EXPERIMENTAL PRODUCTION OF PNEUMOCOCCUS PNEUMONIA IN MICE BY THE INHALATION METHOD.

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(Received for publication, July 28, 1924.)

Many attempts have been made to produce pneumonia in animals with the pneumococcus since Fraenkel (1) and Weichselbaum (2) described this organism as the etiological agent of croupous pneumonia. It early became evident that there were other factors concerned in the production of the disease besides the introduction of the organisms into the respiratory tract. The instances in which experimental lobar consolidation of the lung has been successfully produced by this means are open to the criticism that large amounts of culture material were injected into the respiratory tract under more or less artificial conditions, and that in some instances additional gross injury to the respiratory epithelium was resorted to in order to render the experimental animal susceptible to infection. The experiments here to be reported were planned to obviate as far as possible these objections.

In preceding papers (3) it has been shown that virulent pneumococci generally disappear from the lungs of normal mice within a few hours following inhalation and rarely give rise to a generalized infection. In mice intoxicated with alcohol, however, pneumococci persist in the lungs for a longer period and a fatal septicemia frequently follows. It has further been shown (4) that a high degree of immunity is produced in mice following repeated inhalations of living pneumococci and that resistance is only slightly increased in these animals by repeated exposures to a spray of dead organisms.

It seemed possible that localization of the infection in the lungs might be effected by allowing partially immunized mice to inhale virulent pneumococci while under the influence of alcohol, and the experiments were planned with this in view. We shall here report upon
pulmonary lesions encountered (1) in normal mice which were killed or died while under the influence of alcohol, (2) in normal mice which were alcoholized and allowed to inhale pneumococci while intoxicated, and (3) in partially immune mice which were exposed to an atmosphere of the pneumococci while intoxicated. In this latter group of partially immune mice are included only animals which were immunized by the inhalation method and they fall into three groups: (a) mice which had recovered from several exposures to an atmosphere containing living pneumococci, (b) mice which had in like manner been exposed to atmospheres containing heat killed pneumococci, and (c) mice which had survived, while intoxicated, one or two previous exposures to atmospheres in which living pneumococci were present. There are also included experiments in which a different type of pneumococcus was used for immunization than that employed in the final exposure.

In the course of the study no percentage figures on the incidence of pneumonia produced by the inhalation method were obtained since only animals which died were examined, and it is conceivable that others became infected but recovered.

**EXPERIMENTAL.**

*Method.*—The organisms used were virulent strains of Pneumococcus Type I and Type II and a slightly virulent strain of an atypical Type IIc. The latter strain was used instead of a Group IV pneumococcus as the atypical Type II strains belong in the group of organisms which are encountered frequently in normal mouths but which rarely incite disease in man.

The mice to be immunized were placed in the spray chamber previously described (3) and the air inside was repeatedly sprayed at intervals of 2 to 3 days with 50 cc. of either (1) broth culture of virulent pneumococci, (2) broth cultures of pneumococci which had been killed by heating to 60° for 30 minutes, or (3) killed pneumococci suspended in salt solution.

The mice were alcoholized by intraperitoneal injection of 1.5 cc. of a 10 per cent solution of alcohol in saline. 1 hour after the administration of the alcohol the animals were sprayed with 50 cc. of the pneumococcus culture and allowed to remain in the spray box for 1 hour. In the case of death of the animals, cultures were made in broth from the lungs and from the heart's blood. At least one lobe of the lung was fixed in Zenker's fluid for histological study.
Histological Examination of the Lungs of Normal Mice after Alcohol Administration.

In an accompanying paper (5) the pulmonary lesions most commonly found in normal mice, killed by cranial compression, are described in detail. Suffice it to say here that congestion and a very occasional spontaneous pneumonia of predominating mononuclear type were the only lesions noted. In a series of mice which died of acute alcohol poisoning or were killed within a period of 2 hours following the administration of alcohol the lungs were examined microscopically. Congestion was present but no more frequently than in the normal controls and, in fact, no other lesions were observed which did not also occur in the normals. It is necessary to bear this in mind in order to interpret the lesions to be described below as occurring in the lungs of mice which were allowed to inhale pneumococci.

Infection by the Inhalation Method.

The pulmonary lesions encountered in these animals ranged from a simple congestion to the outspoken lesions of a serofibrinous pleurisy, interstitial inflammation, and red and gray hepatization. A detailed discussion of the pathological changes will be given in an accompanying paper (5). For present purposes the interstitial inflammation and red and gray hepatization alone will be considered as affording evidence of localization of the infection in the lungs.

1. Pulmonary Lesions in Non-Immune Mice.—Of a total of 246 normal mice which were alcoholized and exposed in an atmosphere containing virulent Type I pneumococci, forty-one or 16 per cent died of pneumococcus septicemia. The lungs of thirty-three of these forty-one mice were examined histologically. In only one of these were the organs normal. Congestion occurred in nineteen, congestion and serofibrinous pleurisy in eleven, and interstitial inflammation in two, in one of which pleurisy was also present.

In this experiment only two of thirty-three non-immune mice which died of pneumococcus septicemia following inhalations of Type I pneumococci while intoxicated showed any evidence of localization of the infection in the lungs. In these two instances there was an early interstitial inflammation but neither red nor gray hepatization
occurred. On the other hand, a serofibrinous pleurisy associated with the presence of pneumococcus was noted in eleven instances without demonstrable intrapulmonary lesion in the lobe examined.

2. Pulmonary Lesions in Immunized Mice.—In order to determine the effect of similar treatment in partially immunized animals, mice which had survived one exposure to pneumococci while alcoholized were subsequently treated with alcohol and allowed to inhale an atmosphere of pneumococci. Of the 163 mice so treated, eighteen, or 11 per cent died of a pneumococcus septicemia. Histological examination showed the lungs to be normal in three instances; congestion occurred nine times, pleurisy and congestion three times, interstitial inflammation twice, red hepatization once. In other words, fifteen or 83 per cent of the fatal cases showed no evidence of attempts at localization, while three or 16 per cent showed definite localized pulmonary lesions. In one instance the lesion had advanced to the stage of red hepatization.

With a view of demonstrating whether a greater percentage of pulmonary localization would occur in more highly immunized mice, seventy-eight mice which had survived two exposures to a pneumococcus spray while intoxicated were again alcoholized after a period of 10 days and exposed a third time to virulent Type I pneumococci. Seven of these mice died with pneumococcus septicemia. Histological examination of the lungs of these animals revealed congestion in two instances, interstitial inflammation associated with pleurisy occurred once, red hepatization once, and gray hepatization in three instances. In this more highly immune series five, or 71 per cent of the fatal cases, showed marked local reaction in the lungs, as contrasted with 16 per cent in the less immune animals mentioned above.

In a further demonstration of the point that a greater percentage of pulmonary localization occurs in partially immunized than in normal mice, yet another series of 250 mice which had been immunized by spraying from two to ten times with living or heat-killed cultures of pneumococci were alcoholized and exposed to an atmosphere of virulent pneumococci. In each instance, the final exposure was made 10 days after the last “immunizing” spray. Of these 250 partially immunized mice, forty-five or 18 per cent died of pneumococcus septicemia. The lungs of the forty-one mice which were examined his-
From these experiments it would appear that normal mice infected with pneumococci by the inhalation method die of an overwhelming septicemia without marked cellular reaction in the lungs, whereas partially immune mice which are similarly infected by inhaling pneumococci while alcoholized present in their lungs at death definite inflammatory reactions. It also seems evident that as the mice become more immune there is a progressive decrease in the number of deaths from pneumococcus septicemia without pulmonary localization, and a reciprocal percentage of the fatalities shows pulmonary localization.

3. Pulmonary Lesions in Mice Immunized with One Type of Pneumococcus and Infected by the Respiratory Route by Organisms of a Different Type.—In order to ascertain whether the immunity acquired by the inhalation method is type specific, mice which had been previously immunized by this procedure were later exposed to an infecting spray of virulent organisms of a heterologous type. Since pulmonary localization of an infection occurring by way of the respiratory tract is an evident expression of the degree of immunity acquired by previous exposure, the extent and nature of the local reactions in the lungs were utilized as criteria in these cross-infection experiments.

Nineteen mice were sprayed eight to ten times with a slightly virulent atypical Type IIc, and thirty-two mice were sprayed with the same organism while intoxicated. After a 10 day interval these 51 mice were intoxicated and sprayed with a Type I pneumococcus culture. Nine or 17 per cent of these animals died. Upon histological examination of the lungs two showed congestion, one congestion and pleurisy, two interstitial inflammation, three red hepatization, and one gray hepatization. Of eighteen mice similarly immunized by spraying eight times with Type IIc pneumococcus and subsequently sprayed while alcoholized with virulent Type II pneumococci, six or 33 per cent died. Histological examination of the lungs showed congestion and pleurisy in two instances, interstitial inflammation occurred in two, and in two red hepatization. A total of fifteen of the
69 mice immunized with an atypical Type IIc pneumococcus and exposed to a spray of Type I or Type II pneumococci died. In ten or 66 per cent death was associated with definite pulmonary localization.

In order to see whether mice which had been rendered so immune to Type I pneumococcus that they were no longer amenable to infection by inhalation of the homologous organism, could be infected with a Type II organism, 94 Type I immune mice were again alcoholized and sprayed with a Type II pneumococcus. Histological examination of the lungs of the fifteen mice which died showed them to be normal in one instance, congestion and pleurisy occurred in four, interstitial inflammation was present in two, red hepatization in four, and gray hepatization in four. Thirteen other mice which were so highly immunized against a Type I pneumococcus that they survived an otherwise fatal intraperitoneal injection of 0.000,001 cc. of the same virulent culture were alcoholized and sprayed with Type II pneumococcus. Four of these thirteen more highly immune mice died with septicemia. The lungs of the animals showed, respectively, congestion, congestion and pleurisy, interstitial inflammation, and gray hepatization.

From these experiments it is evident that mice which have been immunized against one type of pneumococcus may show an increased resistance locally when infected by the inhalation method by organisms of another type and, further, that even if they acquire a relatively high degree of immunity to one type of pneumococcus, they may be infected by inhalation with a different type.

The results are summarized in Table I which gives the percentage incidence of the lesions produced in the total 246 non-immunized and 667 immunized mice. Of the thirty-three non-immunized mice which died following exposure to pneumococcus atmosphere while alcoholized, only two showed definite intrapulmonary changes, having the nature of an early interstitial inflammation. On the other hand, in the case of the 100 partially immunized mice which died, definite lobar consolidation resembling the red and the gray stages of lobar pneumonia as seen in man were encountered in thirty instances, the early pulmonary lesion termed interstitial inflammation was found twenty-one times, in six instances associated with a pleurisy, and a
serofibrinous pleurisy occurred thirty-one times without evidence of intrapulmonary inflammation.

It is interesting to note that the localizing pulmonary lesions occurred in only 38 per cent of the mice infected with the same type of pneumococcus as that used for immunization, as contrasted with 64 per cent of localizing lesions in mice infected with a different type of organism from that used for immunization.

**TABLE I.**

**Comparative Incidence of Pathological Lesions in Lungs of Non-Immune and Immune Mice Succumbing to Infection by Inhalation Method.**

<table>
<thead>
<tr>
<th>Lesions</th>
<th>33 non-immunized mice</th>
<th>100 immunized by the inhalation method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Congestion</td>
<td>58</td>
<td>14</td>
</tr>
<tr>
<td>Congestion and pleurisy</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Consolidation: red and gray hepatisation</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

**DISCUSSION.**

A number of workers (6) have succeeded in producing pneumonia in animals by intratracheal injections. In most instances it has been necessary to instill considerable amounts of culture well down into the trachea before positive results were obtained. As early as 1904, Wadsworth (7) was able occasionally to produce pneumonia in partially immunized rabbits by the injection of 1 cc. of pneumococci into the trachea. The non-immunized rabbits, on the other hand, either remained well or died of septicemia. More recently Jones (8) has found that the introduction of a virulent culture of *B. avisepticus* into the trachea of normal rabbits was sufficient to cause a rapidly fatal septicemia. However, if the resistance of the rabbits had been stimulated by subcutaneous vaccination, instead of the rapidly fatal septicemia without lung involvement, consolidations of the more dependent lobes occurred with considerable regularity.

In a previous paper of this series it has been shown that when mice are exposed to an atmosphere containing virulent pneumococci the organisms may be recovered for a period of 3 hours thereafter by culturing small pieces of the periphery of the lung, but that they are too few to be demonstrated histologically. A generalized infection rarely
occurs in such animals, yet the survivors develop a specific general immunity. As previously pointed out, this suggests that they are not removed merely mechanically from the respiratory tract, but that a sublethal dose of organisms must actually gain access to the tissues and be destroyed. In contrast, when mice are intoxicated with alcohol and sprayed with pneumococci the organisms persist in the lungs for a longer period and a fatal septicemia often follows. By combining the method of immunization with a subsequent alcoholizing and spraying of virulent organisms, infection and localization in the lung with lobar pneumonia may be produced. It must be remembered, however, that in this work at least three variables are always present: (1) the natural immunity of the individual mouse; (2) the artificial immunity produced by exposure to pneumococcus; (3) the effect of alcoholic intoxication upon the invasion of the pneumococcus, which differs from individual to individual. To produce the pneumonia a selected population was being employed. Furthermore, in these experiments death was the only token of infection recognized, no clinical data being employed, and it is probable that cases of infection occurred and were missed because the animals recovered. Despite all these variable factors, convincing results were obtained, results which furnish interesting epidemiological data.

The high incidence in the normal mice of serofibrinous pleurisy without demonstrable evidence of intrapulmonary localization is difficult to explain. It must be remembered, however, that lungs which show interstitial inflammation often appear only congested on gross examination, and consequently lesions may easily have been present in some one of the lobes which was not included in the portion studied microscopically.

The fact that mice immunized by exposure to a spray of dead organisms showed increased local resistance to subsequent infection by the inhalation method is all the more interesting since it has previously been found that mice immunized by this method present little general immunity to intraperitoneal inoculation. This may be due to a local active immunity in contrast to the instances in which immunization is effected with living organisms when the animals show a general immunity. Of even greater suggestive value is the observation that
mice which have been immunized by inhalation of one type of pneumococcus show evidence of increased immunity against organisms of a heterologous type when infection occurs by inhalation. This would seem to indicate that there is an antigenic fraction of the pneumococcus cell common to all types of pneumococci.

The fact that mice with a relatively high general immunity which have recovered from an intraperitoneal injection of 0.000,001 cc. of a virulent Type I culture may be infected by the inhalation of Type II pneumococcus would lead one to suspect that just as there may be an antigenic factor common to all types of pneumococci, so there may be a specific substance in the various types of pneumococci which plays an important rôle in determining infection. The ability to infect mice which are highly resistant to one type of pneumococcus with another type by inhalation may, in part, explain why in man one attack of pneumonia apparently confers no immunity against subsequent infections.

The results obtained support and extend Wadsworth's contention that to produce pneumonia there must be a subtle equilibrium between virulence of the infecting organism on the one hand, and susceptibility of the host on the other.

CONCLUSIONS.

1. Non-immune mice which are alcoholized and die of septicemia following exposure to a spray of virulent pneumococci rarely show any localization of infection in the lung.

2. Mice which have been partially immunized by previous inhalations of living or killed pneumococci and which while alcoholized are exposed to an atmosphere of virulent pneumococci often develop a pneumococcus lobar pneumonia.

3. Mice which have been rendered relatively immune to one type of pneumococcus may frequently develop lobar pneumonia after the inhalation of pneumococci of another type.

BIBLIOGRAPHY.