

STUDIES ON PNEUMOCOCCUS IMMUNITY.
II. ACTIVE IMMUNIZATION OF MONKEYS AGAINST PNEUMOCOCCUS
TYPES II, III, AND IV PNEUMONIA WITH THE HOMOLOGOUS
PNEUMOCOCCUS VACCINE.*

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In a previous communication (1) it has been shown that the subcutaneous or intravenous injection of monkeys with Pneumococcus Type I vaccine confers on them a complete immunity against experimental Pneumococcus Type I pneumonia. In this article the results of similar experiments with Pneumococcus Types II, III, and IV will be reported. Two species of monkeys have been employed in these experiments, the Philippine *Macacus syrichtus* and the South American *Cebus capucinus*. Both species are quite susceptible to pneumococcus and when inoculated intratracheally with a virulent culture practically always develop pneumonia.

Methods.

Ordinary saline pneumococcus vaccine was used for the experiments. The method of preparation has been described in Paper I of this series (1). In all instances the monkeys received three subcutaneous injections of vaccine at intervals of 5 to 7 days, the total dosage varying from 120 to 200 billion pneumococci. The first dose was usually small, the second and third much larger. As in the previous experiments, the injections of vaccine were made in the back or abdomen and excited only a mild grade of local reaction.

In testing the immunity of the vaccinated monkeys, the same pneumococcus strains were used for the intratracheal injections of living culture that had been employed in making the vaccines. These tests were carried out 2 or 3 weeks after the completion of vaccination. The technique of intratracheal inoculation

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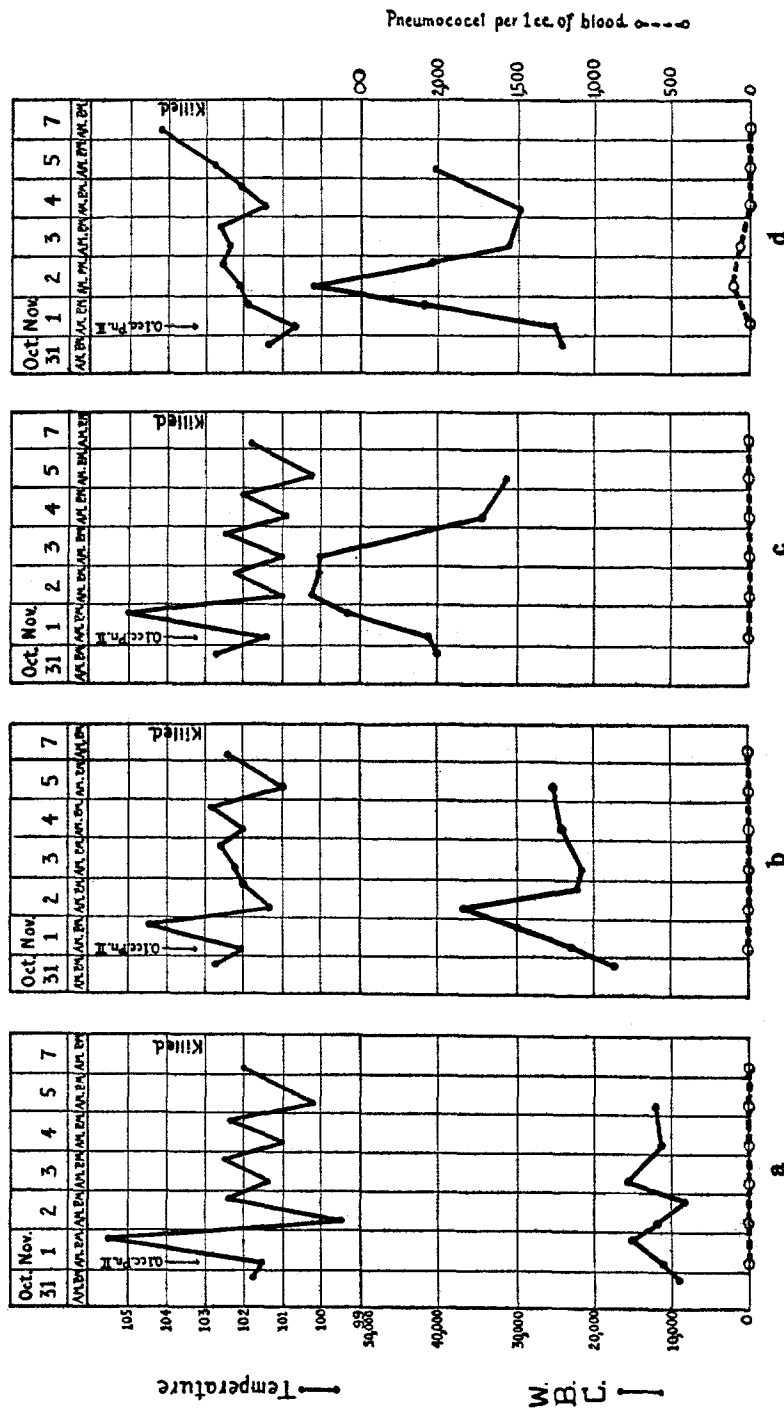
has also been previously described. The dose of culture injected in these experiments varied from 0.1 to 0.000001 cc. for each monkey, depending on the virulence of the strain. In every experiment an unvaccinated control was inoculated simultaneously with the vaccinated monkeys, and in every case the control came down promptly with pneumonia. Following the inoculations each monkey was examined daily, usually for 7 or 8 days. The rectal temperature was taken twice daily and leucocyte counts and blood cultures were made once a day. The monkeys that did not die following inoculation with cultures were killed at the conclusion of the experiment. Complete autopsies were performed on all animals and microscopic sections from the lungs were studied. Routine postmortem cultures were taken from the lungs and heart's blood.

Active Immunity against Pneumococcus Type II Pneumonia Following Three Subcutaneous Injections of Pneumococcus Type II Vaccine.

The first experiment in this study was carried out with Pneumococcus Type II. A virulent culture, fatal for mice in doses of 0.00000001 cc. of broth culture, was used for the preparation of the vaccine and also for the subsequent immunity tests.

Experiment 1.—Three Philippine monkeys were vaccinated subcutaneously as follows: Sept. 3, 1921. No. 1 (1,654 gm.), No. 2 (1,395 gm.), and No. 3 (2,167 gm.) received each 10 billion (1 cc.) of Pneumococcus Type II vaccine subcutaneously. Sept. 10. Each monkey received 80 billion (2 cc.) of Pneumococcus Type II vaccine subcutaneously. Sept. 17. Each monkey received 100 billion (2 cc.) of Pneumococcus Type II vaccine subcutaneously. There was no general reaction noticeable after any of the injections. Monkeys 1 and 2 each developed a small hard nodule at the site of the third inoculation. These nodules disappeared after about 1 week. Oct. 1 (2 weeks after the third injection of vaccine). The three vaccinated monkeys and a control monkey, No. 4 (2,412 gm.), each received intratracheally 0.1 cc. of a broth culture of virulent Pneumococcus Type II.

The results of this experiment are shown in Text-fig. 1. The vaccinated monkeys reacted with a sudden rise in temperature, which reached its maximum about 6 hours after inoculation. The next morning the temperatures of all three vaccinated monkeys were normal and remained so. In each instance this rise of temperature was accompanied by a temporary increase in the number of leucocytes. The monkeys were healthy and lively throughout the experiment and their blood cultures remained sterile. The control monkey became ill shortly after inoculation with the Pneumococcus Type II culture and ran a typical course of lobar pneumonia with marked leucocytosis and pneumococcemia. All four monkeys were killed on the 7th day after the infecting dose was given. At autopsy none of the vaccinated monkeys showed any evidence of consolidation. In two



TEXT-FIG. 1, a to d. Active immunity against Pneumococcus Type II pneumonia following vaccination with Pneumococcus Type II vaccine. (a), (b), and (c) Monkeys 1, 2, and 3; each received 190 billion Pneumococcus Type II vaccine subcutaneously. (d) Monkey 4; control.

of the vaccinated animals a few small petechial hemorrhages were found in the lungs, but microscopic sections from the foci revealed only red blood corpuscles in the alveoli, with no exudation of leucocytes. Cultures from the hemorrhagic areas were sterile. The control monkey showed complete consolidation of the right lower lobe, with typical pneumonic exudate from which *Pneumococcus* Type II was isolated in pure culture.

Protection Tests.—Blood was taken from the three vaccinated monkeys just before their active immunity was tested to determine the protective power of their serums in mice. No protective action could be demonstrated against *Pneumococcus* Type II in any of the serums, even when doses as minute as 0.0000001 cc. of culture were used.

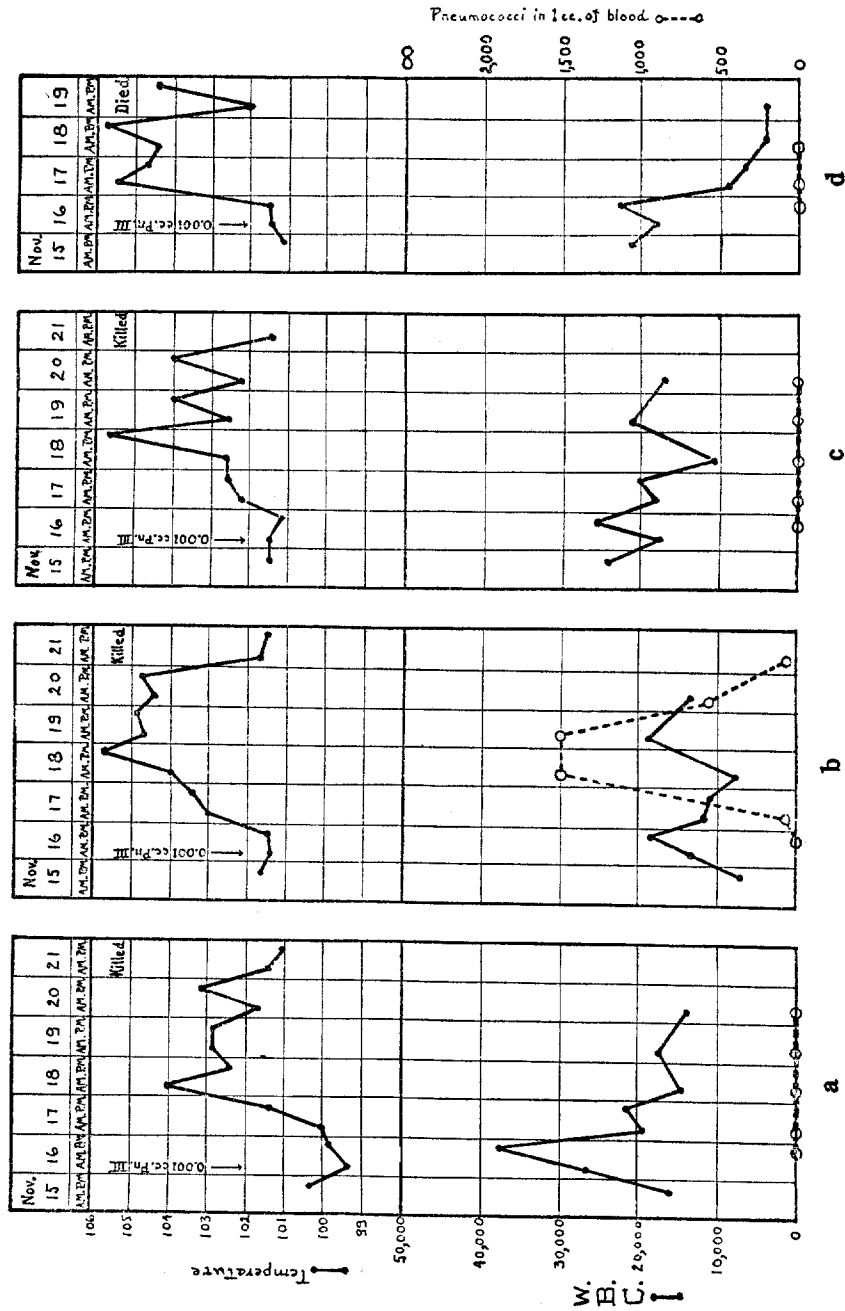
From this experiment it may be inferred that three subcutaneous injections of *Pneumococcus* Type II vaccine afford complete protection to monkeys against *Pneumococcus* Type II pneumonia.

Active Immunity against Pneumococcus Type III Pneumonia Following Three Subcutaneous Injections of Pneumococcus Type III Vaccine.

The next problem was to determine the effectiveness of prophylactic vaccination against *Pneumococcus* Type III pneumonia. The technique in the Type III experiments was similar in every respect to that employed in the *Pneumococcus* Type II experiments.

Experiment 2.—Oct. 18, 1921. Three *Cebus capucinus* monkeys, No. 5 (1,500 gm.), No. 6 (1,250 gm.), and No. 7 (1,432 gm.), received each 20 billion (0.5 cc.) *Pneumococcus* Type III vaccine subcutaneously. Oct. 25. Each monkey received 40 billion (1 cc.) *Pneumococcus* Type III vaccine subcutaneously. Nov. 1. Each monkey received 60 billion (1.5 cc.) *Pneumococcus* Type III vaccine subcutaneously. The monkeys developed small firm nodules at the sites of these injections, but no other reaction. Nov. 15 (2 weeks after the last injection of vaccine). Each of the three vaccinated monkeys received 0.001 cc. of an 18 hour broth culture of *Pneumococcus* Type III (Hughes) intratracheally. An unvaccinated control monkey, No. 8 (1,450 gm.), received the same amount of culture intratracheally.

The results of the experiment are shown in Text-fig. 2. All four monkeys showed a rise of temperature following the inoculation with *Pneumococcus* Type III culture, but in the vaccinated monkeys the temperature returned to normal, on the 5th or 6th day after inoculation, whereas the control monkey ran a high fever until death occurred on the 4th day. The vaccinated monkeys showed a moderate leucocytosis following the intratracheal inoculation. In the control monkey a marked leucopenia developed almost at once. One of the vaccinated monkeys, No. 6, had pneumococci in the blood; in the other two the blood cul-



TEXT-FIG. 2, a to d. Active immunity against Pneumococcus Type III pneumonia following vaccination with Pneumococcus Type III vaccine. (a), (b), and (c) Monkeys 5, 6, and 7; each received 120 billion Pneumococcus Type III vaccine subcutaneously. (d) Monkey 8; control.

tures remained sterile. Strangely enough, no pneumococci were found in the blood of the control monkey. The most significant thing in this experiment was the difference in appearance between the vaccinated monkeys and the control. The former remained lively and well during the entire period of observation. The control became very sick at once and died on the 4th day.

The vaccinated monkeys were killed on the 6th day. Autopsies showed normal lungs in Monkeys 5 and 7. Several small patches of interstitial pneumonia were found in the right lower lobe of No. 6. The control monkey had a frank consolidation of the right middle and lower lobes. Postmortem cultures were negative in Monkeys 5 and 7. Cultures from the right lower lobe of No. 6 yielded *Pneumococcus* Type III. Cultures from the control were overrun with *B. bronchisepticus*, but a few Gram-positive diplococci were seen in microscopic sections stained for bacteria.

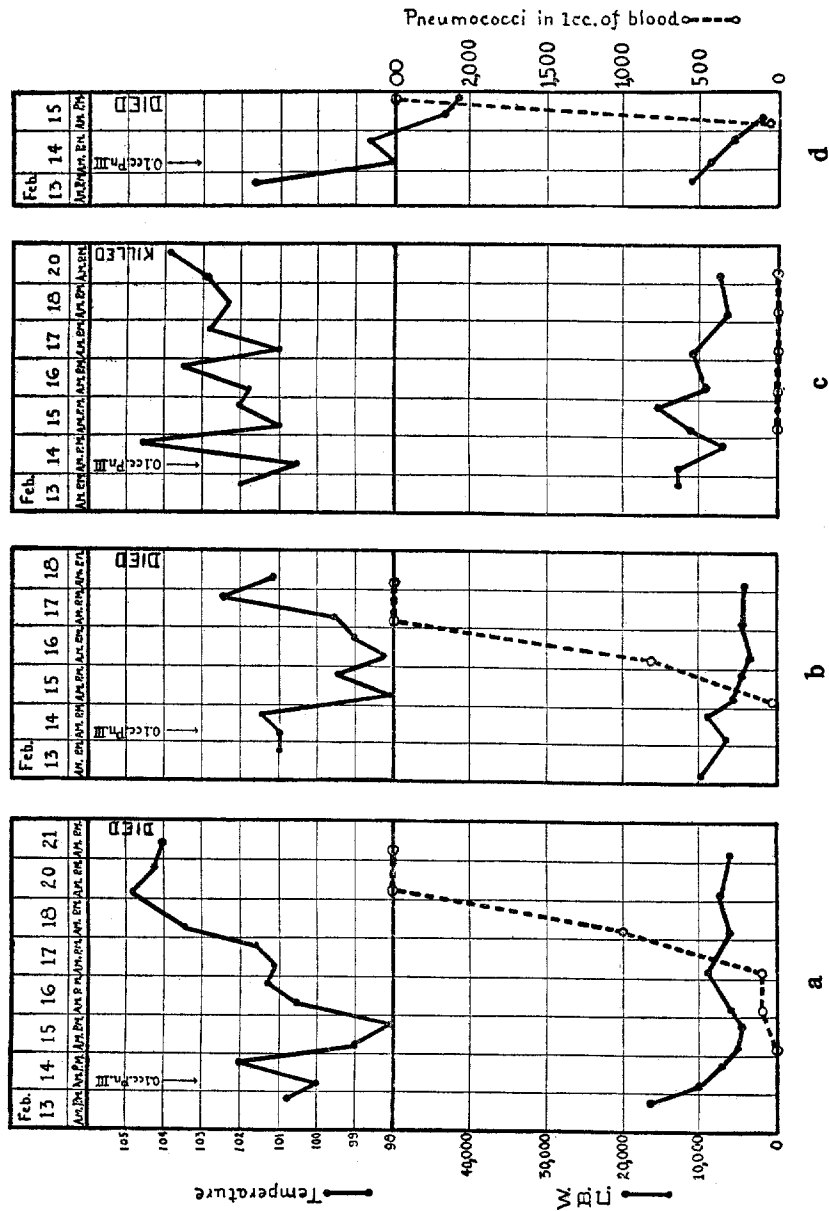
Protection Tests.—Blood from the three vaccinated monkeys showed no protective bodies against *Pneumococcus* Type III in mice.

In this experiment complete protection against *Pneumococcus* Type III was obtained in two of the vaccinated monkeys, partial protection in the third. It is hard to explain why these vaccinated monkeys, though immune to pneumonia, sometimes show a temporary rise in temperature following inoculation with the living culture. In some instances an acute bronchitis is excited which, however, does not develop into pneumonia. In others the fever may be the expression of a foreign protein reaction.

A second experiment was carried out with *Pneumococcus* Type III vaccine in the hope of obtaining complete protection in all the vaccinated monkeys. In this experiment larger doses of vaccine were employed.

Experiment 3.—Jan. 23, 1922. Three *Cebus capucinus* monkeys, No. 9 (1,328 gm.), No. 10 (1,360 gm.), and No. 11 (1,244 gm.), received each 1 billion (1 cc.) *Pneumococcus* Type III vaccine (Hughes) subcutaneously. Jan. 28. Each monkey received 80 billion (1 cc.) *Pneumococcus* Type III vaccine subcutaneously. Feb. 1. Each monkey received 120 billion *Pneumococcus* Type III vaccine subcutaneously. Feb. 13. No. 9 has developed a small ulcer at the site of the last inoculation. The other monkeys show only the usual small nodules. Feb. 14 (2 weeks after the third injection of vaccine). Each monkey received 0.1 cc. of an 18 hour broth culture of *Pneumococcus* Type III (Hughes) intratracheally. An unvaccinated monkey, No. 12 (1,840 gm.), received the same amount (0.1 cc.) of culture intratracheally.

The results are shown in Text-fig. 3. Monkey 11 remained lively and well. There was a temporary rise in temperature, but the leucocytes were normal and



TEXR-Fig. 3, a to d. Active immunity against Pneumococcus Type III pneumonia following vaccination with Pneumococcus Type III vaccine. (a), (b), and (c) Monkeys 9, 10, and 11; each received 200 billion Pneumococcus Type III vaccine subcutaneously. (d) Monkey 12; control.

the blood culture remained sterile. The other two vaccinated monkeys became ill, developed a leucopenia and heavy pneumococcemia and died, one on the 5th and the other on the 7th day of the disease. The control monkey died the day after inoculation with an overwhelming pneumococcemia.

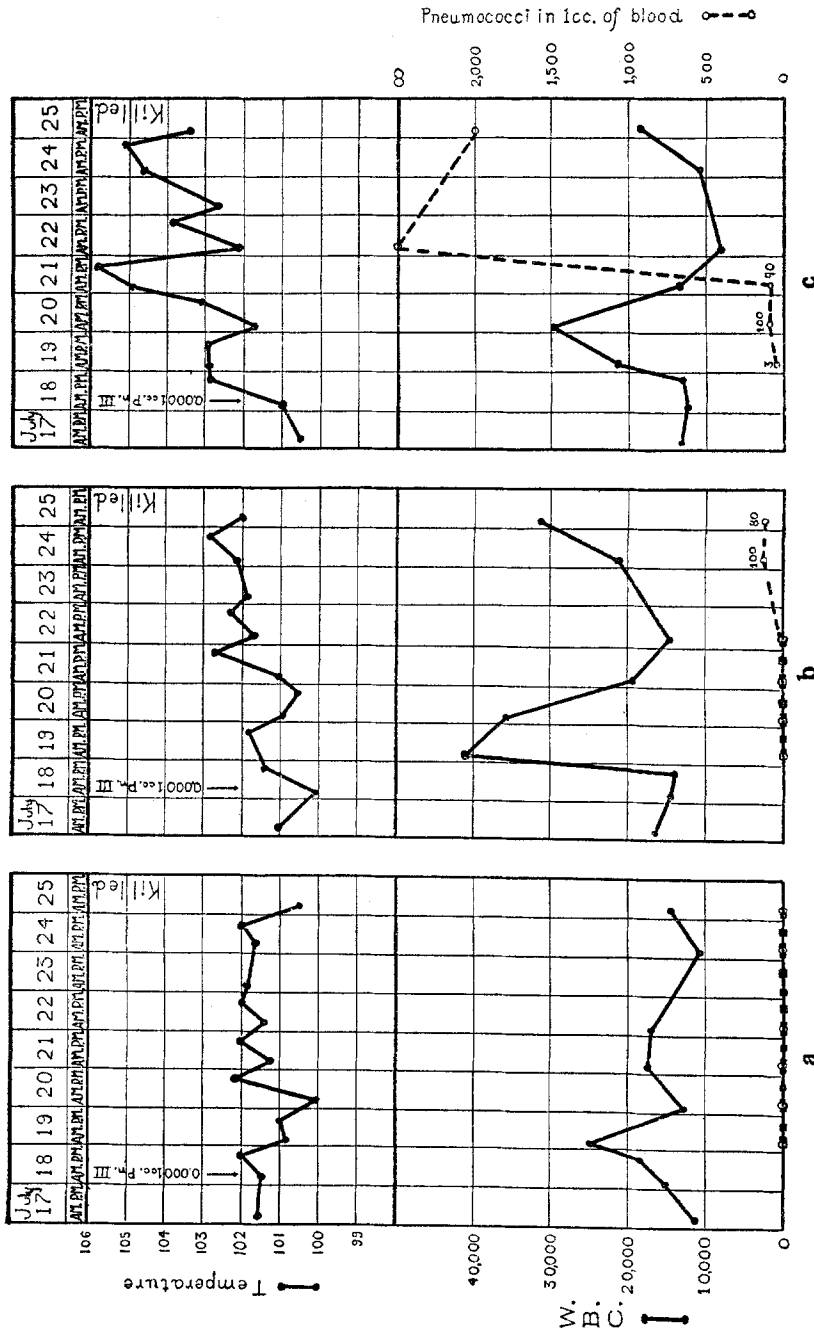
Autopsies were performed on all four monkeys. Monkey 11 showed a few petechial hemorrhages in the lungs, but no consolidation. Monkeys 9 and 10 both had lobar pneumonia, the former in the right lower lobe, the latter in both the right and left lower lobes. The control monkey, No. 12, showed an early lobar pneumonia in the right lower lobe and several partly healed foci of pulmonary tuberculosis. Postmortem cultures from the lungs and heart's blood of Monkeys 9, 10, and 12 yielded *Pneumococcus* Type III. Cultures from Monkey 11 were negative, except for a few colonies of *Pneumococcus* Type III which were found on cultures from the trachea.

In this experiment only one of the three vaccinated monkeys resisted infection with *Pneumococcus* Type III. The other two developed a *Pneumococcus* Type III pneumonia. A careful study of the protocols indicates that too large an infecting dose was used in this experiment. This is shown by the fulminating course which the infection pursued in the control. As it was, the two vaccinated monkeys that developed pneumonia lived several days longer than the unvaccinated monkey.

One more Type III experiment was undertaken, this time with another strain of pneumococcus.

Experiment 4.—June 16, 1922. Two *Cebus capucinus* monkeys, No. 13 (1,200 gm.) and No. 14 (1,550 gm.), received each 12 billion (1 cc.) *Pneumococcus* Type III (Bowen) vaccine subcutaneously. June 23. Each monkey received 50 billion (2 cc.) *Pneumococcus* Type III vaccine subcutaneously. June 30. Each monkey received 138 billion (2 cc.) *Pneumococcus* Type III vaccine subcutaneously. July 18 (2½ weeks after the last injection of vaccine). The two vaccinated monkeys and a control monkey, No. 15 (2,600 gm.), each received 0.001 cc. of an 18 hour broth culture of *Pneumococcus* Type III (Bowen) intratracheally.

Monkey 13 remained lively and well, but Monkey 14 became moderately sick. The control monkey, No. 15, became quite sick, and probably would have died had it not been killed on the 8th day. Text-fig. 4 shows the temperature, leucocyte, and blood culture curves. Monkey 13 showed no changes in temperature or leucocyte count, and the blood remained sterile. Monkey 14 developed no fever, but the leucocytes rose, and pneumococci appeared in the blood in moderate numbers on the 7th day of observation. The control monkey ran a high fever, the leucocytes rose to 29,600, and a heavy pneumococcemia appeared on the 4th day following inoculation.



TEXT-FIG. 4, a to c. Active immunity against Pneumococcus Type III pneumonia following vaccination with Pneumococcus Type III vaccine. (a) and (b) Monkeys 13 and 14; each received 200 billion Pneumococcus Type III vaccine subcutaneously. (c) Monkey 15; control.

All three monkeys were killed on the 8th day. No. 13 showed normal lungs; No. 14 presented several small patches of consolidation in both lower lobes. The control, No. 15, showed an interstitial pneumonia of the right lower lobe. Post-mortem cultures from the lungs and heart's blood of Monkey 13 were sterile. Monkey 14 and the control both yielded *Pneumococcus* Type III from the heart's blood and also from the consolidated areas in the lungs.

Protection Tests.—Protection tests with blood from the two vaccinated monkeys were carried out on mice, but no protective substance could be demonstrated in either monkey.

This experiment, like the two preceding ones with *Pneumococcus* Type III, was not entirely successful in that one of the vaccinated monkeys was not sufficiently immune to resist infection. In view of the difficulties experienced by previous workers in immunizing animals against *Pneumococcus* Type III, we had expected to meet with less success with Type III than with the other types. Altogether, eight monkeys were vaccinated against *Pneumococcus* Type III. Four of them resisted infection and four developed Type III pneumonia.

Active Immunity against Pneumococcus Type IV Pneumonia Following Three Subcutaneous Injections of Pneumococcus Type IV Vaccine.

Pneumococcus Type IV is really not a type but a heterogeneous group of pneumococci that do not fall into any one of the three fixed types. The so called Type IV strains rarely cross-agglutinate or cross-protect, but it seemed desirable to carry out one Type IV experiment on monkeys, using the same strain of pneumococcus for vaccine and for testing the immunity.

Experiment 5.—May 29, 1921. Three *Macacus syrichtus* monkeys, No. 16 (1,775 gm.), No. 17 (1,430 gm.), and No. 18 (1,410 gm.), received each 20 billion (0.5 cc.) *Pneumococcus* Type IV vaccine subcutaneously. June 4. Each monkey received 40 billion (1 cc.) *Pneumococcus* Type IV vaccine subcutaneously. June 11. Each monkey received 60 billion (1 cc.) *Pneumococcus* Type IV vaccine subcutaneously. June 28 (2½ weeks after the last injection of vaccine). Each monkey received 0.00001 cc. of an 18 hour broth culture of *Pneumococcus* Type IV intratracheally. At the same time, an unvaccinated control, No. 19 (2,300 gm.), received the same amount of culture intratracheally.

The results are shown in Text-fig. 5. The three vaccinated monkeys remained perfectly well. There was no noteworthy rise in the temperature or leucocyte count, and the blood cultures remained sterile. The control monkey showed a slight rise in temperature, the leucocytes jumped from 17,000 to 32,000 and the

blood cultures yielded Pneumococcus Type IV on the 3rd and 4th days of the disease. During this period the control was very quiet but did not appear acutely ill.

On the 6th day all four monkeys were killed. The three vaccinated monkeys were found to have normal lungs. The control monkey showed small patches of interstitial pneumonia in the right lower and the left upper lobes. Postmortem cultures from the lungs of the vaccinated monkeys were sterile. Cultures from the patches in the lungs of the control yielded Pneumococcus Type IV.

Protection Tests.—Serum was collected from the three immunized monkeys just before the immunity test was made, and protection tests against the homologous strain of Pneumococcus Type IV were carried out on mice. No protective bodies could be demonstrated in any of the three serums.

This experiment would have been more convincing if the control had shown a more extensive pneumonia. However, pneumonia was present in the control and absent in the three vaccinated monkeys,—evidence enough to show that active immunity could be established against Pneumococcus Type IV as well as against the three fixed types.

DISCUSSION.

It was reasonable to suppose that if monkeys could be immunized against Pneumococcus Type I, they could also be immunized against the other three types, for the pneumococci all belong to the same biological species and have almost identical morphological and cultural characteristics. It is only in respect to immunological traits that they vary among themselves.

It is more difficult to obtain a high immunity against Pneumococcus Type III than it is against the other types. It is possible that the thick capsule which characterizes this type may interfere in some way with the release of the antigen and the consequent stimulation of the mechanism which produces the immune substance. The same factor that prevents the development of a high active immunity may also be responsible for the high death rate in Type III pneumonia in man. In the one instance, killed pneumococci, in the other, living pneumococci, fail to stimulate a sufficiently sharp response by the immune mechanism.

Monkeys tolerate large doses of pneumococcus vaccine remarkably well. In the present study the local reactions were insignificant and constitutional reactions were slight or entirely absent.

In the experiments with Pneumococcus Type I, previously reported, three subcutaneous injections of monkeys with Pneumococcus Type I vaccine often stimulated the production of specific protective substance in the blood of the vaccinated monkeys. In a similar way, protective bodies could be demonstrated after three intravenous injections of Type I vaccine. In the experiments with Pneumococcus Types II, III, and IV, three subcutaneous injections of vaccine failed in every instance to produce protective substance in the blood, even when the monkey was immune to infection. Evidently it is more difficult to stimulate protective bodies against Types II, III, and IV than against Type I. Judging from experience with other animals, however, protective bodies against all types could be developed in monkeys if repeated injections of vaccine were administered either subcutaneously or intravenously.

CONCLUSIONS.

1. Three subcutaneous injections of Pneumococcus Type II vaccine confer on monkeys a complete immunity against experimental Pneumococcus Type II pneumonia. A similar protection can be bestowed on monkeys against Pneumococcus Type IV pneumonia by three subcutaneous injections of a vaccine prepared from the same strain of pneumococcus.

2. The subcutaneous injection of monkeys with three doses of Pneumococcus Type III vaccine confers a complete immunity against this type in only 50 per cent of cases (four out of eight monkeys vaccinated).

3. In spite of the immunity induced in monkeys by three subcutaneous injections of Pneumococcus Types II, III, and IV vaccine, specific protective bodies against the homologous types are not demonstrable in their serums when the vaccine is so administered.

BIBLIOGRAPHY.

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