EXPERIMENTAL PNEUMONIA IN MICE FOLLOWING THE INHALATION OF STREPTOCOCCUS HÆMOLYTICUS AND OF FRIEDLÄNDER'S BACILLUS.

By ERNEST G. STILLMAN, M.D., and ARNOLD BRANCH, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

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In preceding papers (1) it has been shown that the rate at which inhaled bacteria disappeared from the lungs varied with the species of microorganism employed. *Streptococcus hæmolyticus* may be quite regularly recovered from the lungs of normal mice for a period of 6 hours following inhalation, and occasionally may persist as long as 7 days. Following exposure a large proportion of normal mice die of a generalized streptococcus infection. *Bacillus influenzæ* may be regularly recovered from the lungs of normal mice for 24 hours after spraying, while pneumococci generally disappear within a few hours following inhalation and rarely give rise to a generalized infection. In intoxicated mice, however, pneumococci persist in the lungs for a longer period, and a fatal septicemia frequently results, a course of events which resembles that occurring when normal mice are sprayed with *Streptococcus hæmolyticus*. The non-immune mice which die following exposure while alcoholized to an atmosphere containing pneumococci in suspension rarely show any localization of the infection in the lung. Localization of the pneumococcus infection in the lungs only occurs in partially immunized mice (2).

In the present paper are reported (1) a further study of the rate of disappearance of inhaled hemolytic streptococci from the lungs of mice and of the pneumonia produced by them, (2) a comparative study made with *Bacillus friedlænderi*, and (3) a comparison of the mortality and incidence of pneumonia in the mice following inhalation of pneumococci, hemolytic streptococci, and *Bacillus friedlænderi*.

EXPERIMENTAL.

Method.—White mice were used as test animals. They were allowed to inhale an atmosphere containing a fine bacterial mist produced by spraying a culture of the organism to be studied. The organisms used were virulent *Streptococcus hæmolyticus* and *B. friedlænderi*. The animals were placed in the spray chamber previously described (1) and 50 cc. of an 18 hour broth culture of the test organism were sprayed into it. As a rule, the mice were removed from the spray box after an exposure of 1 hour and placed in glass jars in batches of ten. At daily intervals following spraying, three mice were killed by chloroform anesthesia and after immersion in a solution of lysol were autopsied with sterile instruments. As routine, a piece of the lung and a few drops of heart's blood were separately cultured in plain broth. The positive cultures were plated on blood agar for further identification. All sprayed mice were kept under observation for a period of 30 days following exposure.

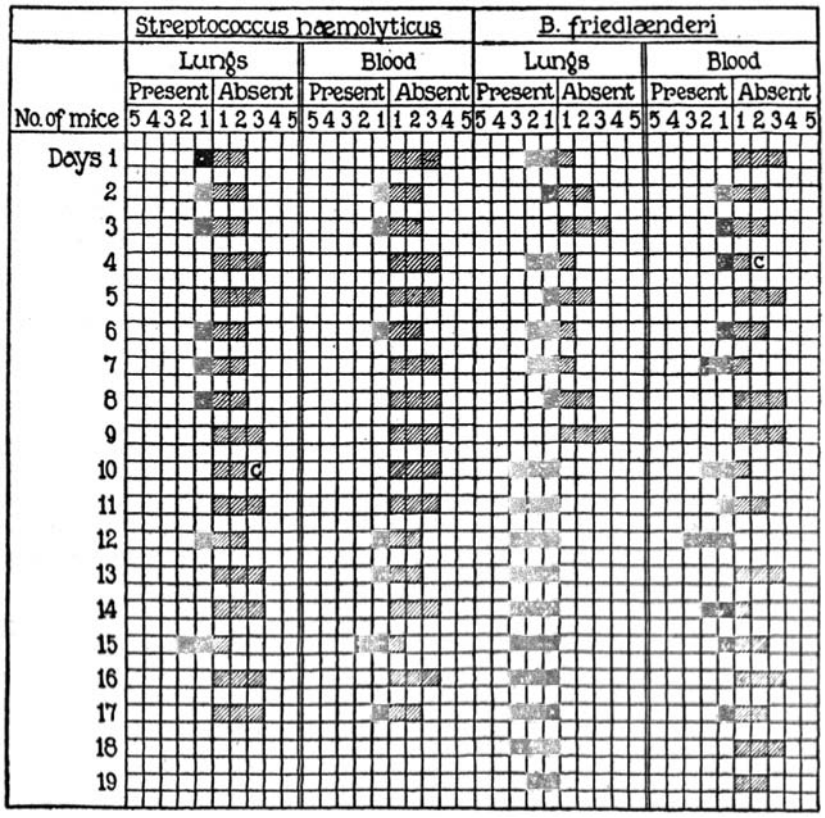
Persistence of Streptococcus hæmolyticus in the Lungs of Mice Following Inhalation.—Text-fig. 1 shows in graphic form the results obtained by culturing the lungs and heart's blood of 51 mice killed at daily intervals for 17 days following spraying.

Each black square represents a single mouse from which a positive culture was obtained, either from the lungs or heart's blood. Each cross-hatched square represents a single mouse from which the test organisms were not recovered. In a few instances, especially in the cultures from the lungs, the identification of the sprayed organism was not possible because of contamination by other bacteria. All lung or blood cultures so contaminated are indicated in the text-figures by the letter *c*.

It will be seen that the recovery of *Streptococcus hæmolyticus* proved quite irregular. The organisms may be recovered as late as the 17th day after exposure. As a rule both the lung and blood cultures were positive, but in two instances the organism was recovered only from the lungs, and in two other animals from the blood only. If more than one lobe had been cultured, the number of positive lung cultures would probably have been greater. It is difficult to explain, nevertheless, a negative lung culture in the presence of a positive blood culture.

Tests for Contact Infection.—The above experiment shows that hemolytic streptococci may persist in the lungs of mice for long periods following inhalation. Since the ability to recover the bacteria

from the lungs might be accounted for by cross-infection from animals confined in the same jar rather than by the persistence of the organisms originally inhaled, the following experiment was planned to determine the actual case.



TEXT-FIG. 1.

Forty mice were sprayed with hemolytic streptococci. Twenty were then placed in separate jars, and the remaining twenty were equally divided between two jars. The mice were kept under observation for a period of 30 days. In the latter group, *i.e.* the animals which were not isolated, fourteen of the twenty mice died. Hemolytic streptococci were recovered from the heart's blood in nine instances, that is, in 45 per cent of the number exposed. In the case of the isolated animals fifteen died and *Streptococcus hæmolyticus* was recovered from the heart's blood in thirteen, or 65 per cent, of the twenty isolated mice.

From this experiment it would appear that contact infection played no part in the mortality from streptococcus septicemia since that was even greater among the animals separately isolated.

Infection with Streptococcus hæmolyticus Following Inhalation.—It has been shown above that *Streptococcus hæmolyticus* may persist in the lungs of mice for a period of 17 days after inhalation and that cross-infection apparently will not account for this. It seemed advisable, therefore, to inquire whether actual infection of the lung occurs in these animals. A total of 216 normal mice were exposed to a spray containing hemolytic streptococci, and kept under observation for a period of 30 days. Of this number 127 died, and hemolytic streptococci were recovered from the heart's blood in 108, that is to say in 50 per cent infection had occurred. In 30 of these animals definite consolidation in the lung was present on gross inspection. A detailed discussion of the pathological lesions will be given in a subsequent paper.

Persistence of Bacillus friedlænderi in the Lungs of Mice Following Inhalation.—Since *Bacillus friedlænderi* is an organism which infects the respiratory tract and occasionally causes pneumonia in man an attempt has been made to determine the length of time these organisms persist in the lungs of susceptible animals exposed to infection by the inhalation method.

In Text-fig. 1 is shown the number of positive cultures obtained from the lungs or heart's blood of 56 normal mice killed at daily intervals during a period of 19 days following inhalation of *Bacillus friedlænderi.* It is seen that while this organism is recovered only irregularly in the mice killed during the first 9 days following exposure, it is invariably present in the lungs after the 9th day.

Tests for Contact Infection.—From the preceding experiment it is evident that *Bacillus friedlænderi* persists in the lungs of mice for a number of days following inhalation. To learn whether the organisms recovered during this period actually represent a persistence of the bacteria originally acquired, or whether following the initial exposure reinfection occurs as the result of contact infection the following experiment was carried out.

Thirty-eight mice were sprayed with a culture of *B. friedlænderi.* Nineteen of these were placed in individual jars and the remaining nineteen were divided

into two groups and placed in two jars and observed for 30 days. All the animals in the individual jars died of a *B. friedlænderi* septicemia and only six of the nineteen mice in the group jars. Here also contact infection did not seem to play any part in the infection which it would seem must have occurred at the preliminary exposure.

Infection with Bacillus friedlænderi.—The preceding experiments have established the fact that *Bacillus friedlænderi* may persist in the lungs of mice for a period of 3 weeks following inhalation of these organisms. That organisms acquired in this way give rise to actual infection has been brought out by a study of the occurrence and nature of the lesions induced. A total of 169 normal mice have been exposed to an atmosphere of *Bacillus friedlænderi.* Of these 76, or 44 per cent, died of a Friedländer bacillus septicemia. Gross consolidation of the lung was present in 32, or 18 per cent. The pathological histology will be given in a subsequent paper.

The results of the experiments on the inhalation of hemolytic streptococci or Friedländer's bacilli by normal animals shows that these organisms,—which gain entrance to the respiratory tract by methods involving no mechanical injury—not only become implanted on the respiratory mucosa, but give rise to pulmonary lesions. A large number of the exposed mice succumb to a septicemia, and gross consolidation at one or more lobes is not infrequent.

In view of the great difference in the rate of disappearance from the mouse lung of pneumococci, hemolytic streptococci, and Friedländer's bacilli following inhalation, a comparison of the relative mortality and incidence of the induced pneumonia is of interest.

In Table I is given a summary of the total number of mice exposed, the number of which died with septicemia, and the incidence of gross consolidation of one or more lobes. It is seen that only 1 per cent of normal mice exposed to a spray of pneumococci died of septicemia. If, on the other hand, the mice are first alcoholized and exposed during the stage of intoxication the mortality rises to 31 per cent. Under neither of these circumstances, however, is consolidation of the lungs ever encountered. In the case of alcoholized mice previously rendered partially immune to pneumococcus, although the mortality is only 20 per cent, owing no doubt to some persistence of immunity despite the alcoholism, pneumonia occurs in 30 per cent

of those dying. In the case of the normal mice exposed to either hemolytic streptococci or *Bacillus friedlænderi*, there is a mortality of 50 and 44 per cent, respectively, which exceeds that occurring in

TABLE I.

Comparative Mortality of Mice Exposed to Inhalation of Pneumococci, Hemolytic Streptococci, and B. friedlænderi.

Organism.	Mice.	No. exposed.	No. died of septicemia.	Died.	No. with pneumonia.	Consolidation.	No. survived.
				per cent		*per cent*	
Pneumococcus.	Normal.	326	4	1	0	0	322
	Alcoholized normal.	189	51	31	0	0	138
	Alcoholized immune.	486	99	20	35	7	387
Streptococcus.	Normal.	216	108	50	30	13	108
B. friedlænderi.	"	169	76	44	32	18	93

TABLE II.

Comparative Rate of Death Following Inhalation of Pneumococci, Hemolytic Streptococci, and B. friedlænderi.

Organism.	Mice.	Died.	Percentage deaths.					
			Days.					
			1–5	6–10	11–15	16–20	21–25	25–30
Pneumococcus.	Normal.	4	75	25	—	—	—	—
	Alcoholized normal.	51	74	21	3	—	—	—
	Alcoholized immune.	99	90	6	3	—	—	—
Streptococcus.	Normal.	108	39	33	12	5	5	3
B. friedlænderi.	"	76	22	44	18	9	3	1

alcoholized mice sprayed with pneumococcus, and pneumonia not infrequently develops.

Another interesting feature is to be seen in the time of death after

inhalation of different organisms. In Table II is shown the number of deaths occurring in 5 day periods following inhalation of the three organisms. From this table it is seen that while 80 per cent of the mice dying of pneumococcus septicemia succumb within the first 5 days, only 39 per cent of the streptococcus and 22 per cent of the Friedländer mice die within the first 5 days, the remainder succumbing irregularly after periods up to 30 days following the inhalation. The greatest mortality among the mice receiving *Bacillus friedlænderi* occurs from the 6th to the 10th day.

SUMMARY.

The results of these experiments support the previous observations that mice react in different ways to the inhalation of different bacteria. If normal mice are sprayed with hemolytic streptococci or Friedländer's bacillus, these organisms may be recovered from the lungs and heart's blood for a number of days after exposure. A large number of mice so exposed die of a septicemia during the 30 days following spraying. Consolidation of one or more lobes is not rare. If normal mice are exposed to an atmosphere of pneumococci, however, the organisms rapidly disappear from the lungs and only an occasional mouse succumbs to pneumococcus septicemia. In order to cause the pneumococcus to invade, the mice must first be alcoholized. If mice are first alcoholized and then exposed to a pneumococcus atmosphere, the pneumococci persist for a longer time in the lungs and a number will die of septicemia, a state of affairs which resembles that seen after the administration to normal mice of the other two organisms studied. In order to produce pneumococcus lobar consolidation, however, the mice must be first partially immunized and then allowed to inhale the organisms while under the influence of alcohol. A large percentage of both the normal and immune mice so alcoholized die within 5 days of exposure.

There also appears to be a definite relation between the mortality and the length of time inspired bacteria persist in the lungs. Whether this lag in removal allows multiplication of the bacteria and massive infection to occur has not been determined. Following inhalation of pneumococcus, the organisms were either removed rapidly and without apparent detriment to the animal, or, as in the case of the

alcoholized mice, invasion occurred, in which event the mouse usually succumbed rapidly, localization in the lung only occurring in partially immune animals. In the case of the hemolytic streptococci and *Bacillus friedlænderi*, however, the whole process of removal and infection seems to be slower. Infection and pneumonia occurred more often. These organisms seem to possess much greater invasive power when inhaled than the pneumococcus, though on intraperitoneal or subcutaneous inoculation, pneumococcus is highly virulent for normal mice.

CONCLUSIONS.

1. The reaction of mice to inspired bacteria varies according to the kind of organism inspired.

2. Mortality and actual infection bear a direct relationship to the length of time the inspired bacteria persist in the lungs following inhalation.

3. Hemolytic streptococci and *Bacillus friedlænderi* which have reached the lungs of normal mice following inhalation persist for several days. A fatal septicemia with or without pulmonary localization is frequent, and death may not occur for several days following exposure.

4. Pneumococci on the other hand, rapidly disappear from the lungs of normal mice following inhalation and rarely cause a septicemia. In alcoholized mice, however, the pneumococci persist in the lungs for a longer period, a fatal septicemia is frequent, and pulmonary localization of the infection only occurs in partially immunized animals. Death occurs early, generally within 5 days.

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