STUDIES ON PNEUMOCOCCUS IMMUNITY.

I. ACTIVE IMMUNIZATION OF MONKEYS AGAINST PNEUMOCOCCUS TYPE I PNEUMONIA WITH PNEUMOCOCCUS TYPE I VACCINE.*

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In an article recently published on the results of prophylactic vaccination against pneumococcus pneumonia in monkeys, Cecil and Blake1 found that subcutaneous inoculation with Pneumococcus Type I vaccine in doses comparable to those employed in man did not protect monkeys against subsequent attacks of Pneumococcus Type I pneumonia, either spontaneous or experimental. Vaccination did, however, modify the course of the disease. Invasion of the blood stream by the pneumococcus was usually slight or absent in vaccinated animals, and the proportion of recoveries was considerably higher for vaccinated than for unvaccinated monkeys. In the experiments of Cecil and Blake, comparatively small doses of vaccine were used. In view of the failure, however, of small doses of vaccine to give complete protection against pneumonia, it seemed desirable to determine the effect of large doses of pneumococcus vaccine.

In the following experiments, three species of monkeys have been employed. Macacus rhesus was used in three of the experiments, Macacus syrichtus in one, and the Cebus capucinus in one. Macacus rhesus is less susceptible to pneumococcus infection than the other two species, and rarely if ever develops a true lobar pneumonia. The infection is more apt to be of the interstitial or confluent lobular type. The Philippine macaque (Macacus syrichtus) is the preferable animal for studying experimental pneumococcus pneumonia, as it develops a

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true lobar consolidation. At the time these experiments were carried out, however, it was not possible to obtain this species in any considerable number. *Cebus capucinus* occupies an intermediate position in respect to susceptibility to pneumococcus pneumonia. In some cases it may present a typical lobar consolidation, but, more often, this species, like the *rhesus*, develops only an interstitial or patchy consolidation, which is usually associated with a heavy pneumococcus septicemia.

**Methods.**

The vaccine employed for these experiments was prepared from a stock laboratory strain of Pneumococcus Type I. This organism was highly virulent, killing a mouse in quantities as small as 0.0000001 cc. of a 24 hour broth culture. The vaccine was prepared as follows: 18 to 24 hour glucose broth cultures were centrifuged, the sediment was washed with normal salt solution, and then resuspended in normal salt solution in such a concentration that 0.5 cc. equalled 20 billion pneumococci. The suspension was heated to 55° C. for 1 hour to kill the pneumococci, and 0.25 per cent tricresol added as a preservative. With one exception (Experiment 3), the same dosage of vaccine was employed in all the experiments. Three subcutaneous injections were given at intervals of 1 week, the first dose consisting of 20 billion pneumococci, the second of 40 billion, and the third of 60 billion. The injections were made in the abdominal wall, and caused very little local or general reaction.

The culture used for testing the immunity of the vaccinated monkeys was the same strain of Pneumococcus Type I from which the vaccine had been prepared. The method of producing experimental pneumonia in monkeys has been previously described by Blake and Cecil.² A small quantity of an 18-hour broth culture of pneumococcus is introduced with a Luer syringe through the skin into the trachea. In testing for resistance to pneumonia following the injection of pneumococcus vaccine, the intratracheal inoculations were made in most cases 2 to 3 weeks after the completion of vaccination. The dose of culture injected varied from 0.01 to 0.000001 cc. for each monkey. A further test of the immunity of each vaccinated monkey was made

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by withdrawing some blood just before intratracheal inoculation, and carrying out protection tests in mice with the serum of the monkey.

In most cases the monkeys died or were killed at the conclusion of the experiment. Complete autopsies were performed in each instance and microscopic sections studied from the various lobes of the lungs.

Active Immunity in Macacus rhesus Following Vaccination with Large Doses of Pneumococcus Type I Vaccine.

The first experiment with large doses of Pneumococcus Type I vaccine was carried out on rhesus monkeys.

Experiment I.—Oct. 21, 1919. Three Macacus rhesus monkeys (Nos. 1 to 3) received each 20 billion (0.5 cc.) Pneumococcus Type I saline vaccine subcutaneously. Oct. 22. Mild local reactions. Oct. 28. Each monkey received 40 billion (1 cc.) Pneumococcus Type I vaccine subcutaneously. Nov. 4. Each monkey received 60 billion (1.5 cc.) Pneumococcus Type I vaccine subcutaneously. Nov. 18. 2 weeks after last injection of vaccine, each of the three monkeys received 0.000001 cc. of an 18 hour broth culture of Pneumococcus Type I intratracheally. An unvaccinated control monkey (No. 12) received the same amount of culture intratracheally.

The results are shown in Text-fig. 1. The three vaccinated monkeys remained perfectly well, showed practically no change in temperature or leucocyte count, and had negative blood cultures throughout the period of observation. The control was sick for 3 days, developed a leucocytosis of 47,000, and had a transient pneumococcus septicemia. All four animals were killed on the 5th day following inoculation. The lungs in the vaccinated monkeys were entirely free from pneumonia. In the control a small patch of interstitial pneumonia was found in the left lower lobe. Cultures from the lungs and heart's blood were sterile in all the monkeys.

Protection Tests.—The serums from the three vaccinated monkeys were tested for specific protective bodies against Pneumococcus Type I. On November 18, just before intratracheal inoculation, the three vaccinated monkeys were bled for protection tests in mice. The serum of Monkey 1 developed a moderate amount of protective substance, protecting a mouse against 0.000001 cc. of Pneumococcus Type I culture. The serum of Monkey 2 protected mice against 0.0001 cc. of Pneumococcus Type I culture. The serum of Monkey 3 showed practically no protective power.
Text-Fig. 1, a to d. Active immunity against Pneumococcus Type I pneumonia following vaccination with Pneumococcus Type I vaccine. (a), (b), and (c) Monkeys 1, 2, and 3; each received 120 billion Pneumococcus Type I vaccine subcutaneously. (d) Monkey 12; control. Br.+., Broth culture positive for Pneumococcus Type I.
It is evident from this experiment that three large subcutaneous doses of Pneumococcus Type I vaccine conferred complete protection against pneumonia. The result, however, was unsatisfactory in one respect; the control developed only a small patch of interstitial pneumonia and was not seriously ill. The experiment was therefore repeated on the same species with larger infecting doses of Pneumococcus Type I culture.

*Experiment 2.*—Nov. 24, 1919. Three *Macacus rhesus* monkeys (Nos. 13 to 15) received each 20 billion (0.5 cc.) Pneumococcus Type I saline vaccine subcutaneously. Nov. 27. Small nodules at site of vaccine injection. Dec. 1. Each monkey received 40 billion (1 cc.) Pneumococcus Type I vaccine subcutaneously. Dec. 6. Small nodules at site of previous inoculation. In Monkey 13 the nodule has broken down and is discharging sterile pus. Dec. 7. Each monkey received 60 billion (1.5 cc.) Pneumococcus Type I vaccine subcutaneously. Dec. 17. Each monkey received 0.01 cc. of an 18 hour broth culture of Pneumococcus Type I intratracheally, and an unvaccinated control monkey (No. 21) received the same amount (0.01 cc.) of culture intratracheally.

The results are shown in Text-fig. 2. The three vaccinated monkeys remained lively and well, and showed no noteworthy changes in temperature or leucocyte count. The blood remained sterile in the three vaccinated monkeys. The control monkey developed the signs and symptoms of pneumonia and recovered by crisis on the 6th day. One of the vaccinated monkeys (No. 15) and the control monkey were killed. The vaccinated monkey showed normal lungs. The control showed a resolving pneumonia of the interstitial type involving the right upper, middle, and lower lobes and the left upper lobe. Cultures taken at autopsy from the lungs and heart's blood of both monkeys were sterile.

*Protection Tests.*—Blood was taken from the veins of the three vaccinated monkeys just previous to the intratracheal inoculations for the purpose of determining the protective power of their serums. In spite of the high degree of active immunity conferred by the vaccine, none of the serums showed any protection for mice.

This experiment corroborated the findings in Experiment 1 and indicates that three large subcutaneous injections of Pneumococcus Type I vaccine confer complete protection against the homologous type of pneumonia.
TEXT-FIG. 2, a to d. Active immunity against Pneumococcus Type I pneumonia following vaccination with Pneumococcus Type I vaccine. (a), (b), and (c) Monkeys 13, 14, and 15; each received 120 billion Pneumococcus Type I vaccine subcutaneously. (d) Monkey 21; control.
It should be noted that in these experiments, as in the previously reported experiments of Cecil and Blake, the amount of protective substance in the serum of vaccinated monkeys varies widely with different individuals and apparently bears no close relation to local immunity in the lungs, a phenomenon which appears to be fairly constant following vaccination.

Active Immunity Following Intravenous Vaccination with Small Doses of Pneumococcus Type I Vaccine.

In order to determine the effect of intravenous injections of pneumococcus vaccine, three rhesus monkeys were injected intravenously with small doses of Pneumococcus Type I vaccine and their immunity was tested by intratracheal inoculation of virulent pneumococci as in the preceding experiments.

Experiment 3.—Oct. 27, 1919. Three Macacus rhesus monkeys (Nos. 4, 5, and 10) received each 300 million Pneumococcus Type I saline vaccine intravenously. Nov. 3. Each monkey received 400 million Pneumococcus Type I vaccine intravenously. Nov. 4. Monkeys lively and well. Nov. 10. Each monkey received 800 million Pneumococcus Type I vaccine intravenously. Nov 11. Animals lively and well. Nov. 17. Each monkey received 1,600 million Pneumococcus Type I vaccine intravenously. Dec. 2. Each monkey received 0.01 cc. of an 18 hour broth culture of a Pneumococcus Type I intratracheally. An unvaccinated control monkey (No. 17) received the same amount (0.01 cc.) of culture intratracheally.

The results are shown in Text-fig. 3. The three vaccinated monkeys remained lively and well. Monkey 10 had a sharp leucocyte reaction but showed no other signs of infection. The control monkey became ill and showed symptoms of pneumonia. One of the vaccinated monkeys (No. 10) and the control monkey (No. 17) were killed. Monkey 10 showed an acute bronchitis but no pneumonia. The control monkey presented a complete consolidation of the right upper lobe, and, microscopically, resolving pneumonia of the interstitial type was demonstrated. Cultures taken at autopsy from the lungs and heart's blood of these two monkeys were in both cases sterile.

Protection Tests.—The vaccinated monkeys were bled previous to the intratracheal inoculations in order to test the protective power of their serums. Monkey 10 showed a high degree of protective
Text—Fig. 3, a to d. Active immunity against Pneumococcus Type I pneumonia following vaccination with Pneumococcus Type I vaccine. (a), (b), and (c) Monkeys 4, 5, and 10; each received 3 billion Pneumococcus Type I vaccine intravenously. (d) Monkey 17; control.
substance in its serum which protected mice against 0.0001 cc. of Pneumococcus Type I culture; in Monkey 4 there was a small amount of protective substance, the mouse receiving 0.0000001 cc. of Pneumococcus Type I surviving. The serum of Monkey 5 showed no protective power at all.

This experiment demonstrates that small doses of Pneumococcus Type I vaccine injected intravenously confer an immunity equivalent to that obtained by large doses of pneumococcus vaccine administered subcutaneously. This is in harmony with the observations of many immunologists that immunity can be obtained more readily by intravenous than by subcutaneous inoculations.

Active Immunity in Philippine Monkeys Following Vaccination with Large Doses of Pneumococcus Type I Vaccine.

In view of the fact that most of the experiments carried out by Cecil and Blake in their study of prophylactic vaccination against pneumonia in monkeys were performed on *Macacus syrichtus*, it seemed desirable to control the result obtained in the above described experiments by an experiment on Philippine monkeys.

Experiment 4.—Feb. 27, 1920. Two Philippine monkeys (Nos. 34 and 35) received each 20 billion (0.5 cc.) Pneumococcus Type I saline vaccine subcutaneously. Feb. 28. Monkeys lively and well. Mar. 5. Each monkey received 40 billion (1 cc.) Pneumococcus Type I vaccine subcutaneously. Mar. 12. Each monkey received 60 billion (1.5 cc.) Pneumococcus Type I vaccine subcutaneously. Mar. 30. Each monkey received 0.000001 cc. of an 18 hour broth culture of Pneumococcus Type I intratracheally. An unvaccinated control monkey (No. 36) received the same amount (0.000001 cc.) of culture intratracheally.

The results are shown in Text-fig. 4. The two vaccinated monkeys remained lively and well. Their blood remained free from bacteria and the temperature and leucocytes showed no significant variation. The control monkey developed pneumonia and an overwhelming pneumococcus septicemia and died on the 5th day of the disease. The two vaccinated monkeys were killed on the 5th day following inoculation. Monkey 34 showed several clusters of small gray tubercles but there was no pneumonic consolidation. The presence of tuberculosis in this monkey explains the morning and evening varia-
tions in temperature (Text-fig. 4). Monkey 35 also showed a patch of encapsulated tuberculosis in the left upper lobe but there was no fresh consolidation. The control monkey showed an early pneumococcus pneumonia (stage of engorgement), involving the right and left lower lobes. Cultures at autopsy from the lungs and heart’s blood of the vaccinated monkey were sterile. Films from the tuberculous foci showed tubercle bacilli. Cultures from the lungs and heart’s blood of the control monkey gave Pneumococcus Type I.
Protection Tests.—The serums of the two vaccinated monkeys were tested for protective substances. Monkey 34 showed a definite amount of protective substance in its serum, the mice receiving 0.0000001 and 0.00001 cc. of Pneumococcus Type I both surviving. With the serum from Monkey 35 only one mouse survived—the one receiving 0.0000001 cc. of culture.

This experiment shows that even in the case of the Philippine macaque, a monkey peculiarly susceptible to pneumonia, an adequate immunity against Pneumococcus Type I pneumonia may be obtained by the administration of three large subcutaneous injections of Pneumococcus Type I vaccine.

Active Immunity in Cebus capucinus Following Vaccination with Three Large Doses of Pneumococcus Type I Vaccine.

A final test of Pneumococcus Type I vaccine was carried out on the small South American ringtail. This species was being used for other pneumococcus experiments and it seemed desirable to determine whether an efficient immunity against pneumococcus pneumonia could be obtained in this species by means of three subcutaneous inoculations of pneumococcus vaccine.

Experiment 5.—Oct. 29, 1920. Two Cebus capucinus monkeys (Nos. 82 and 83) received each 20 billion (0.5 cc.) Pneumococcus Type I saline vaccine subcutaneously. Nov. 3. Small nodules at site of previous inoculation. Nov. 4. Each monkey received 40 billion (1 cc.) Pneumococcus Type I vaccine subcutaneously. Nov. 11. Each monkey received 60 billion (1.5 cc.) Pneumococcus Type I saline vaccine subcutaneously. Nov. 26. Each monkey received 0.0001 cc. of a 24 hour broth culture of Pneumococcus Type I intratracheally. An unvaccinated control monkey (No. 86) received the same amount (0.0001 cc.) of culture intratracheally.

The results are shown in Text-fig. 5. Monkey 82 remained lively and well and showed no signs of infection. Monkey 83 remained well until the 4th day following inoculation, when he unfortunately contracted distemper. An epidemic of this disease had broken out among the stock monkeys and, in spite of the fact that the subjects under experiment were isolated in separate cages, Monkey 83 contracted the disease and was quite ill when he was killed on the 5th day following inoculation. Autopsy showed lobar pneumonia of the
right middle and left lower lobes, and *Bacillus bronchisepticus* was obtained in pure culture from the lungs. There was no evidence of a pneumococcus infection at any time during the experiment. Monkey 82 was also killed on the 5th day following inoculation and the

Text-Fig. 5, a to c. Active immunity against Pneumococcus Type I pneumonia in Capuchin monkeys following vaccination with Pneumococcus Type I vaccine. (a) and (b) Monkeys 82 and 83; each received 120 billion Pneumococcus Type I vaccine subcutaneously. (c) Monkey 86; control.

lungs were found free from consolidation. Monkey 86, the control, died with an overwhelming pneumococcus septicemia on the 3d day following inoculation. At autopsy the lungs showed engorgement and patches of incipient pneumonia in the right middle and left lower
lobes, and cultures taken from the lungs and from the heart's blood showed a pure growth of Pneumococcus Type I.

**Protection Tests.**—Blood was taken from the two vaccinated monkeys just before the intratracheal inoculations and was tested on mice for the presence of specific protective substance against Pneumococcus Type I. Contrary to the usual experience, no protective bodies could be demonstrated in either of the vaccinated monkeys.

This experiment corroborates the results obtained in the previous experiments and shows that in Capuchin monkeys three large subcutaneous injections of pneumococcus Type I vaccine confer a high degree of active immunity against the homologous type of pneumonia.

**DISCUSSION.**

The experiments reported in this paper establish a fact which has at least a theoretical importance. They demonstrate that monkeys can be completely protected against Pneumococcus Type I pneumonia by means of three subcutaneous injections of Pneumococcus Type I vaccine, provided sufficiently large doses are administered. The total dosage of vaccine employed in these studies averages about ten times as large as that used by Cecil and Blake in their unsuccessful attempts to vaccinate monkeys against pneumonia. It may be argued that the dosage employed in the present study could not be made use of in man without exciting severe local and general reactions. It is doubtful, however, whether such large doses would be necessary in the case of man. The average, healthy human being probably possesses more resistance to pneumonia than the average monkey by reason of long continued exposure to pneumococcus infection. Furthermore, when monkeys are infected artificially by intratracheal inoculation, a large number of pneumococci (400 to 1,000,000) attack the organism at once, whereas, in spontaneous infection in man, the number of pneumococci attacking at the onset of pneumonia is probably very small. It would be desirable, however, to administer large doses of pneumococcus vaccine to man if the toxic element in the vaccine could be removed without injury to the antigenic element.

The results of the protection tests carried out with the serum of the vaccinated monkeys are in harmony with the results reported in the studies of Cecil and Blake. Protective substances may or may
not be present in the serum of vaccinated monkeys and appear to play little or no part in active immunity to pneumococcus infection in the lung itself.

The intravenous administration of pneumococcus vaccine induces a higher immunity than subcutaneous injection. The intravenous method however would be much more time-consuming and this would be an important consideration where large groups of individuals were to be vaccinated.

CONCLUSIONS.

1. The subcutaneous inoculation of monkeys with three large doses of Pneumococcus Type I vaccine confers on them a complete immunity against experimental Pneumococcus Type I pneumonia.

2. The intravenous inoculation of small doses of Pneumococcus Type I vaccine also confers complete immunity against the homologous type of pneumonia.

3. Specific protective bodies may or may not be present in the serum of monkeys vaccinated against Pneumococcus Type I. There appears to be no intimate relation between active immunity against pneumonia and the presence or absence of protective substances in the serum of the vaccinated animal.