STUDIES ON RESPIRATORY DISEASES

XXXV. THE PATHOLOGY OF PNEUMOCOCCUS INFECTIONS IN MICE

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In the preceding paper was reported a study of the influence of some extracts and filtrates of the pneumococcus and of a few chemical reagents on the virulence of pneumococci. It was shown that purpura developed in mice inoculated with the extract prepared by repeatedly freezing and thawing the pneumococci. If the mice received the extract twenty-four hours preceding the inoculation of a pneumococcus culture of borderline virulence, they were protected against the infection. This protection persisted only as long as purpura was observable. It was also reported that when fresh filtrates of virulent pneumococci were inoculated simultaneously with the culture of low virulence a large number of the mice at autopsy showed a fibrinopurulent pleuritis. The most potent filtrates were obtained when the cultures grew in the presence of salts that lower the electrophoretic potential of microorganisms. Complete autopsy records of the mice were kept and the tissues from a representative number were prepared for histological examination. The present paper comprises a study of the pathological lesions observed in these mice.

Among the early reports on the pathological changes produced by the pneumococcus in mice is the report by Sprunt and Leutscher (1912). They studied about eighty mice and found, in those dying several days after injection of living and dead pneumococci, acute degeneration of the walls of some of the large blood vessels and local hemorrhage. They did not find this de-
generation in mice that died from other bacterial diseases. One year previously Leutsher (1911) had reported the finding of large blood clots in one, or in many, pleural regions in mice dying from pneumococcus infections. He stated that the occurrence of the hemorrhage was more frequent with the smallest doses because the duration of life was longer.

In a study of pneumococcus lobar pneumonia in mice, Branch and Stillman (1924) state that the primary lesion is an interstitial inflammation of the alveolar walls and that the infection spreads in the interstitial tissue like a cellulitis.

Julianelle and Reimann (1926) observed hemorrhagic areas in the lungs and voluntary muscles of mice following the injection of the frozen and thawed extract.

In a preliminary report on the pathology of the lungs produced by a filtrate Olson (1925) states that there was intense congestion, interstitial hemorrhage with decreased air content of the alveoli, and interalveolar extravasation but no filling of the alveoli with exudate or blood. There was some cellular reaction along the blood vessels and parenchyma and there was some degeneration of the alveolar epithelium.

The following report of the pathological lesions observed in mice after they had received intraperitoneal inoculations of pneumococci or pneumococcus products, has been divided into five parts.1

I. THE PATHOLOGICAL LESIONS NOTED IN THE TISSUES OF MICE KILLED AFTER AN INOCULATION OF AN EXTRACT OF PNEUMOCOCCI

In the preceding paper it was reported that, when white mice were inoculated intraperitoneally with an extract prepared by repeatedly freezing and thawing pneumococci, purpura developed on all or a part of the external surface of the mouse that is free from hair. At autopsy it was observed that the injected blood vessels and hemorrhagic areas were not limited to the external surface but that they were present in nearly every organ and tissue of the mouse. The following description of the gross lesions

1 The detailed tabulations are not included in this report. They are contained in the Doctoral Dissertation of M. J. Pittman, University of Chicago, Libraries.
observed in a mouse which had been killed with chloroform eleven hours after an inoculation of 0.7 cc. of the extract, is in general true for all mice that were killed while purpura was visible. The subcutaneous blood vessels were injected; this was particularly noticeable in the regions of the inguinal and cervical lymph glands. Several hemorrhagic areas were present in the subcutaneous fascia and all the blood vessels of the peritoneum were distended with blood. The liver, spleen and kidney were very dark red in color; and the surfaces of the ovaries and the uterus were a bright red. The blood vessels of the stomach and the omentum were injected. The margins of the lungs were very bright red and extending from the margin towards the hilus were red lines. On the pleura there were many bright red spots, 0.5 to 1 mm. in diameter. The blood vessels of the brain were not injected and the brain appeared to be normal.

The histological study\(^2\) of nine mice, killed after they were inoculated with the extract (table 7 of preceding paper), revealed the same picture of engorged and hemorrhagic blood vessels that had been observed grossly. In the lungs nearly all the blood vessels were distended with blood and there were many areas of hemorrhage and edema. In many, the alveoli were filled with a pink-staining granular exudate and a few leucocytes. These cells were never abundant. There was a slight mononuclear cellular increase in the lungs of all the mice which were killed twenty-four hours after the inoculation, while only one which had received the extract less than twenty-four hours before being killed, showed a mononuclear cellular increase. Compensatory emphysema was present in a third of the lungs.

The other tissues that were examined histologically were the ear, spleen, kidney, adrenals, salivary and cervical lymph glands. The blood vessels of practically all seemed to be distended with blood and some had hemorrhagic areas. The increase in blood was especially noticeable in the sinuses of the spleen and the glomerular tufts of the kidney. The livers from a third of the mice showed an infiltration of polymorphonuclear cells and one had a few necrotic areas.

\(^2\) The tissues were fixed in Zenker's solution embedded in celloidin sectioned and stained with Delafield's hematoxylin and eosin.
II. PATHOLOGICAL LESIONS OBSERVED IN MICE WHICH DIED AFTER THE INOCULATION OF PNEUMOCOCCI AND A PURPURA-PRODUCING PNEUMOCOCCUS EXTRACT

This division included the study of the gross pathological lesions that were observed in 38 mice and the histological study of the tissues of 9. These mice, which had been inoculated intraperitoneally with the extract and a culture of low virulence, simultaneously or at different time intervals, are from experiments 2, 3, 4, 5, 6, and 8 of the preceding paper and two other experiments not published in detail.

At autopsy, two-thirds of the mice showed external purpura and internally they presented the same picture of engorged and hemorrhagic blood vessels that was observed in the mice killed after they had received the extract alone. In table 1 the frequency of the occurrence of the pathological lesions in these mice may be contrasted. The most marked differences are observed in the hemorrhagic appearance of the lungs and the presence of fibrinopurulent growths. The lungs of 97 per cent of the mice that had received the culture and extract showed hemorrhagic areas grossly while 55 per cent of the mice inoculated with the extract alone showed hemorrhage. The low percentage observed in the latter group is partially due to the fact that external purpura had blanched or was blanching on two of the nine mice before they were killed. Forty-five per cent of the culture extract mice showed a fibrinopurulent pleuritis.

The lungs showed more extensive involvement than the other organs. The picture of the lung was one of marked hemorrhagic areas, edema, consolidation, distention of the capillaries with blood; while some of the larger blood vessels were filled with thrombi. The consolidation which was present in two-thirds of the mice was largely due to edematous fluid, fibrin and a small number of leucocytes.

Again it was observed that there was a polymorphonuclear cellular increase in the liver. Zenker’s waxy degeneration was present in the rectus abdominis muscle of the one mouse in which this organ was examined. This muscle was not examined in the mice which received the extract alone.
III. PATHOLOGICAL LESIONS OBSERVED IN MICE THAT RECEIVED FILTRATES OF PNEUMOCOCCUS CULTURES

Ten mice were used in this study. Eight were inoculated intraperitoneally with 2 cc. of a fresh filtrate prepared from an eighteen-hour-old broth culture of a highly virulent type I pneumococcus strain. These mice were killed with chloroform, thirty, forty-eight, sixty and ninety hours after the inoculation. At autopsy all showed that the organs were slightly injected with blood. No marked pathological lesions were noted, grossly or microscopically. The liver and kidney showed more hyperemia than any other tissue and the respiratory muscle, rectus abdominis, of three that were given the filtrate showed slight waxy degeneration. In the other mice this muscle was not examined. The lungs of the filtrate mice showed slightly more consolidation, edema, fibrin, leucocytic exudation, hemorrhage and capillary distention than the control mice, but these lesions were never marked in any of the mice. Compensatory emphysema was present in the lungs of all except one control; and, since this latter condition is frequently observed in normal mice, it is thought that the chronic condition might be partially responsible for the mild pathology observed in the mice.

IV. PATHOLOGICAL LESIONS NOTED IN MICE INOCULATED WITH PNEUMOCOCCI AND PNEUMOCOCCUS FILTRATES

The tissues were taken from 16 mice used in the experiments of Part III of the preceding paper.

At necropsy the subcutaneous blood vessels and the internal organs showed an increase in blood and some hemorrhage but the hyperemic condition was not as marked as when the mice received the purpura-producing extract.

The most noticeable pathological lesions were the fibrino-purulent growths on the pleurae and to a lesser extent on the peritoneum. Seventy-one per cent of the mice showed a pleuritis, and 33 per cent a peritonitis. The pleuritis was more frequent in the mice which received the pneumococcus filtrate which contained some substance, trivalent salts or serum, that lowers the
electrophoretic potential of microorganisms, than in those which were inoculated with the filtrate of the culture grown in buffered broth or broth containing glucose. Nine of ten mice that died after the inoculation of the former filtrate had a pleuritis.

The pathological lesions of the lungs were more extensive than were those observed in the other groups of mice. The lungs of all showed hemorrhage, consolidation and edema. All except one, showed a moderate amount of leucocytic exudation. The majority showed fibrin and capillary distention while half had thrombi in a number of the larger blood vessels. Interstitial inflammation was not infrequently observed.

Other than the fibrinopurulent growth, the pathological lesions of the peritoneal organs, liver and kidney excepted, were about the same as was reported for those mice that had died after the inoculation of extract and culture. Fifty per cent of the mice had necrotic areas in the liver. The muscle rectus abdominalis which was examined from only two mice, showed waxy degeneration.

V. Pathological Lesions Found in Mice Which Died After They Had Been Inoculated with Pneumococci of Varying Degrees of Virulence of With Pneumococci Grown in the Presence of Substances That Lower the P.D. of Microorganisms

The virulence of the three strains of pneumococci measured in cubic centimeters per MLD for white mice were $1 \times 10^{-8}$, $1 \times 10^{-3}$ and 1.0 cc. The tissues from only two mice which succumbed to the highly virulent strain were studied microscopically. This number is not sufficient to warrant a generalized statement but it should be mentioned that hyperemia was observed in practically every organ and that the lungs of one showed a picture which closely resembled early lobar pneumonia. A fibrinopurulent pleuritis was not present, nor was it observed at the autopsy of more than 50 other mice which died following an inoculation of this culture.

Of the seven mice which succumbed to the culture of medium virulence (Pn B orig.), more than a third had a fibrinopurulent
pleuritis. The other pathological lesions are very similar to those that were observed in mice that died after they had been inoculated with the culture of lowest virulence (Pn C orig.). From among the mice which died after receiving the latter culture, the tissues of 19 were studied histologically. Again, the same generalized hyperemia was observed as in all mice which received pneumococcus cultures. The lungs showed some edema, consolidation, fibrin, leukocytic exudation, hemorrhage, capillary distention, and some thrombotic blood vessels. Twenty-one per cent of them had a fibrinopurulent pleuritis. To be contrasted with these lesions are those observed in mice which died following the inoculation of the same culture after it had been grown in the presence of substances that lower the P.D. of microorganisms. The substances that were used were cerium and lanthanum nitrate and serum. The latter mice showed more extensive involvement of the lungs and pleuritis was more than twice as prevalent, occurring in 50 per cent of the animals. The lesions of the latter group of mice closely resembled those observed in mice which died from the culture-filtrate inoculations.

DISCUSSION AND SUMMARY

This work includes the study of the pathological lesions observed in 138 mice that were killed or died after they had been inoculated with pneumococcus products, pneumococci or the two substances combined. The products of the pneumococci used were filtrates and an extract produced by repeatedly freezing and thawing pneumococci. The pneumococcus cultures, which were selected from strains that varied in virulence, were grown in buffered broth or in broth which contained substances that lower the P.D. of microorganisms.

The gross pathological lesions observed in the majority of the mice are summarized in table 1. On inspection of the table it will be noted that the blood vessels of a very large number of the mice were injected or hemorrhagic and that this condition was most marked in those mice which had been inoculated with broth culture and extract. The extract alone when introduced into mice produced marked hemorrhagic lesions.
### TABLE 1

**Summary of macroscopical findings**

<table>
<thead>
<tr>
<th>SUBSTANCE INOCULATED</th>
<th>NUMBER OF ANIMALS</th>
<th>SUBCUTANEOUS BLOOD VESSELS</th>
<th>INGUINALLYMP NODES</th>
<th>CERVICALLYMP NODES</th>
<th>PERITONEUM BLOOD VESSELS</th>
<th>PLEURAL CAVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injected</td>
<td>Hemorrhagic</td>
<td>Injected</td>
<td>Injected</td>
<td>Injected</td>
<td>Fluid</td>
</tr>
<tr>
<td>Extract</td>
<td>9</td>
<td>88*</td>
<td>33</td>
<td>33</td>
<td>55</td>
<td>44</td>
</tr>
<tr>
<td>Ext. + cult.</td>
<td>38</td>
<td>87, 80</td>
<td>8</td>
<td>33</td>
<td>24</td>
<td>43</td>
</tr>
<tr>
<td>Filtrate</td>
<td>10</td>
<td>40, 20</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Filt. + cult.</td>
<td>14</td>
<td>79, 50</td>
<td>29</td>
<td>29</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>PhC cult.</td>
<td>19</td>
<td>94, 73</td>
<td>21</td>
<td>31</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td>PhC + salts</td>
<td>21</td>
<td>100, 63</td>
<td>10</td>
<td>5</td>
<td>36</td>
<td>47</td>
</tr>
<tr>
<td>PhB cult.</td>
<td>7</td>
<td>100, 66</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>66</td>
</tr>
<tr>
<td>PhB + salts</td>
<td>7</td>
<td>100, 83</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>83</td>
</tr>
</tbody>
</table>

* The figures under pathological headings indicate percentage.
Another finding which is considered significant may also be noted in the table. This is the presence of fibrinopurulent pleuritis among the several groups of mice. The percentages of mice with pleuritis which succumbed to the borderline culture (PnC), the culture plus extract, the culture grown in the presence of substances that lower the P.D. of organisms, and the cultures plus filtrates, are respectively 21, 43, 50 and 71. This suggests that the extract influences to some extent the production of pleuritis, that salts which lower the P.D. are more influential and that filtrates exert the greatest influence. When the mice which received both the culture and the filtrate prepared with a broth that contained substances that lower the P.D., were considered alone, it was noted that 90 per cent of the mice had a fibrinopurulent pleuritis. Since this percentage is so much higher than is observed among the mice which died after receiving large amounts of the culture that had been grown in the presence of these substances, it is thought that there was some other substance in the filtrate influencing the invasion and localization of the pneumococci. Yet this factor is very transient, inasmuch as it had no influence on the virulence of the culture if it preceded or followed the culture inoculation by two hours (table 9 of preceding paper). It may be argued that the salts lowered the virulence of the organisms, thereby giving the pneumococci more time to localize. Yet the same amount of culture when inoculated alone or with broth equivalent to the amount of filtrate, rarely caused death or even an invasion of the blood stream. It should also be mentioned in this connection that the mice frequently died within twenty-four hours (table 8 of preceding paper) and that this interval was less than was sometimes the case when the mice were given high dilutions of the virulent culture. Pleuritis was never observed in mice which died from the effects of the highly virulent culture. It was noted that the culture of medium virulence caused the production of pleuritis in 3 out of 7 mice.

The sections prepared from the lungs of a mouse which had received the highly virulent culture showed a picture that more closely resembled an early stage of lobar pneumonia than was observed in the lungs of other mice. An insufficient number of
mice that died after the inoculation of the virulent culture were examined, however, to draw the conclusion that the most virulent culture produces lobar pneumonia while the ones of lesser virulence produce bronchial pneumonia or localized growths. This conclusion has been drawn by Gaskell (1928), Whittle (1928) and Armstrong and Gaskell (1921). Since no mice showed a complete picture of lobar pneumonia, although the picture at times was lacking only in the massive number of polymorphonuclear cells and heavy fibrin deposits, it is thought that the resistance of the host plays as great a part in the production of lobar pneumonia as the virulence of the culture. The latter theory is supported by the work of Blake and Cecil (1920), Permar (1923), Stillman and Branch (1924), Nakajima (1926) and Pittman and Falk (1930). Stillman and Branch were unable to produce lobar pneumonia in mice unless the mice had a partial immunity to the pneumococcus. They think that interstitial inflammation is the first stage in the development of lobar pneumonia in mice. This lesion was frequently observed in our mice which succumbed to the culture filtrate combination.

A generalized summary of the lesions found in the lungs of mice after they had received pneumococci or pneumococcus products is distention of the capillaries, thrombi in the large blood vessels, edema of the alveolar walls and alveoli filled with an edematous fluid, fibrin and a few leucocytes, and pleuritis. The severity of the lesions found in each group of mice is discussed under the different divisions of the pathological findings. The lungs of that group of mice which were killed after the inoculation of filtrate showed the mildest reaction and the lesions which at no time were marked were never comparable to those reported by Olson. Hemorrhage was most severe in those mice which succumbed to the inoculation of culture and extract; and pleuritis was most frequent in those mice which had been inoculated with culture and filtrate.

It is significant that the most extensive lesions were observed in the lungs when the inoculations were made intraperitoneally. The liver frequently showed an increase in polymorphonuclear cells and the spleen and kidney were engorged with blood. Zenker's waxy degeneration was observed in the respiratory
muscle of all animals examined. This condition is frequently observed in fatal cases of pneumonia and other inflammatory processes (Forbus, 1926 and Wells, 1927).

The experiments have not adequately explained the phenomenon that mice are protected from at least two MLD of a culture of border-line virulence if they have received the purpura-producing extract twenty-four hours before the inoculation. The only difference noted in the tissues of mice that were killed from 24 hours to 5 days after the extract inoculation and in the tissues of those that were killed in less than twenty-four hours after the inoculation, was a slight increase in mononuclear cells in the lungs. Stuppy, Cannon and Falk (1929) and Gay and Clark (1929) have noted a mononuclear cell reaction in the lungs of immune rabbits when they were inoculated with pneumococci. Although our mice had no permanent immunity it might be suggested that this temporary increase in mononuclear cells protected the mice from infection.

As the most extensive hemorrhagic lesions were observed in those mice which died after they received an inoculation of the endocellular substance followed within twenty-four hours by an inoculation of culture, and since the frequency of fibrinopurulent pleuritis was most marked in the mice which died after receiving an inoculation of the culture and the exo-product, it is suggested that both products may function in the pathogenesis of pneumococcus infections. It has been stated (Julianelle and Reimann, 1927) that since purpura is rarely observed in humans or animals with pneumococcus infections this purpura-producing extract is not liberated by the pneumococcus \textit{in vivo}. An argument against this theory was observed in the preceding paper: if the mouse had a pneumococcus infection at the time of the inoculation, or if the extract was given simultaneously with the culture, the external purpuric reaction was less severe or entirely absent.

CONCLUSIONS

1. When mice were inoculated with an extract of the pneumococcus produced by repeatedly freezing and thawing the pneumococcus, they developed marked hemorrhagic lesions on all or a part of the external surface that was free from hair, and at autopsy,
after they were killed, hemorrhagic areas could be found in practically every tissue of the mouse.

2. When mice died after they were inoculated with extract followed several hours later with a culture inoculation, they had more marked hemorrhagic lesions than any other group of mice that were studied.

3. Fresh filtrates of virulent pneumococci produced very slight pathological reaction in mice but, when the filtrates were inoculated with a culture of low virulence, 71 per cent of the mice at autopsy showed a fibrinopurulent pleuritis.

4. Pleuritis was twice as frequent in mice which died from pneumococci that had grown in the presence of salts that lower the P.D. as it was in mice which had died from the same strain grown in plain broth.

5. Mice which died or were killed after inoculation of pneumococci or pneumococcus products showed pathological lesions in the lungs, liver, respiratory muscles and possibly in the kidneys.

6. The relation of the extract and filtrates to the pathogenesis of pneumococcus infections is discussed.

The authors wish to express their appreciation to Professor H. G. Wells for assistance with the pathological diagnoses.

REFERENCES