PERSISTENCE OF INSPIRED BACTERIA IN THE LUNGS OF ALCOHOLIZED MICE.

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In a preceding paper (1) on the presence of bacteria in the lungs of mice following inhalation, the rate of disappearance of the inhaled bacteria was shown to depend upon the variety of the microorganism used. Pneumococci are disposed of within a few hours and do not give rise to a general infection. Hemolytic streptococci, on the other hand, persist in the lungs for a much longer period of time and generalized septicemia frequently occurs. In the present study, an attempt has been made to determine the conditions under which pneumococci which have reached the lung may induce a local or general reaction. As mice rarely succumb with a generalized infection following inhalation of pneumococci, it seemed desirable to see what effect alcoholic intoxication would have on the penetration of inspired bacteria.

There is a general belief that alcohol exerts a harmful influence on the natural defences of the body. The fact that alcoholic intoxication increases the susceptibility of animals to a number of infections is already recognized. Abbott (2) found that the normal resistance of rabbits is markedly diminished when alcohol is administered daily to the stage of acute intoxication. Rubin (3) states that intoxicated rabbits succumb more readily to pneumococcus and streptococcus infection and that the leucocytes in the peritoneal exudate of alcohol-intoxicated rabbits phagocyte fewer carmine granules than do leucocytes from normal rabbits. According to Friedenwald (4), except for a slight increase in the eosinophils, there is little difference between the blood of normal and the blood of intoxicated rabbits. Paulson (5) states that half the minimal lethal dose of acetonitrile is sufficient to kill guinea pigs which have received moderate quantities of alcohol.

In the present study, a comparison has been made in normal and intoxicated mice of the time of disappearance from the lungs of inspired pneumococci, hemolytic streptococci, and *Bacillus influenzæ*,

and the relative occurrence of generalized infection in the two groups of animals following the inhalation of pneumococci.

Method.

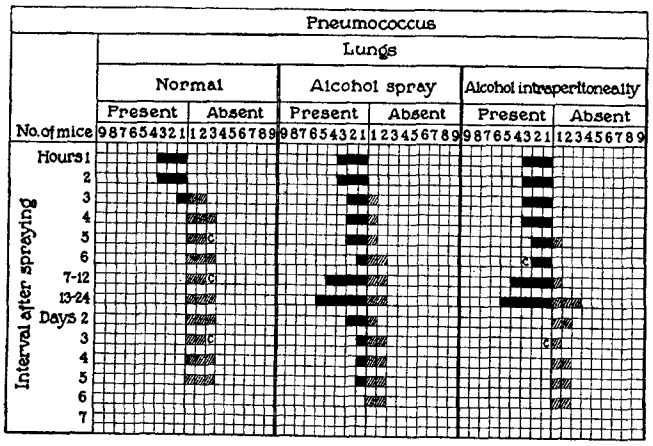
Mice were used as test animals. They were allowed to inhale an atmosphere in which a fine bacterial mist had been produced by spraying a culture of the organism to be studied. The organisms used were Pneumococcus Type I, *Streptococcus hæmolyticus*, and *B. influenzæ*. The animals were placed in the spraying chamber previously described (1), and were sprayed with 50 cc. of broth culture of the test organism. As a rule, the mice were removed from the spray box after an exposure of 1 hour. At various intervals following spraying, individual mice were killed. In order to prevent the possibility of aspiration of bacteria from the upper to the lower respiratory tract at the time of death, the mice were killed instantaneously by clamping the trachea and spinal cord with sponge forceps. The mice were then immersed in a solution of lysol and autopsied with sterile instruments. As a routine procedure, small pieces of the lung and a few drops of the heart's blood were separately cultured in plain broth. All positive cultures were plated on blood agar for further identification.

The mice were intoxicated either by inhalation of an alcoholic spray or by intraperitoneal injection of 1.5 cc. of 10 per cent alcohol in salt solution. In the first procedure, the mice were allowed to remain in the spray box 30 to 45 minutes after spraying with alcohol by which time they were under the influence of it. This method, however, gave irregular results. Some of the mice were relatively unaffected while others died later from the acute alcohol poisoning. For this reason the second procedure, in which the alcohol was administered intraperitoneally, was adopted. By the injection method, the average 20 gm. mouse becomes completely stupefied within 3 minutes and remains so for from 3 to 4 hours. The tolerance of individual mice to alcohol varies greatly. As a rule, however, mice show little evidence of intoxication on the day following the administration of alcohol.

In Text-figs. 1 to 4 each black square indicates one mouse from the lungs or heart's blood of which the sprayed bacteria were recovered. The cross-hatched squares represent those mice 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*.

EXPERIMENTAL.

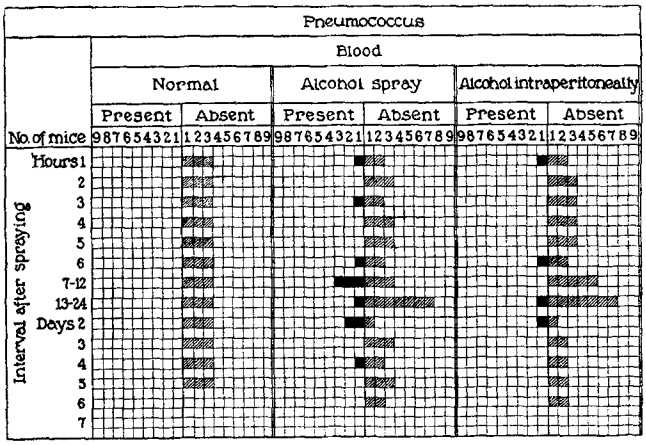
In Text-fig. 1 are shown the number of times pneumococci were recovered from the lungs of mice which were killed at stated intervals following spraying pneumococci into the air breathed by the animals. It is seen that while pneumococci were almost invariably recovered from the lungs within 3 hours following spraying, after 4 hours they could no longer be demonstrated by cultural methods. In the alcohol-treated mice, however, the sprayed bacteria were recovered from the lungs quite regularly during the first 24 hours, and in one instance at least were demonstrated in the tissue after 5 days. From this



TEXT-FIG. 1. Recovery of pneumococci from the lungs of normal and intoxicated mice following spraying. Each black square represents one mouse from whose lungs the sprayed bacteria were recovered. The cross-hatched squares represent those mice from whose lungs the sprayed bacteria were not recovered.

experiment, it is clear that pneumococci may be recovered from the lungs of normal mice only for a short period following exposure to an atmosphere containing pneumococci in finely divided droplets. On the other hand, if mice are intoxicated with alcohol, the normal mechanism for the disposal of the inspired bacteria is interfered with and in consequence of this the period of their persistence in the lungs is much prolonged.

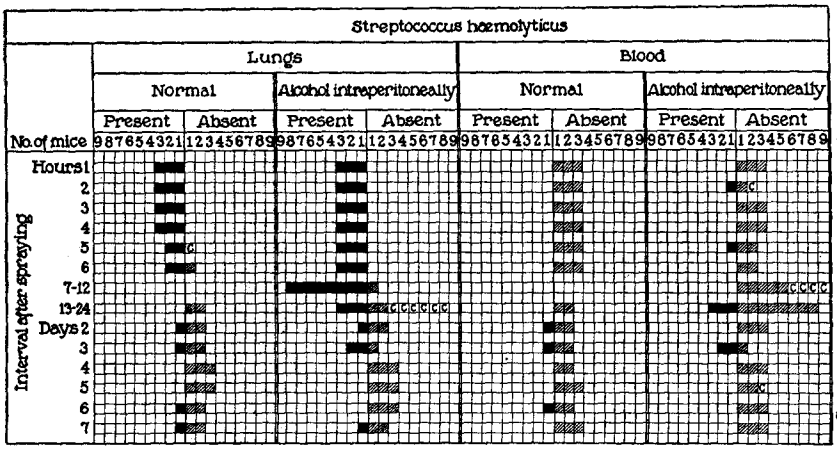
This inhalation experiment shows that pneumococci which are implanted on the lung tissue remain viable for several days in the lungs of intoxicated mice. In order to determine whether the inspired bacteria were merely implanted on the surface of the bronchi, or if invasion of the lungs had actually occurred, cultures were made of the heart's blood in each instance. The results of the cultures of the heart's blood of the 36 normal and 87 intoxicated mice is shown in Text-fig. 2. The blood cultures of the 36 normal mice remained sterile. Of the 87 mice treated with alcohol previous to their exposure, fourteen, or 16 per cent, showed the presence of the type



TEXT-FIG. 2. Recovery of pneumococci from the blood of normal and intoxicated mice following spraying. Each black square represents one mouse from whose blood the sprayed bacteria were recovered. The cross-hatched squares represent those mice from whose blood the sprayed bacteria were not recovered.

organisms in their blood. It is surprising to note that in at least two instances the blood was invaded within 1 hour after spraying. This experiment demonstrates that when normal mice are allowed to breathe an atmosphere in which pneumococci have been suspended by spraying, the inspired bacteria rarely invade the tissue in sufficient numbers to cause septicemia. If, however, mice are intoxicated previous to the inhalation of pneumococci, the inhaled bacteria are able, in a considerable number of instances, to invade the host in sufficient numbers to cause septicemia.

In order to determine the influence of alcohol upon the occurrence of infection, a number of experiments were performed, in which the technique was similar to that already described. In these experiments, however, the mice were not killed, but were kept under observation for a period of at least 14 days to contrast the mortality from infection in the two groups of animals. Of the 54 normal mice only two succumbed during this period, and in both instances pneumococci were recovered from the heart's blood. 50 of the 102 intoxicated mice died. From the blood of 45, or 44 per cent, pneumococci were recovered, while in five the blood remained sterile. From these experiments it is seen that alcohol



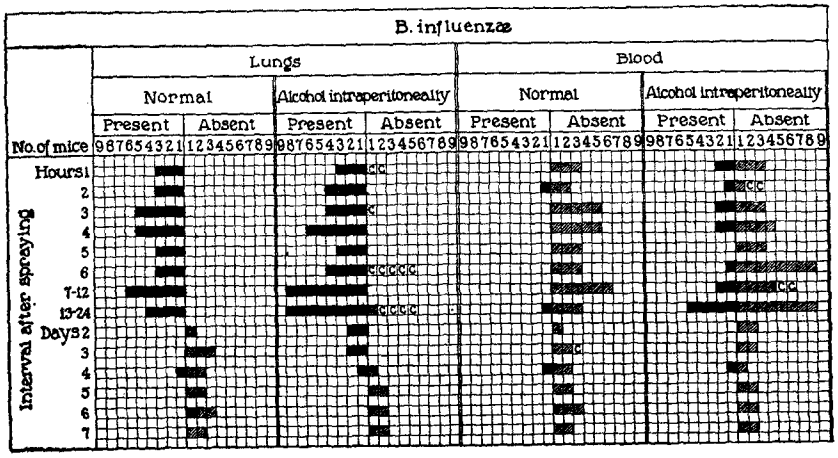
TEXT-FIG. 3. Recovery of *Streptococcus hæmolyticus* from the lungs and blood of normal and intoxicated mice following spraying. Each black square represents one mouse from whose lungs or blood the sprayed bacteria were recovered. The cross-hatched squares represent those mice from whose lungs or blood the sprayed bacteria were not recovered.

intoxication so alters the natural defensive mechanism of the body that inspired pneumococci may invade in sufficient numbers to cause death by septicemia.

In order to determine how alcohol would affect the persistence of other bacteria, inhalation experiments were performed with *Streptococcus hæmolyticus* and *Bacillus influenzæ*.

The results of spraying 38 normal and 57 intoxicated mice with *Streptococcus hæmolyticus* are seen in Text-fig. 3. Hemolytic streptococci were recovered from the lungs of sixteen of the eighteen normal

mice killed within 6 hours after spraying but only in four, or 20 per cent, of the twenty mice killed from 1 to 7 days following exposure. In the case of intoxicated mice, hemolytic streptococci were cultured from the lungs of twenty-six, or 96 per cent, of the twenty-seven mice killed within 12 hours of spraying and from seven of the thirty mice killed from 1 to 7 days after spraying. It is remarkable that in two instances this organism was recovered from the lungs on the 7th day after spraying. There is little difference in the rapidity of disappearance of this organism from the lungs of normal and intoxicated mice. The hemolytic streptococcus, however, was demonstrated in the blood of only three, or 7 per cent, of the 38 normal



TEXT-FIG. 4. Recovery of *B. influenzæ* from the lungs and blood of normal and intoxicated mice following spraying. Each black square represents one mouse from whose lungs or blood the sprayed bacteria were recovered. The cross-hatched squares represent those mice from whose lungs or blood the sprayed bacteria were not recovered.

mice and did not appear in the blood until the 2nd day following spraying. In the 57 intoxicated mice, however, this organism was recovered from the blood in seven instances (12 per cent) and appeared in the blood as early as 2 hours after spraying.

In Text-fig. 4 are seen the results of spraying 46 normal and 66 intoxicated mice with *Bacillus influenzæ*. This organism was recovered from the lungs of all the 32 normal mice killed within 24

hours of spraying and was present only once in the lungs of the remaining fourteen mice. In the intoxicated mice, *Bacillus influenzæ* was likewise recovered from the lungs of all animals killed within 12 hours. The blood was invaded in only three, or six per cent, of the normal mice, while septicemia occurred in sixteen, or 24 per cent, of the intoxicated animals. Not only was the incidence of septicemia higher in the intoxicated mice, but the organism persisted in the lungs for a longer period.

The results as regards the persistence in the lungs and invasion of the blood stream are not so striking with hemolytic streptococci and *Bacillus influenzæ* as in the case of pneumococci. The organisms first mentioned are not so rapidly removed from the lungs of normal mice and occasionally invade the blood even in normal animals. However, even with these organisms there is a greater tendency to blood invasion in the intoxicated animals, as evidenced by the increased number of positive blood cultures.

DISCUSSION.

The results of these experiments support the previous observations that in mice exposed to a dense spray containing bacteria in suspension, the inhaled organisms penetrate to the smaller bronchi. The further history of the bacteria which have been implanted in the lungs by inhalation depends not only upon the kind, number, and invasive quality of the bacteria, but also upon the normal defensive mechanism of the host. The difference in the invasive powers of the bacteria studied, and the variations in the number of bacteria gaining entrance to the respiratory tract following spraying are not subject to experimental control. The varying results of the present experiments are in part dependent upon these facts. The ability of the host to resist infection may, however, be somewhat depressed by experimental procedures. In the present instance the depressive influence of alcohol was successfully used to alter the normal defensive mechanism of the body against bacterial invasion.

Pneumococcus is an organism which is highly virulent for normal mice when given by *intraperitoneal* or subcutaneous inoculation. When this organism, however, is implanted on the respiratory mucosa

of normal mice by inhalation, it disappears from the lungs within a few hours and rarely gives rise to infection. If the normal mechanism of disposal, however, is interfered with by the administration of alcohol, inspired pneumococci persist in the lungs of mice for a longer time and frequently invade the blood. Thus, in the inhalation experiments less than 3 per cent of the normal mice died of pneumococcus septicemia, while over 40 per cent of intoxicated animals succumbed to fatal blood infection. Hemolytic streptococci and *Bacillus influenzæ* persist in the lungs of normal mice for a much longer time, and the difference between intoxicated and normal mice is not so distinct. However, in intoxicated mice the blood is apparently not only more rapidly, but also more frequently, invaded even by these organisms.

These experiments throw no light on whether the inspired bacteria are killed *in situ*, are taken up by leucocytes, or are carried to other parts of the body. Neither do they show in what manner the alcohol exerts its harmful effects, or whether alcohol intoxication causes local changes in the permeability of the respiratory mucosa, or acts adversely on the systemic resistance. The experiments therefore fail to shed any light on the exact mode of natural infection of the lungs with pneumococci. But they do show that alcohol delays the removal of inspired bacteria from the lungs and favors the invasion of the blood stream.

CONCLUSIONS.

1. When mice are exposed to an atmosphere containing certain bacteria in the form of a fine mist, the bacteria may be recovered from the deeper respiratory passages.

2. Pneumococci which have reached the lungs of normal mice as a result of this procedure usually disappear within a few hours and give rise to no generalized infection. In mice intoxicated with alcohol, on the other hand, pneumococci persist in the lungs for a longer period and fatal speticemia frequently follows.

3. Hemolytic streptococci and *Bacillus influenzæ* generally persist in the lungs for about 24 hours. In intoxicated mice these organisms do not disappear so rapidly from the lungs and generalized infection is much more frequent.

4. The experiments yield no evidence as to how alcoholic intoxication renders the lungs more permeable to inspired bacteria.

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