

SUSCEPTIBILITY OF RABBITS TO INFECTION BY THE  
INHALATION OF VIRULENT PNEUMOCOCCI.

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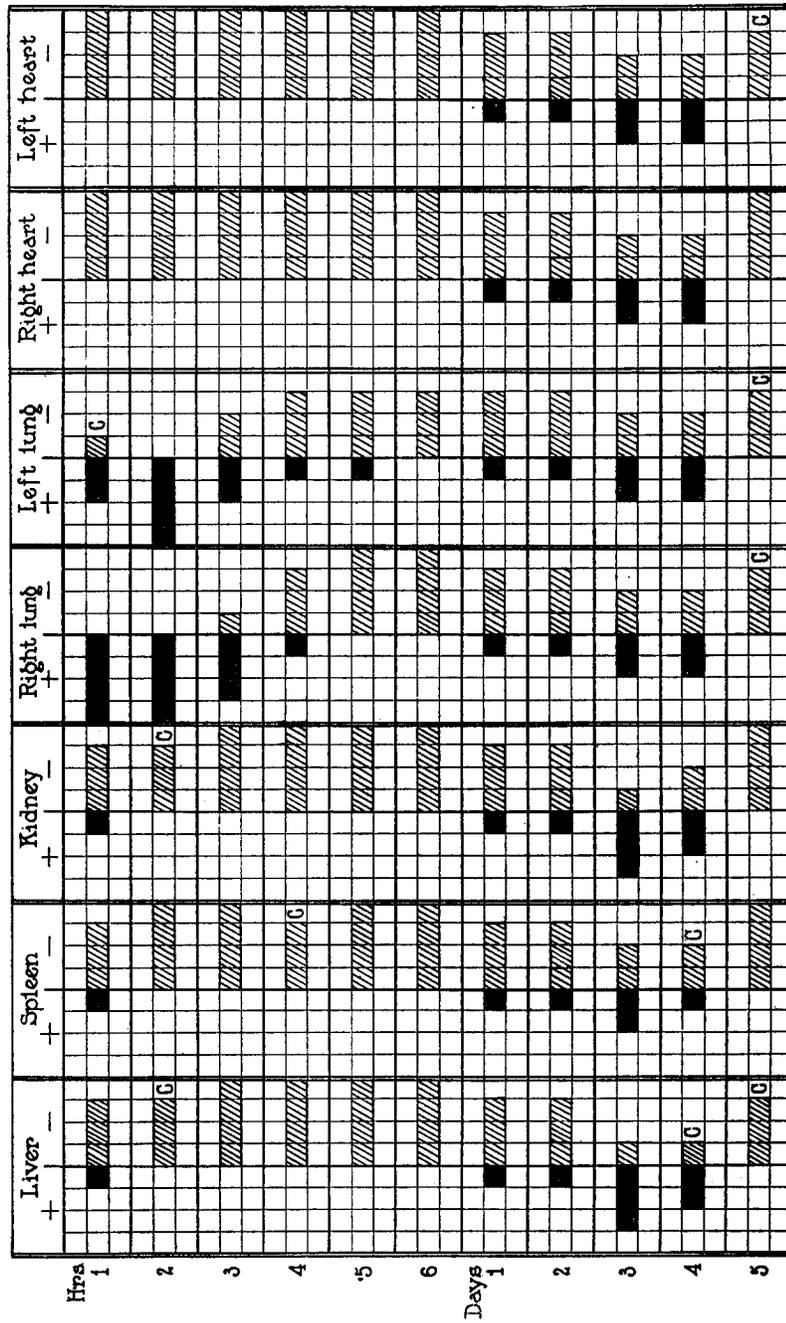
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In preceding papers (1, 2) it has been shown that following inhalation, virulent pneumococci generally disappear from the lungs of normal mice within a few hours, that infection rarely occurs, and that in only a few instances does death result from septicemia. It has further been shown that a high degree of active immunity may develop in mice following repeated inhalations of living pneumococci. However, mice are not suitable for tracing serologically the varying degrees of immunity developed by the repeated inhalations. Since rabbits may be easily and repeatedly bled, they were chosen for this work.

In the present paper are reported (1) the distribution of pneumococci in the organs of rabbits following spraying with pneumococci, (2) the occurrence of positive blood cultures, (3) the mortality of the exposed rabbits, and (4) the pathology of the pulmonary lesions in the rabbits which died. The immunity following upon the inhalation of pneumococci will be dealt with in a subsequent paper.

*Method.*

The rabbits were placed in a large spray chamber similar to that already described (2) and exposed to a spray of virulent Type I pneumococci. 150 cc. of an 18 hour broth culture was used for each spraying. The animals were exposed at 10 day intervals. Before each spraying, a sample of blood was obtained by bleeding the animal from the ear vein. At varying intervals after the spraying, blood cultures were taken from the ear vein. In the case of the animals which were killed and autopsied, broth cultures were made from both sides of the heart, and small pieces of the periphery of both lungs, and the kidney, liver, and spleen were placed directly in tubes of broth. Heart's blood cultures alone were made from the rabbits which died. All cultures were plated on blood agar for further identification. The animals killed with chloroform were immersed in a solution of lysol and opened with sterile instruments.



TEXT-FIG. 1. Distribution of pneumococci in organs following spraying. The black squares indicate organs in which cultures showed pneumococci to be present, the cross-hatched squares represent organs in which cultures remained sterile.

*Distribution of Pneumococci in the Organs.*

In Text-fig. 1 is shown the distribution of pneumococci in the organs of 44 sprayed rabbits which were killed at intervals after spraying. It will be seen from the figures for the 24 rabbits killed in from 1 to 6 hours that pneumococci were generally recovered from the periphery of the lungs for the first 3 hours after the spraying but that later they rapidly disappeared. In only 1 rabbit were pneumococci recovered from the liver, spleen, and kidney. The heart's blood cultures from this animal remained sterile. Of the remaining 20 rabbits killed from 1 to 5 days following exposure, 6, or 30 per cent, showed a general pneumococcus septicemia. In 1 rabbit killed on the 3rd day, pneumococci were recovered only from the liver and kidney. This experiment shows how readily normal rabbits may become infected following inhalation of pneumococci. It further indicates that a transient pneumococcus septicemia may be present following spraying. In order to gain further knowledge of this point daily blood cultures were taken from the ear vein of a few rabbits.

*Blood Cultures.*

From Table I it is seen that following a first exposure positive blood cultures were obtained in 15 out of 44 rabbits. Of the 15 rabbits which developed a septicemia 5, or 33 per cent, continued to have a positive blood culture until death. The other 10 rabbits had merely a transient septicemia and survived. In some instances the septicemia could be detected during the first 24 hours but in others the organisms were first recovered as late as the 6th or 7th day.

*Mortality of Rabbits Following Exposure to a Pneumococcus Spray.*

Table II shows the number of normal rabbits which succumbed during the course of spraying with virulent pneumococci. From this table it is shown that out of 231 rabbits 80, or 38 per cent, died of a pneumococcus septicemia following their first exposure. Although some rabbits escaped infection at the first exposure, 7, or 6 per cent, died after the second spraying, and an occasional rabbit died after the third and fourth exposures. Of the 112 rabbits which died, the blood of 13, or 11 per cent, was sterile. These rabbits probably succumbed

TABLE I.

*Days on Which Positive Blood Cultures Were Obtained Following a Single Exposure to a Pneumococcus Spray.*

Rabbit No.	Days. 1	2	3	4	5	6	7	8	9	Course
1	c	c	-	-	-	+				Survived
2	-	+	+	-	-					"
3	+	+	+	+						Died 6th day
4	+	-	-	-	-	-				Survived
5	-	+	c	c		+				Died 7th day
6	-	+	+	c	c					" " "
7	-	-	-	c	+		-			Survived
8	+		c			-				"
9	-	-	-	+						Died 6th day
10	-	c	+	+						" 5th "
11	+	c	-	-	-					Survived
12	+	+	-		-	-	-	-	-	"
13	+	-	-		-	-	+	c	-	"
14	-	-	-		-	-	+	-	-	"
15	-	-	-		c	-	+	-	-	"

Blood cultures in 29 rabbits were negative.

A cross indicates a positive culture of pneumococcus, a dash a sterile culture, and "c" a contaminated culture.

TABLE II.

*Fate of Rabbits\* after One to Ten Exposures to Inhalations of Pneumococci.*

No. of times exposed to virulent pneumococci	No. of rabbits exposed	No. dying	Pneumococcus recovered from heart's blood	Pneumococcus not recovered from heart's blood
1	231	86	80	6
2	111	9	8	1
3	98	11	9	2
4	71	4	2	2
5	50	1	-	1
6	39	1	-	1
7	34	-	-	-
8	31	-	-	-
9	31	-	-	-
10	27	-	-	-

\* The discrepancy between the total numbers and the sum of the numbers in the individual groups is due to the fact that certain of the animals that survived were used for intraperitoneal tests or for other experiments.

to suffocation from being enclosed in the spray boxes too long. This technical error was corrected in later experiments. The table also brings out the fact that the greatest number of deaths occurred after the first exposure. After each successive spraying the mortality tended to lessen until after the fourth exposure no animals succumbed. In other words, those rabbits which were most susceptible to pneumococcus died early. As will be shown in a subsequent paper, the surviving rabbits were those which not only had the greatest natural resistance but also had acquired a certain degree of immunity as a result of repeated exposures to pneumococcus.

The interval elapsing between the time of spraying and death from pneumococcus septicemia is shown in Table III. From this it is seen that the greatest number of animals died within the first 7 days fol-

TABLE III.

*Number of Days Elapsing between Spraying and Death of Rabbits from Pneumococcus Septicemia.*

	Days..1	2	3	4	5	6	7	8	9	10
Spray I.....	3	12	16	8	14	12	8	5	1	1
" II.....	—	2	—	3	1	—	—	—	1	1
" III.....	2	—	2	—	—	1	2	2	—	—
" IV.....	—	—	1	—	—	1	—	—	—	—

lowing the initial exposure, although an occasional rabbit survived longer. The time of death of the rabbits which had survived the first exposure is much more diverse, and these rabbits have a tendency to die after a longer interval.

#### *Pathology.*

In no instance was there any gross evidence of pneumonia, although serous and serofibrinous pleurisy and serofibrinous pericarditis were common. A histological examination was made of the lungs of 77 rabbits which died of pneumococcus septicemia following spraying. The lungs appeared normal in 20. Congestion and pleurisy were noted in 7 instances, edema in 25, congestion in 38, and hemorrhagic extravasation into the alveoli in 19. In only 1 instance was there any histolog-

ical evidence of an attempt at localization of the infection. The lungs of this rabbit which died on the 4th day following the first exposure showed an interstitial inflammation (3).

#### DISCUSSION.

From the foregoing experiments it appears that rabbits are even more susceptible than mice to infection with pneumococci following inhalation. For a period of 3 hours after spraying the organisms may be demonstrated at the periphery of the lungs. It is unfortunate that their subsequent course deeper into the lungs cannot be followed. But the great number of other organisms which normally inhabit the bronchi of rabbits, as Jones (4) has shown, renders cultural studies of the lungs difficult. Histological detection of pneumococci in the tissues is also unreliable when the organisms are present only in small numbers.

In all probability the majority of the organisms are destroyed either when they first localize or deeper in the lung tissue by the endothelial leucocytes or by polymorphonuclear leucocytes. In any case a few organisms occasionally filter through into the blood stream. Gaskell (5) believes that invasion of the blood stream by pneumococci probably always takes place in the early stages of an air-borne infection of the lung. The actual invasion of the blood stream must be much more common than is supposed. Not only are there probably few organisms free at any one time in the circulating blood but these few bacteria may even be within leucocytes and not in reality multiplying in the blood.

The occasional organisms which reach the blood are probably rapidly filtered out of the blood stream by the organs and locally destroyed. It is evident, however, that following the apparent disappearance of the pneumococci from the periphery of the lungs, they again appear, this time in the blood, and as a result may be recovered from all the organs. The point of this reinvasion is as yet uncertain.

Following the initial spraying with pneumococci the majority of susceptible rabbits die of an overwhelming septicemia without any attempt on the part of the body to localize the infection.

## CONCLUSIONS.

1. Rabbits are very susceptible to infection by inhalation of Type I pneumococci.
2. When rabbits are exposed to a pneumococcus spray the bacteria readily penetrate into the lower respiratory tract. The pneumococci which reach the periphery of the lungs as a result of this procedure usually disappear within a few hours but a generalized and fatal septicemia frequently later appears. Pneumococci may then be recovered from the periphery of the kidney, liver, and spleen. In the animals which die pleurisy and pericarditis are common but pneumonia does not occur.
3. Rabbits may recover from pneumococcus septicemia.

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