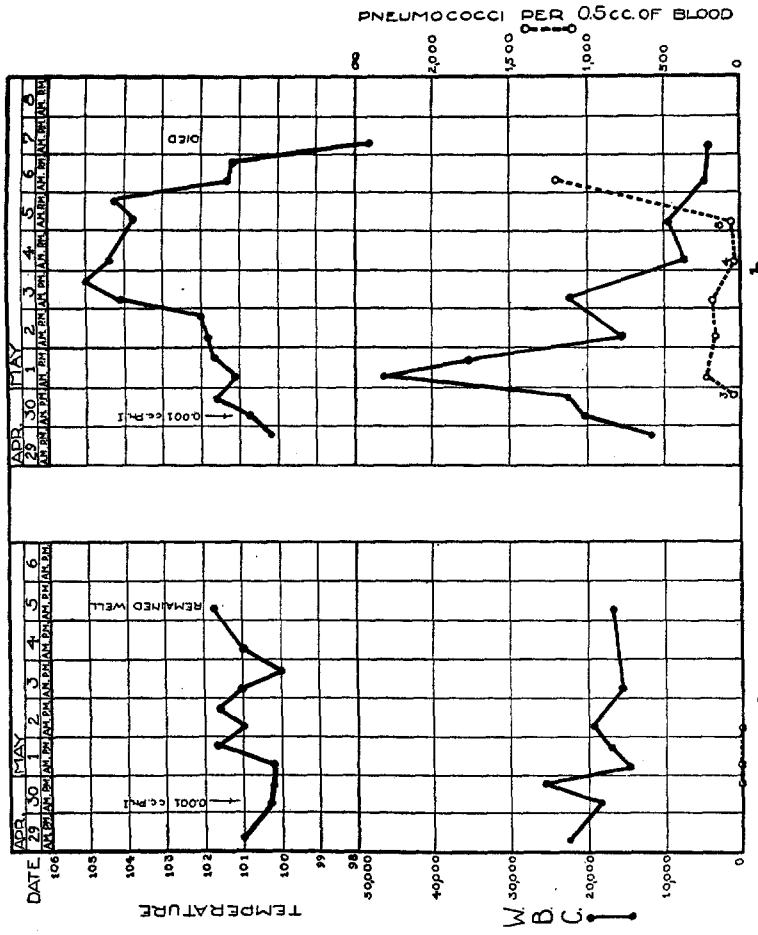
One of the *Macacus* monkeys (No. 48) which had been vaccinated with living virulent pneumococci (0.001 cc.) was next tested.[7]

Experiment 4.—Apr. 30, 1919. Monkey 48, vaccinated on Apr. 15 with 0.001 cc. of living Pneumococcus Type I, and Monkey 83, control, were each injected intratracheally with 0.001 cc. of Pneumococcus Type I broth culture.

The protocols (Table III and Text-fig. 4) show that the vaccinated monkey remained active and well. The control developed a typical lobar pneumonia and died on the 8th day with the usual pneumococcus septicemia. As in the previous experiment, the vaccinated monkey showed no rise in temperature and did not even react with a leucocytosis.

There was, however, a difference between the vaccinated monkey in Experiment 3 and the one in Experiment 4. The former developed a considerable amount of protective substance in the blood following vaccination; the latter showed none. Yet both were immune to Pneumococcus Type I pneumonia.

[7] Monkey 47 will be considered later.



TEXT-FIG. 4, *a* and *b*. Active immunity against Pneumococcus Type I following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 48; received 0.001 cc. of living culture subcutaneously. (*b*) Monkey 83; control.

TABLE III.

Active Immunity Following Subcutaneous Injection of a Living Culture of Virulent Pneumococcus Type I.

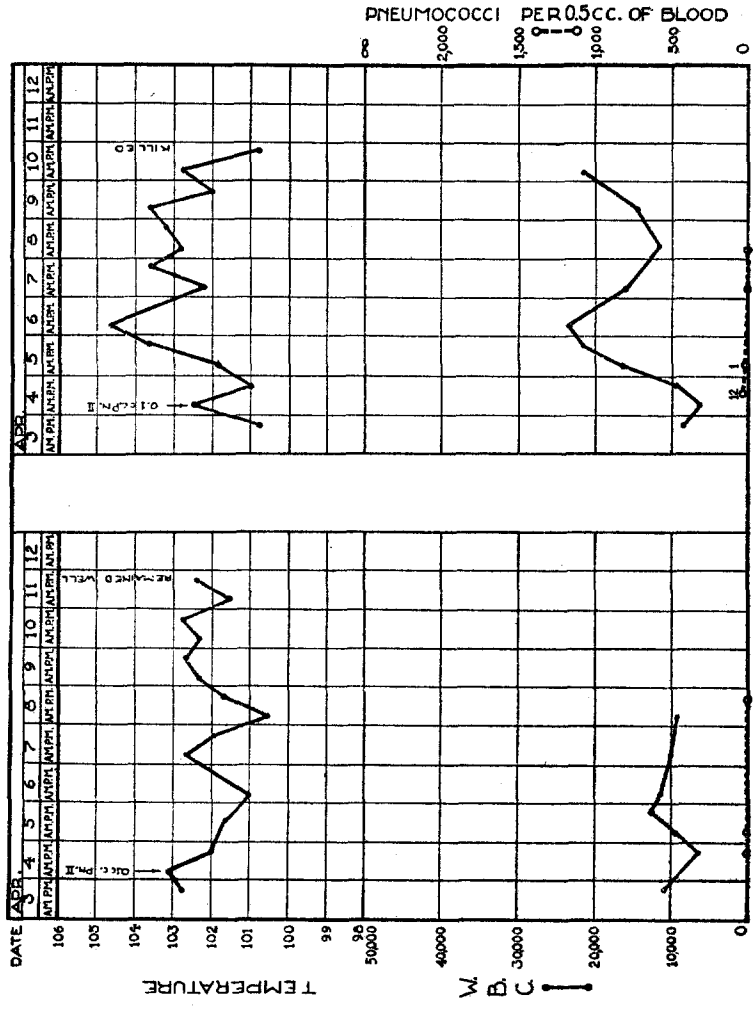
Monkey No.	Weight.	Apr. 15. Broth culture of Pn. I subcutaneously.	Apr. 30. Broth culture of Pn. I intratracheally.	May 2. X-ray.	Result.	Autopsy.	Autopsy cultures.	
							Lung.	Heart's blood.
	gm.	*cc.*	*cc.*					
48	3,590	0.001	0.001	Negative.	No signs of pneumonia. Remained well.			
83	2,470	0	0.001		Clinical pneumonia. D. 8th day.	Lobar pneumonia, R. U., R. M., R. L.*	Pn. I	Pn. I

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

Cross-Immunity against Pneumococcus Type II Following Vaccination with Living Virulent Pneumococcus Type I.—The experiments just reported afford ample proof that vaccination with a living virulent Pneumococcus Type I stimulates a high degree of active immunity against the homologous type of pneumococcus. The next step was to determine whether vaccination with living Pneumococcus Type I afforded any cross-immunity against the other types of pneumonia. 1 week, therefore, after the test against Pneumococcus Type I, Monkey 7 was tested against a virulent strain of Pneumococcus Type II.

Experiment 5.—Apr. 4, 1919. Monkey 7, vaccinated against Pneumococcus Type I, and Monkey 25, control, were injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type II (Table IV, Text-fig. 5). Monkey 7 remained perfectly well, with no rise in temperature or leucocytes. Monkey 25, the control, developed lobar pneumonia with leucocytosis and positive blood culture. The control recovered by crisis on the 7th day.

According to this experiment vaccination with a living virulent Pneumococcus Type I had conferred not only immunity against the homologous type but a cross-immunity against Pneumococcus Type II as well. Furthermore, this immunity existed in spite of the absence of protective bodies against Pneumococcus Type II in the serum of the monkey.



TEXT-FIG. 5, *a* and *b*. Cross-immunity against Pneumococcus Type II following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of Pneumococcus Type I living culture subcutaneously. (*b*) Monkey 25; control.

TABLE IV.

Production of Cross-Immunity with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Mar. 31. Serum tests with Pn. II.		Apr. 4. Broth culture of Pn. II intratracheally.	Apr. 7. X-ray.	Result.	Autopsy.	Autopsy cultures.	
		Agglutinins.	Protection.					Lung.	Heart's blood.
	gm.			*cc.*					
7	1,430	0	0	0.1	Negative.	Remained well.			
25	740			0.1	Shadow, R. U.	Clinical pneumonia. Crisis on 7th day. Killed.	Lobar pneumonia, R. U.; gray stage.	Sterile.	Sterile.

Experiment 6.—June 24, 1919. 8 weeks after the test against Pneumococcus Type I, Monkey 48, vaccinated against Pneumococcus Type I, and Monkey 91, control, were injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type II (Table V, Text-fig. 6).

Both monkeys developed Type II pneumonia and both recovered. In the vaccinated monkey, however, the disease ran a mild short course, and the blood was sterile throughout practically the entire course of the disease. The control monkey ran a long course (crisis on the 18th day) and showed a fairly heavy pneumococcus septicemia.

In the case, then, of Monkey 48 successful vaccination against Type I pneumonia did not give sufficient cross-immunity to protect against Pneumococcus Type II pneumonia, though it did apparently moderate the severity of the disease. The inconsistency between the results obtained in Experiments 5 and 6 may be explained in several ways. In Experiment 5, the test for cross-immunity against Pneumococcus Type II was made 1 week after the test against Pneumococcus Type I; in Experiment 6 the test for cross-immunity was carried out 8 weeks after testing for homologous immunity. The duration of pneumococcus immunity is not known, but a high degree of resistance lasts probably not more than a few months. Another ex-

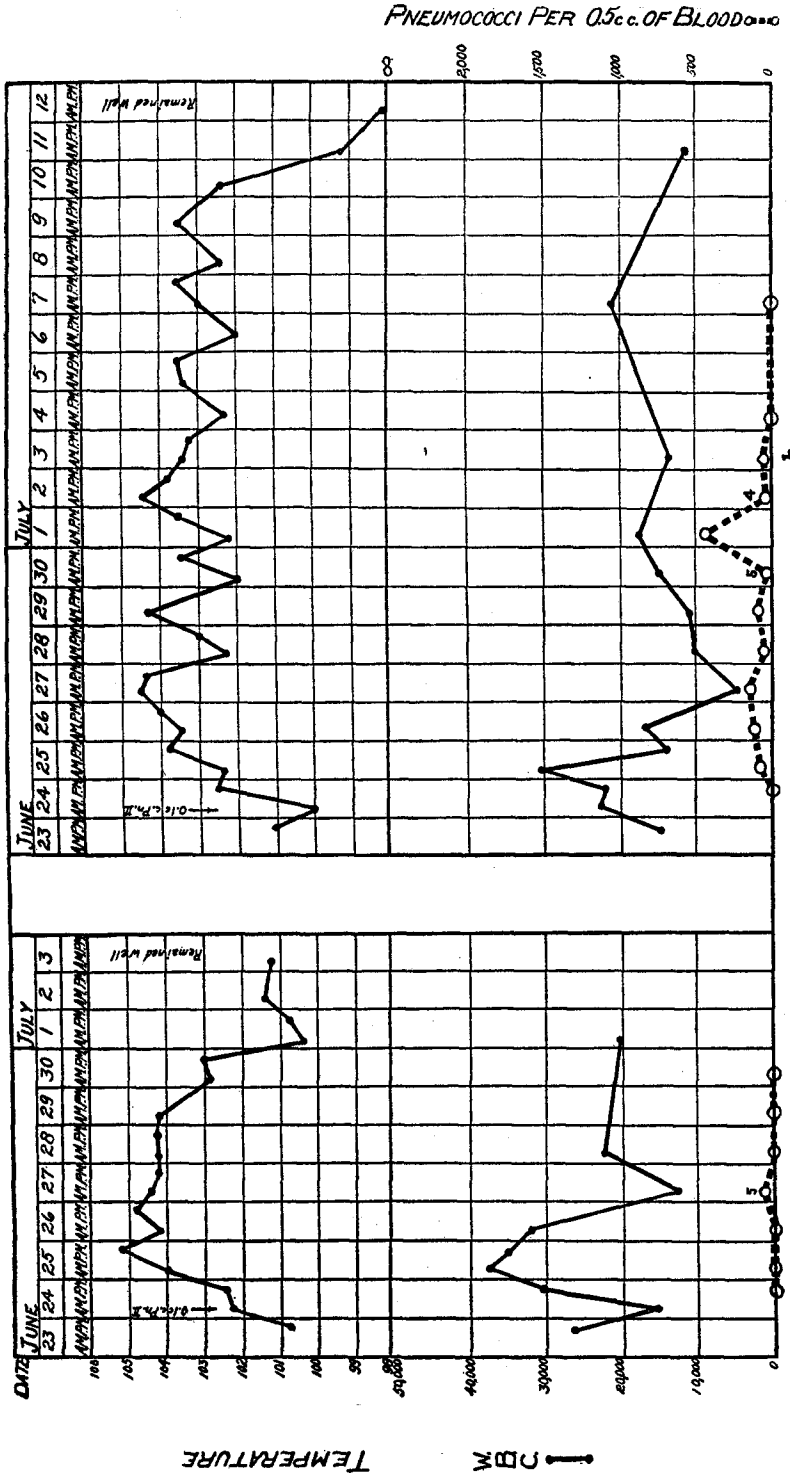
planation may be found in the much severer reaction exhibited by Monkey 7 following vaccination, with a consequent development of a higher grade of immunity. Still another and perhaps more rational explanation would interpret this difference in cross-immunity to individual variation in the monkeys.

TABLE V.

Experimental Pneumococcus Type II Pneumonia Following Vaccination with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Apr. 15. Broth culture of Pn. I subcutaneously.	Apr. 30.	June 23. Serum tests with Pn. II.			June 24. Broth culture of Pn. II intratracheally.	Result.
				Agglutinins.	Protection.	Control.		
	gm.	*cc.*					*cc.*	
48	3,590	0.001	Resisted infection with Pn. I intratracheally.	0	0.000001 cc. S. 0.00001 cc. S. 0.0001 cc. D. 48 hrs. 0.001 cc. D. 48 hrs.	0.000001 cc. D. 48 hrs. 0.00001 cc. D. 20 hrs. 0.0001 cc. D. 18 hrs.	0.1	Clinical pneumonia. Recovery by crisis on 8th day.
91 (control).	4,002	0					0.1	Clinical pneumonia. Recovery on 18th day.

There is one more interesting feature to this experiment. It will be recalled that Monkey 48, though immune to Pneumococcus Type I pneumonia showed no protective substance in the blood. When, however, 8 weeks later a second protection test was carried out on the serum of this monkey, this time against Pneumococcus Type II, a definite though slight amount of protection was demonstrated. We offer no explanation of this phenomenon.



TEXT-FIG. 6, *a* and *b*. Pneumococcus Type II pneumonia following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 48; received 0.001 cc. of living culture of Pneumococcus Type I subcutaneously. (*b*) Monkey 91; control.

Cross-Immunity against Pneumococcus Type III Following Vaccination with Living Virulent Pneumococcus Type I.—The last two experiments (Nos. 5 and 6) gave some evidence of a cross-immunity against Pneumococcus Type II following vaccination with living virulent Type I pneumococci. It was decided, therefore, to determine whether a similar cross-protection existed against Pneumococcus Type III.

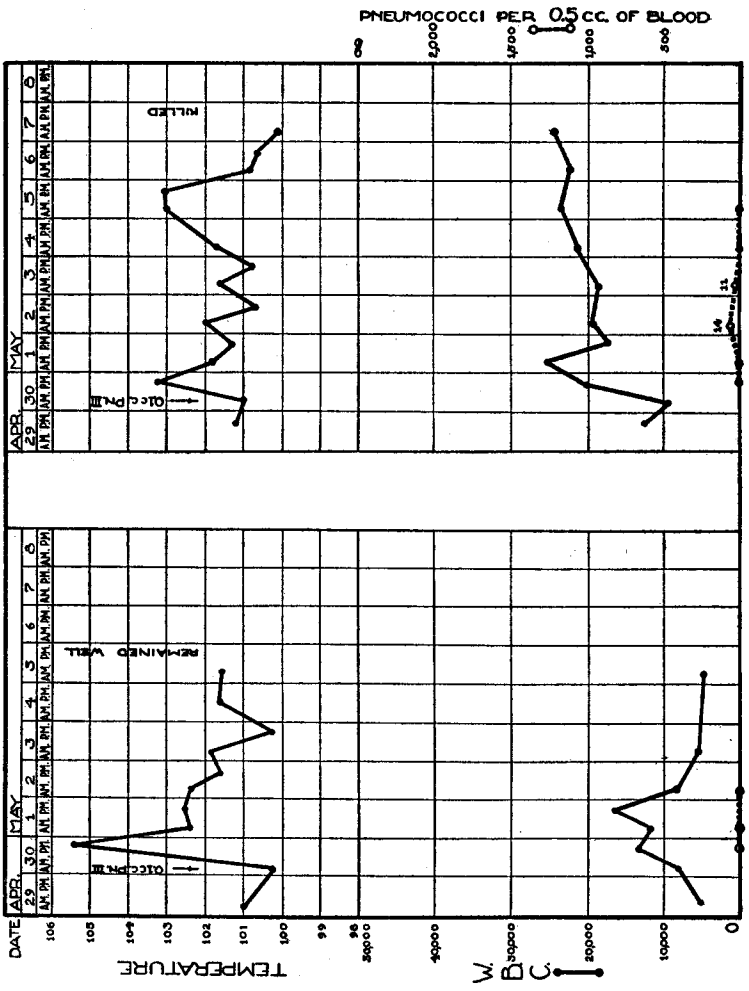
TABLE VI.

Production of Cross-Immunity with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	Apr. 29. Serum tests with Pn. III.		Apr. 30. Broth culture of Pn. III intratracheally.	May 3. X-ray.	Result.	Autopsy.	Autopsy cultures	
		Agglutinins.	Protection.					Lung.	Heart's blood.
	gm.			*cc.*					
7	1,430	0	0	0.1	Negative.	Temporary febrile reaction; no pneumonia.			
82	732			0.1	Shadow, R. U.	Clinical pneumonia. Crisis on 7th day. Killed.	Interstitial pneumonia, R. U	Sterile.	Sterile.

Experiment 7.—Apr. 30, 1919. About 4 weeks after the test against Pneumococcus Type II, Monkey 7, vaccinated with living culture of Pneumococcus Type I, and Monkey 82, control, were injected intratracheally with 0.1 cc. of broth culture of a virulent Pneumococcus Type III. The results are shown in Table VI and Text-fig. 7.

The vaccinated monkey reacted to the injection of Pneumococcus Type III with a sharp but temporary rise of temperature, and a slight increase in leucocytes. The blood culture remained sterile. The monkey appeared sick during the afternoon of the day on which it was injected, but the next morning it was lively and well and remained so. The x-ray was negative, and if any pneumonia developed it must



TEXT-FIG. 7, *a* and *b*. Partial cross-immunity against Pneumococcus Type III following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of living culture of Pneumococcus Type I subcutaneously. (*b*) Monkey 82; control.

have been an exceedingly small patch. On the other hand, the control monkey developed a definite but rather mild attack of Type III pneumonia, with moderate leucocytosis and positive blood culture. The control recovered by crisis on the 7th day and autopsy showed a resolving interstitial pneumonia.

Vaccination with a living virulent Pneumococcus Type I apparently gave Monkey 7 a certain amount of cross-immunity against Pneumococcus Type III as well as against Type II. As with Pneumococcus Type II, the monkey possessed no demonstrable protective bodies in the blood against Pneumococcus Type III.

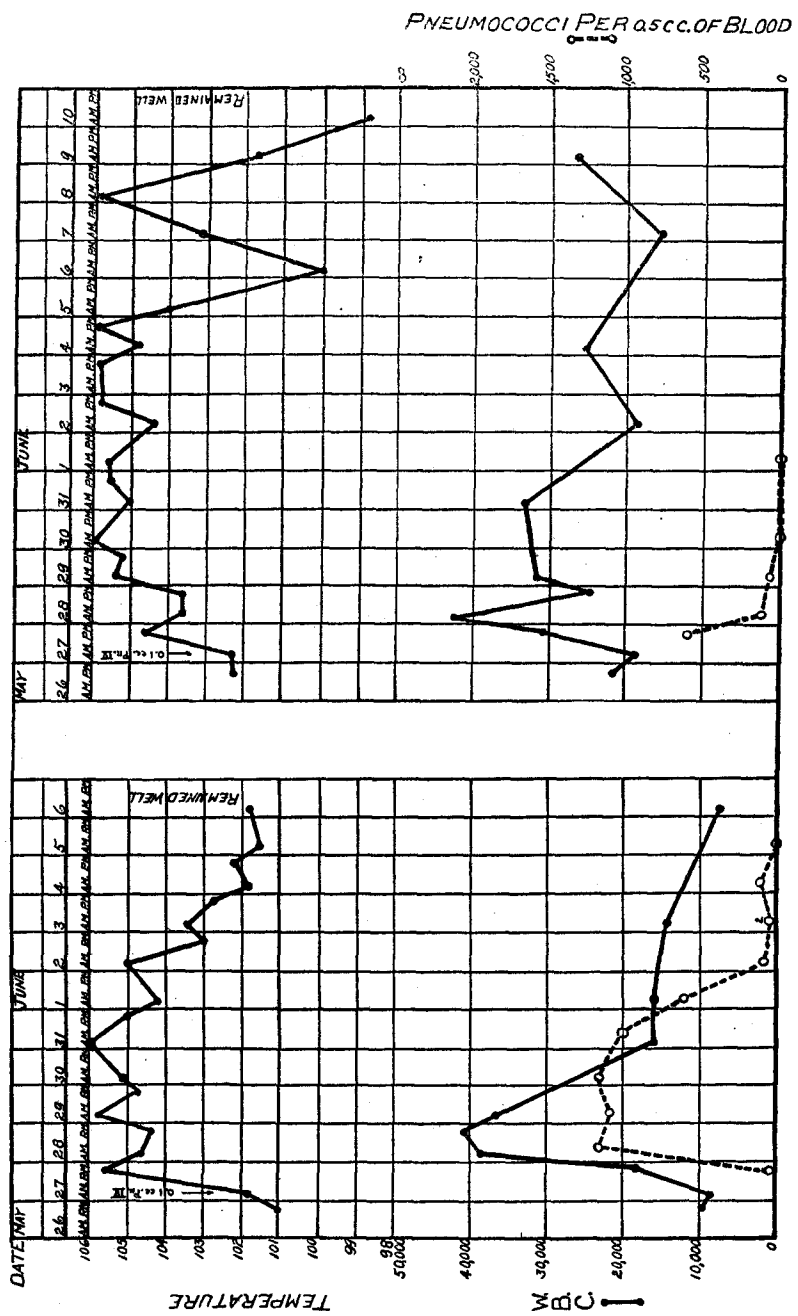
Test for Cross-Immunity against Pneumococcus Type IV Following Vaccination with Living Virulent Pneumococcus Type I.—One of the

TABLE VII.

Experimental Pneumococcus Type IV Pneumonia Following Vaccination with a Living Culture of Virulent Pneumococcus Type I.

Monkey No.	Weight.	May 27. Broth culture of Pn. IV intratracheally.	June 5. X-ray.	Result.	Autopsy.	Autopsy cultures.	
						Lung.	Heart's blood.
	gm.	*cc.*					
7	1,430	0.1	Negative.	Clinical pneumonia. Recovery by lysis on 8th day. Killed on 14th day.	Resolving lobar pneumonia, R. L., L. L.	No growth.	No growth.
111	2,219	0.1	Shadow, R. L.	Clinical pneumonia. Recovery on 14th day.			

vaccinated monkeys (No. 7) had resisted Pneumococcus Type I, Type II, and probably Type III pneumonia. It remained, therefore, only to test it against Pneumococcus Type IV. In this test a strain of Type IV was employed that had been recently isolated from a case of spontaneous Pneumococcus Type IV pneumonia in one of the stock monkeys (No. 97).



TEXT-FIG. 8, *a* and *b*. Pneumococcus Type IV pneumonia following vaccination with living virulent Pneumococcus Type I. (*a*) Monkey 7; received 0.001 cc. of living culture of Pneumococcus Type I subcutaneously. (*b*) Monkey 111; control.

Experiment 8.—May 27, 1919. 4 weeks after being tested for immunity against Pneumococcus Type III, Monkey 7, vaccinated, and Monkey 111, control, were injected intratracheally with 0.1 cc. of broth culture of Pneumococcus Type IV (Strain M 97). Table VII and Text-fig. 8 show the results of the experiment.

Both monkeys developed Type IV pneumonia with high temperature, leucocytosis, pneumococcus septicemia, and final recovery; but the vaccinated monkey was well on the 8th day, while the control did not recover until the 14th day.

Apparently, there was not enough cross-immunity to protect the vaccinated animal against pneumonia, though there was enough, as with Monkey 48 in Experiment 6, to moderate and shorten somewhat the course of the disease. Just why Monkey 7 resisted the three fixed types of pneumococcus and then became infected with a Pneumococcus Type IV it is hard to say. Nearly 3 months had elapsed from the day of vaccination to the day when the monkey was injected with Pneumococcus Type IV, and it may be that by that time the immunity curve was on the downward way. Moreover, the pneumococcus used had recently been isolated from another infected monkey, and was therefore adapted, in a sense, to the species. The fixed strains of pneumococcus used had never been passed through monkeys.

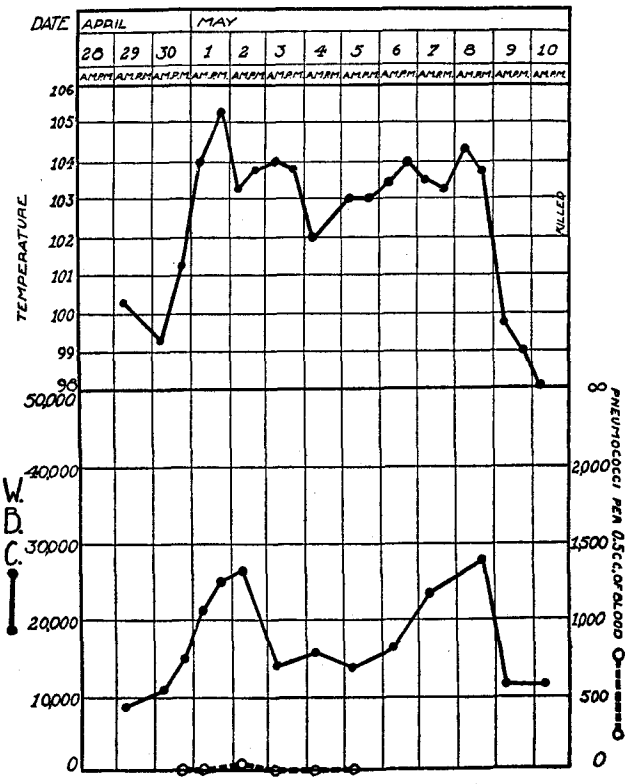
Spontaneous Pneumococcus Type IV Pneumonia Following Vaccination with a Living Virulent Pneumococcus Type I.—The following case corroborates the evidence obtained in the last experiment; namely, that there is not much cross-protection against Pneumococcus Type IV pneumonia in monkeys following vaccination with a living virulent culture of Pneumococcus Type I.

Experiment 9.—Monkey 47. *Macacus syrichtus*, male; weight 2,170 gm. Apr. 15, 1919, 10 a.m. Vaccinated with 0.001 cc. of 18 hour broth culture of Pneumococcus Type I (see Experiment 2). Apr. 30. Well and active. May 1. Looks sick; rapid labored respiration. May 2. Blood culture, Pneumococcus Type IV. May 3. Bronchial breathing in left axilla. May 4. Dyspnea persists; cough. May 5. Condition the same. May 9. Crisis and recovery. May 10. Killed.

Autopsy.—Resolving lobar pneumonia, left middle and lower lobes and right middle lobe; acute fibrinous pleuritis, left.

Text-fig. 9 shows the temperature, leucocyte, and blood culture curves of this case of spontaneous pneumonia. The disease ran a typical course, very similar to that seen in the experimental pneumo-

nia of monkeys. The blood, however, remained practically sterile (only one positive blood culture), and this fact suggests that here as in Monkey 7, Pneumococcus Type I vaccination afforded enough cross-immunity against Pneumococcus Type IV to moderate the severity of the pneumonia caused by the latter organism.

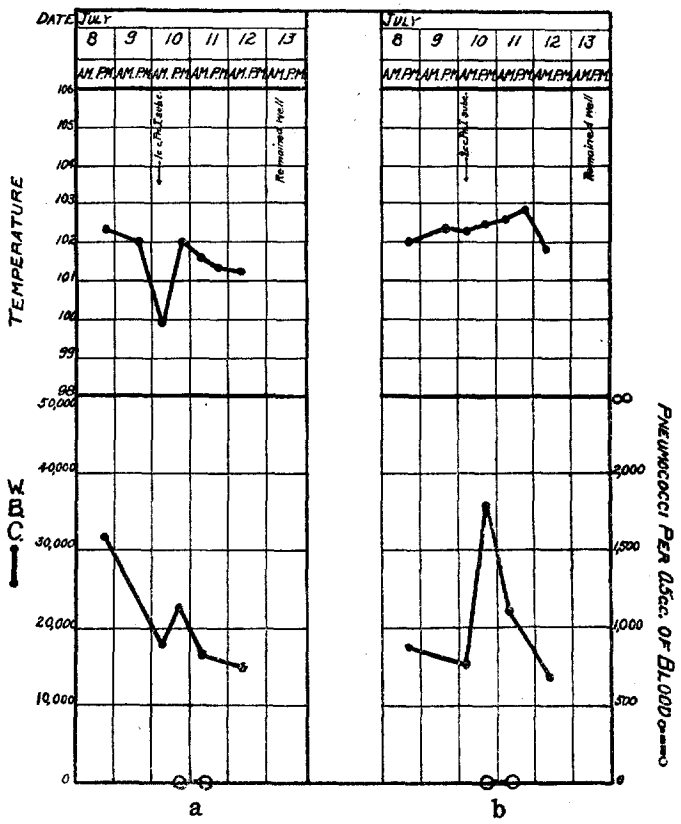


TEXT-FIG. 9. Monkey 47. Spontaneous Pneumococcus Type IV pneumonia in a monkey vaccinated with living virulent Pneumococcus Type I. Received 0.001 cc. of living culture of virulent Pneumococcus Type I subcutaneously.

Vaccination with a Living Avirulent Culture of Pneumococcus.

Up to this point all the experiments reported have shown the results obtained by vaccination with a living virulent pneumococcus. The remainder of the vaccination experiments were carried out with

a living avirulent pneumococcus, in order to determine how important a factor virulence was, on the one hand, and, on the other, how much depended upon the use of living or killed cultures.



TEXT-FIG. 10, *a* and *b*. Reactions following the subcutaneous inoculation of living avirulent Pneumococcus Type I. (*a*) Monkey 117; received 1 cc. of living culture subcutaneously. (*b*) Monkey 118; received 2 cc. of living culture subcutaneously.

The culture used for vaccination in the following experiments was an old stock Pneumococcus Type I which was avirulent for mice in doses of 1 cc. of broth culture. This strain was used for the preparation of the vaccine in the study of vaccination with killed cultures.[8]

[8] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 499.

It was assumed that in view of the lack of virulence a much larger dose of vaccine would be necessary in order to obtain results; consequently, a dose of 1 to 2 cc. of broth culture was employed. The injections were given subcutaneously in the abdominal wall as in the previous experiments.

Experiment 10.—July 10, 1919. Two *Macacus syrichtus* monkeys (Nos. 117 and 118) were injected subcutaneously with an 18 hour broth culture of living avirulent Pneumococcus Type I. Monkey 117 received 1 cc. of culture (about 300 million pneumococci). Monkey 118 received 2 cc. (about 600 millon pneumococci). The reactions are shown in Text-fig. 10.

Monkey 117 developed a small area of induration at the site of inoculation but had no constitutional reaction whatever. Monkey 118 also showed a slight local reaction, and in addition a moderate rise in leucocytes. The blood remained sterile in both monkeys.

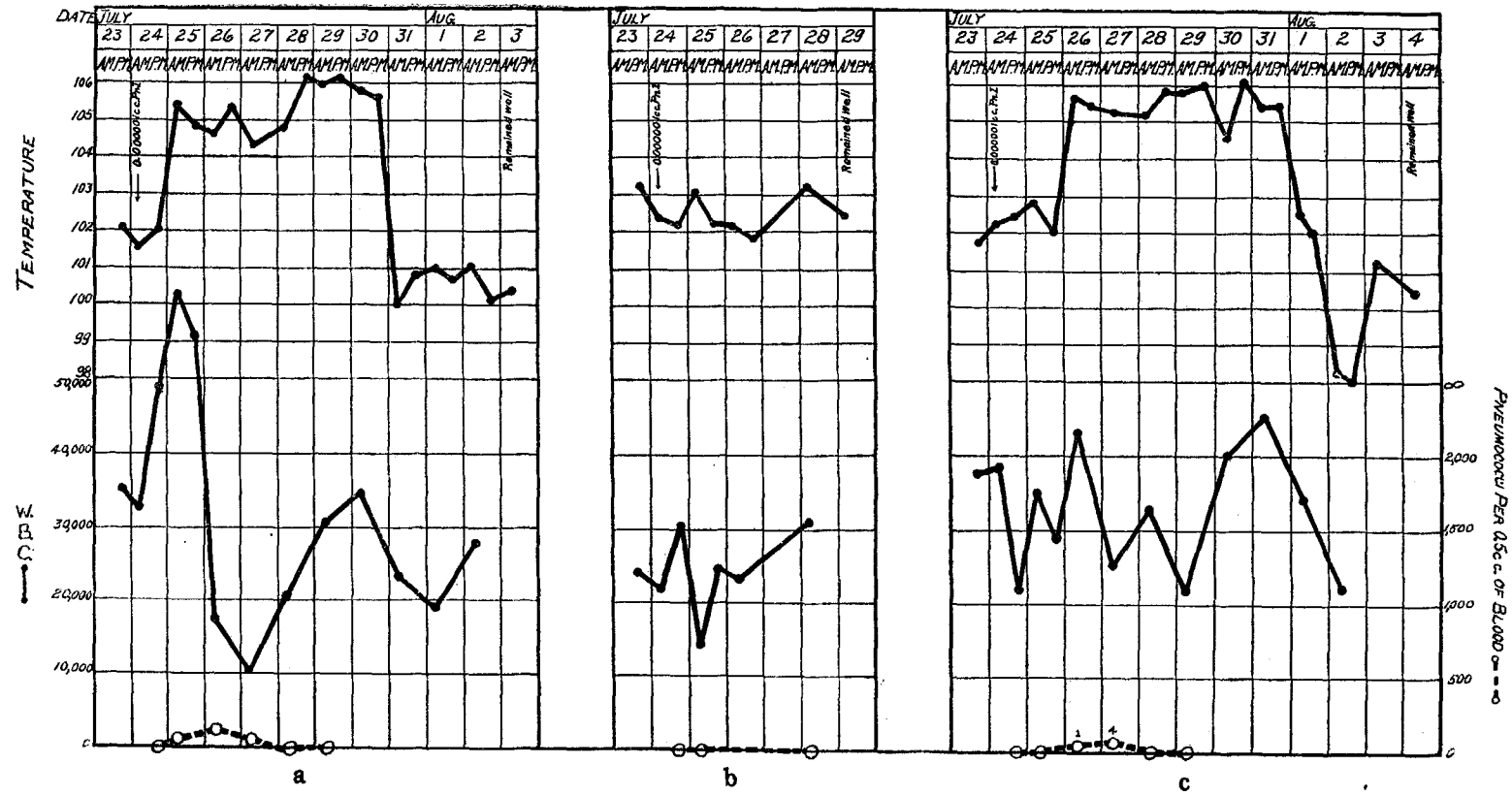
TABLE VIII.

Active Immunity Following Subcutaneous Injection of a Living Culture of Avirulent Pneumococcus Type I.

Monkey No.	Weight.	July 10. Broth culture of Pn. I subcutaneously.	Reaction.	July 24. Serum tests with Pn. I.		July 24. Broth culture of Pn. I intratracheally.	Result.
				Agglutinins.	Protection.		
	gm.	*cc.*				*cc.*	
117	2,565	1	Slight; local.	0	0	0.000001	Clinical pneumonia. Recovery by crisis on 8th day.
118	2,915	2	Slight; local.	0	0	0.000001	Remained well.
127 (control).	2,700	0				0.000001	Clinical pneumonia. Recovery by crisis on 9th day.

This experiment indicated that living avirulent pneumococci could be administered subcutanequsly in fairly large doses to monkeys without producing any serious local or general reaction.

The following experiment was carried out for the purpose of testing the amount of active immunity produced by vaccination with living avirulent pneumococci.



TEXT-FIG. 11, *a*, *b*, and *c*. Active immunity (Monkey 118) against Pneumococcus Type I following vaccination with 2 cc. of living avirulent Pneumococcus Type I; 1 cc. of the same culture failed to protect Monkey 117. (*a*) Monkey 117; received 1 cc. of living avirulent culture subcutaneously. (*b*) Monkey 118; received 2 cc. of living avirulent culture subcutaneously. (*c*) Monkey 127; control.

Experiment 11.—July 24, 1919. Monkeys 117 and 118, both vaccinated on July 10 with living avirulent pneumococci, and Monkey 127, control, were injected intratracheally with 0.000001 cc. of broth culture of Pneumococcus Type I (Table VIII). Monkey 117, which received the small dose (1 cc.) of living vaccine, developed a mild pneumonia and recovered by crisis on the 8th day of the disease. Monkey 118, which received the large dose (2 cc.) of living vaccine, remained perfectly well. The control (Monkey 127) developed pneumonia and ran a typical course with crisis on the 9th day.

Text-fig. 11 shows the temperature, leucocyte, and blood culture curves. Monkey 118 presented no reaction of any kind. Monkey 117 ran a typical lobar pneumonia, with positive blood culture, high leucocytosis, and crisis on the 8th day. Monkey 127 also ran a typical course with crisis on the 9th day.

Living avirulent pneumococci, when injected subcutaneously, appear to excite an immunity equal in degree to that produced by living virulent pneumococci, if a large enough dose is administered. In this experiment 1 cc. of broth culture of living avirulent pneumococci did not confer a sufficiently high degree of immunity to protect the monkey from pneumonia. When 2 cc., however, of the same culture were administered to another monkey, the protection was satisfactory. In other words, the dose is a factor to be considered in vaccinating with living avirulent pneumococci.

DISCUSSION.

The inferences to be drawn from the experiments reported in this study are plain. A high degree of immunity against pneumococcus pneumonia can be induced by the subcutaneous injection of living virulent pneumococci, but the method is too dangerous for any sort of practical application. Vaccination with attenuated living pneumococci could probably be practised with impunity, but the problem of transporting and keeping alive large quantities of pneumococci in the field would be difficult to solve. The fact that a higher degree of immunity is produced by living pneumococci than by dead pneumococci is not surprising in the light of previous observations on vaccination with other bacteria. The degree of cross-immunity, however, which sometimes followed vaccination with living Pneumococcus Type I was surprising and confirms the fact already established that the various types of pneumococci are closely related biologically.

This study had suggested a number of theoretical problems. In the first place, why do living pneumococci confer a more efficient immunity than dead pneumococci? Virulence appears to play some part, but even with avirulent strains good protection can be secured with sufficiently large doses. These animals vaccinated with living cultures are, of course, in a sense infected, and we are accustomed to think of infection as bestowing a more efficacious immunity than mere vaccination with killed cultures.

The question also arises as to the significance of agglutinins and the so called protective bodies in an animal's blood. It has been shown in the experiments reported that the serum of a monkey may be entirely free from these substances, and yet the animal may possess a high grade of immunity against pneumonia. On the other hand, it was pointed out in the study of pneumococcus saline vaccines[1] that the serum of a vaccinated monkey might protect mice against 100 or even 1,000 minimal lethal doses of pneumococci, and still that monkey be susceptible to experimental pneumococcus pneumonia. These facts complicate the whole question of resistance to pneumococcus infection and revive the old problem of humoral *versus* cellular immunity.

These studies also emphasize the fact that "immunity" is a relative term and under any circumstances dependent on a number of factors. Every immunologist is familiar with the great differences which animals manifest in their capacity to produce antibodies. Even with the most ideal methods of vaccination there will always be certain animals that respond poorly to vaccine, and these animals will not possess the same amount of immunity that others will have.

Furthermore, the degree of immunity against a certain microorganism depends in large measure on the virulence of the strain used in testing it. For example, in the experiments on cross-immunity, Monkey 7 resisted infection with Pneumococcus Type III which was not very virulent for monkeys, but could not resist Pneumococcus Type IV which, in this particular instance, happened to be virulent for monkeys. In view of this fact the question arises whether in the experiments with Pneumococcus Type I lipovaccine and saline vaccine immunity would not have been demonstrated if a less virulent strain of Pneumococcus Type I had been used for testing the efficacy of the vaccine.

All that has been said regarding virulence applies with equal force to the size of the infecting dose. Even in well immunized monkeys experimental pneumonia could probably have been induced if a sufficiently large dose of culture had been injected into the trachea. This would have been a most artificial procedure, but the argument holds in spite of that fact. The animal possesses a definite sum of resistance, and when that sum is spent it becomes at once susceptible to infection.

CONCLUSIONS.

1. The subcutaneous injection of small doses of living virulent Pneumococcus Type I stimulates in monkeys a degree of active immunity sufficient to protect them against experimental pneumococcus pneumonia of homologous type.

2. The subcutaneous injection of living avirulent Pneumococcus Type I, if administered in a sufficiently large dose, likewise renders the monkey immune to a subsequent pneumonia of homologous type.

3. Vaccination of monkeys with small doses of living virulent pneumococci may or may not be followed by a severe constitutional reaction, depending on the natural resistance of the individual. The severe reactions are caused by the development of a pneumococcus septicemia. which is either temporary, or leads to a fatal termination. The mild reactions are not accompanied by septicemia, and there are no symptoms other than a slight elevation of temperature and moderate leucocytosis. Vaccination with living avirulent pneumococci does not induce severe reactions and is not accompanied by pneumococcus septicemia.

4. Active immunity against pneumococcus pneumonia, produced by vaccination with living pneumococci, appears to be largely independent of the presence or absence of agglutinins and protective bodies in the serum of the monkey.

5. Vaccination with living cultures of Pneumococcus Type I confers against other types of pneumococci a certain amount of cross-immunity which, however, varies considerably with the individual monkey.

6. Immunity against pneumococcus, like other forms of immunity, is a relative term, and depends upon the capacity of the individual for antibody production, the virulence of the invading microorganism, and the size of the dose injected.