STUDIES ON THE PATHOGENESIS OF EXPERIMENTAL PNEUMOCOCCUS PNEUMONIA IN THE DOG

II. Secondary Pulmonary Lesions. Their Production by Intratracheal and Intrabronchial Injection of Fluid Pneumonic Exudate

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Plates 7 and 8

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In the preceding paper (1) data were presented which suggest that in experimental canine pneumonia the intrabronchial flow of infected edema fluid plays the principal rôle in the transport of pneumococci from one lobe to another and that secondary lobe lesions may result from the penetration of such fluid exudate into the terminal airways. Supporting this concept of the mode of origin of metastatic pulmonary lesions are the following observations. First, the incidence of secondary lobar involvement appeared to be related to the wetness of the primary lesions. Second, the finding that in dogs with edematous primary lesions pneumococci were widely distributed throughout the lungs while in those showing well consolidated and relatively dry initial lesions the remainder of the peripheral lung tissue was sterile. Third, the sequence of interlobar spread was such as one might expect from the anatomical arrangement of the bronchi in respect to the influence of gravity on the distribution of fluid in the air passages. Fourth, the finding of small amounts of pneumococcus-laden edematous exudate in the terminal airways in the otherwise normal lobes of dogs in which secondary lesions were occurring elsewhere in the lung.

If this were the correct interpretation of the mechanism of interlobar spread, it should be possible to induce secondary lesions by the intrabronchial implantation of fluid pneumonic exudate in dogs with primary lesions which ordinarily are not followed by extension to other lobes. Such experiments constitute the subject of the present communication.

Methods and Materials

The pneumonic exudate employed in the following experiments was aspirated from the lung lesions of dogs 24 hours after the induction of experimental pneumonia with

doses of pneumococcus culture which produce a rapidly evolving inflammatory process and a high mortality. Aspiration was performed in the living animals by means of a catheter inserted into a terminal bronchus of the initially infected lobe and in the sacrificed animal by direct withdrawal of fluid from the bronchi with a 5 cc. pipette. The latter method proved to be the more satisfactory of the two since a larger quantity of fluid could be secured with greater ease from the excised lung. Inspection of such excised lungs showed frothy exudate in the main bronchi of the involved lobes. Quantities of fluid exudate varying from 3 to 6 cc. could be withdrawn by gentle suction. Stained films of the exudate showed usually a relatively small number of polymorphonuclear leucocytes and large numbers of pneumococci—almost all extracellular. Estimation of the number of microorganisms by plating serial dilutions of the material yielded colony counts of 5 billion to 50 billion per cc., a very much heavier growth than can be secured in broth culture. The relatively viscid exudates obtained from well localized lesions were of a quite different character. Stained films showed great numbers of polymorphonuclear leucocytes and often a small per cent of macrophages; the pneumococci were fewer and mostly intracellular. Such exudates were used only in the experiments listed under controls.

The dogs were prepared for exudate injection in the same manner as that employed in the production of pneumonia with the starch-broth inoculum, *i.e.* a preliminary injection of morphine and cocainization of the larynx. With the exception of the first two experiments the exudate was injected through a small bore rigid metal catheter inserted through a bronchoscope 1 cm. in diameter. After injection the animals were kept in the same position for 20 to 40 minutes, the longer period of time being used in the later experiments. Daily blood counts, blood cultures, and x-rays were made. Temperatures were taken twice a day. The dogs were sacrificed 24 hours after the introduction of the pneumonic exudate. The extent of pulmonary involvement was charted, cultures of the lungs were made, and sections of the lesions taken for microscopic study.

Production of Pneumonia in Normal Dogs

(*a*) *Injection of Exudate into a Terminal Bronchus*

It was necessary first to determine whether pneumococcus lobar pneumonia could be produced in the normal animal by the intrabronchial implantation of small amounts of fluid pneumonic exudate. Accordingly, 0.25 cc. of exudate, aspirated from the lung lesion of a living animal, was injected into a terminal bronchus of the right lower lobe of a normal dog. 24 hours later the dog had fever, leucopenia, and x-ray showed consolidation of approximately half the right lung field. The findings at autopsy (24 hours after injection) are shown in Text-fig. 1—3-09 T. The right lower lobe was completely and evenly consolidated while patchy consolidation of parts of the right middle, right upper, and postcardiac lobes was present. Pneumococci were cultured from all the lesions. This represents the type of pneumonia which results from a large dose of pneumococcus culture suspended in starch.

Another animal, 3-27 T, was similarly infected with a slightly smaller inoculum, 0.2 cc. The result, consolidation of most of the right lower lobe, is depicted in the chart.

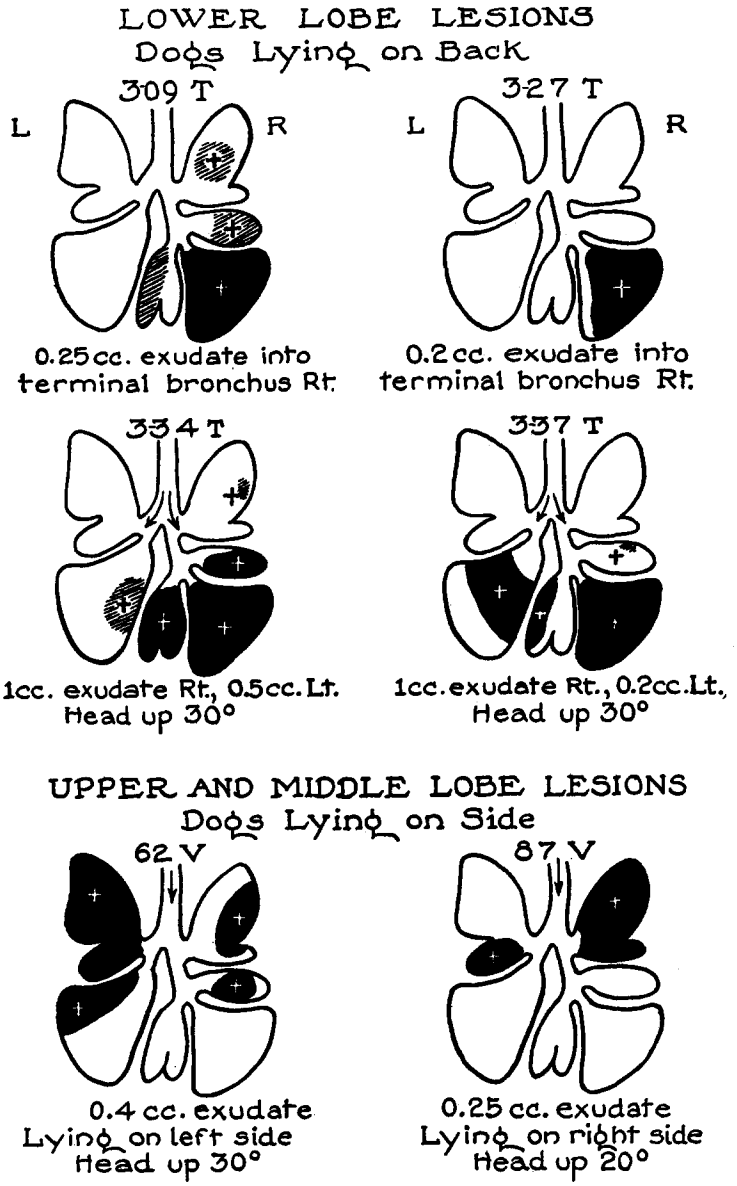
(b) Effect of Gravity on the Production and Distribution of Lesions

Lower Lobe Lesions.—The next step consisted in ascertaining whether or not the simple deposition of infected exudate in the bronchi or trachea would result in pneumonia, provided the position of the dog was such as to favor the flow of fluid into the lobe bronchi. The first experiment was carried out in two animals placed on their backs with the head end of the board elevated about 30°. Reference to the photograph of the dog's bronchial tree (Figs. 1 and 2) shows that in this position exudate following the floor of the right and left main stem bronchi would flow into the lower lobes.[1] One dog, 3-34 T, was given 1 cc. of exudate into the right main bronchus and 0.5 cc. into the left just beyond the carina as indicated on the diagram (Text-fig. 1). At the end of 24 hours an extensive pneumonia involving almost the whole of three lobes of the right side was present, while the left lower lobe showed only a small lesion. It seemed probable that the implanted exudate flowed into all three lobes attached to the right main bronchus since the lesions appeared to be of the same age. A second dog, 3-37 T, similarly injected, exhibited lesions confined chiefly to the two lower lobes. Both animals showed signs of pronounced infection, namely leucopenia and bacteremia.

Upper Lobe Lesions.—With the intent of producing lesions of the upper lobes, dogs were placed on either side with the head elevated 20–30°. The lesions resulting from the deposition of 0.4 cc. of exudate in the trachea of dog 62 V lying on its left side are shown in Text-fig. 1. There were complete consolidation of the left upper lobe, involvement of the upper ⅓ of the lower lobe, and small lesions in the right middle and upper lobes. These latter lesions may represent spread from the left side but were more probably due to infection by the original exudate. A smaller amount of exudate, 0.25 cc., injected into the trachea of dog 87 V lying on its right side, resulted in a pneumonia confined principally to the right upper lobe.

It is evident from these experiments that quite small amounts of fluid exudate from acute pulmonary lesions, when deposited in the trachea or bronchi of normal dogs, will produce typical experimental pneumonia and that the distribution of lobar involvement is influenced largely by gravity.

[1] Whether or not fluid always flows along the most dependent part of the channel in the bronchi is not known. The spiral movement of the cilia may conceivably cause some deviation from this direction.



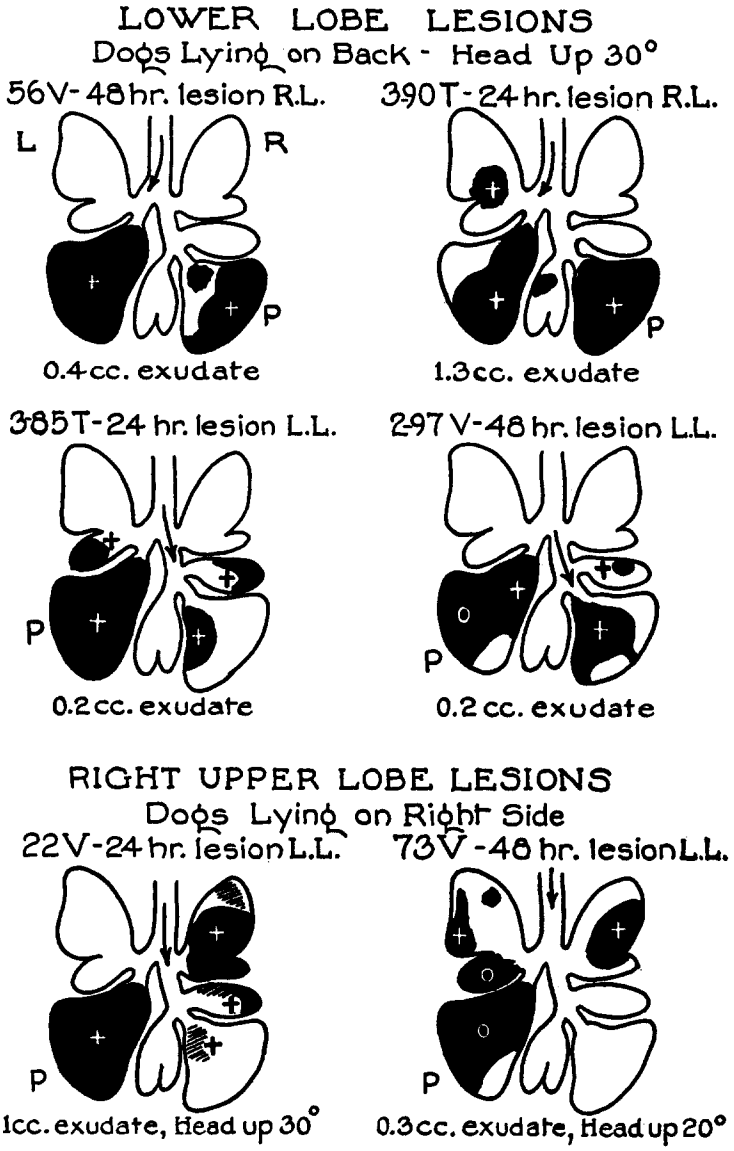
TEXT-FIG. 1. Production of primary lobar lesions in normal dogs by injection of pneumonic exudate into bronchi and trachea. + = pneumococci recovered on culture.

Production of Secondary Lobar Lesions in Dogs with Localized Primary Lesions 24 to 48 Hours of Age

When a fluid pneumonic exudate was similarly injected into dogs during the course of a relatively mild pneumonia, which seldom shows any tendency to spread from the initially involved lobe, secondary lesions were induced regularly in various parts of the lung. For these tests dogs were infected with 0.001 cc. to 0.00001 cc. doses of culture. At the end of 24 hours there is usually complete consolidation of the infected lobe, fever, leucocytosis, but no bacteremia. By 48 hours many of the animals begin to show evidence of beginning recovery. Not more than 5 to 6 per cent of such animals exhibit spread of the infection to other lobes and these lesions are usually quite small. Only those animals whose x-rays revealed a lesion confined to a single lobe, were employed for exudate injection.

Lower Lobe Lesions.—Since bilateral lower lobe lesions in the absence of involvement elsewhere are rare in the experimental disease, the first experiments were carried out with a view to producing such bilobar lesions. With the dog in the dorsal position and tilted head up at an angle of approximately 30°, pneumonic exudate was injected into the main stem bronchus of the side opposite to that of the primary lesion. 24 hours later x-ray and then autopsy revealed secondary lesions in the opposite lower lobe involving a part or the whole of the lobe (Text-fig. 2). Serial x-rays of one such animal are shown in Figs. 4 to 6. The arrows in the diagrams indicate the points at which the exudate was injected. These lesions appeared to be similar to the metastatic processes occurring spontaneously during the course of the more pronounced infections. The dogs showed evidence of increased severity of the disease, often a drop in white count, increase in fever, and sometimes bacteremia. The presence of consolidation in both the middle and lower lobes of dog 3-85 T, which received exudate into the right main stem bronchus, suggested that the implanted exudate flowed into both lobes. In order to avoid this possibility, exudate was injected directly into the right lower lobe bronchus of dog 2-97 V. This resulted in a lesion confined almost entirely to the right lower lobe.

Right Upper and Middle Lobe Lesions.—In dogs with primary left lower lobe lesions, secondary involvement was produced in the right upper lobe by placing the dog on its right side with the head elevated 20–30° so as to bring the opening of the right upper lobe bronchus in the most dependent position (see photograph of model of bronchial tree in Fig. 3). 1 cc. of exudate injected into the right main bronchus of dog 22 V resulted in consolidation of almost the entire right upper lobe with small lesions in the



TEXT-FIG. 2. Production of secondary lobar lesions in dogs with primary lesions 24 to 48 hours of age. 0 = no pneumococci recovered on culture. P = primary lesion.

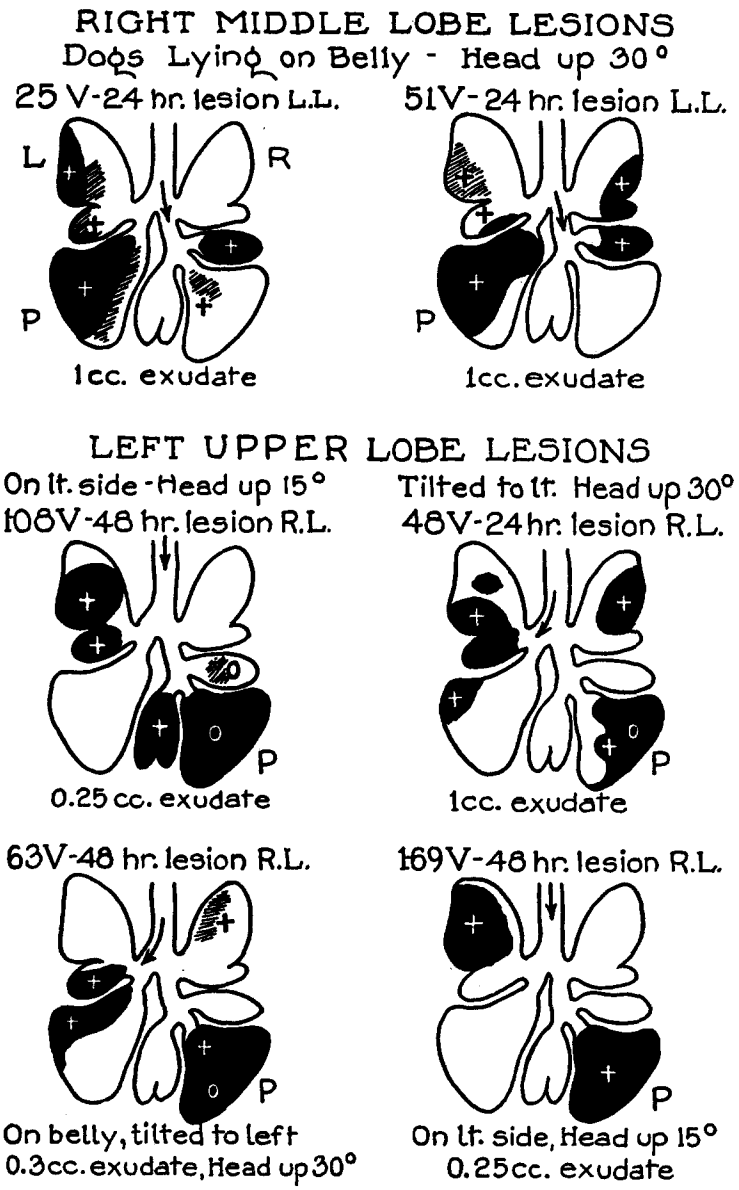
right middle and lower lobes (Text-fig. 2). X-ray of this animal at 48 hours shows a dense shadow in the right upper lung field which was clear before injection of the exudate (Figs. 10 and 11). The small lesions in the

right middle and lower lobes probably represent a spread from the upper lobe as they appeared to be of much more recent origin. A smaller amount of pneumonic exudate (0.3 cc.) injected into the trachea of dog 73 V placed in the same position as 22 V, produced a lesion involving more than half of the right upper lobe. Areas of consolidation were also present in the left upper lobe which appeared normal by x-ray before the exudate injection.

In order to produce secondary infection in the right middle lobe, the animal was placed in the natural position with the head elevated 30° and the exudate deposited in the right main bronchus beyond the opening of the bronchus to the right upper lobe. In each of the two instances shown in Text-fig. 3, consolidation of practically the whole of the right middle lobe resulted. Smaller lesions and mostly much younger ones were found in two of the other lobes in both dogs. It seems more likely that these early metastatic lesions arose from the secondarily induced process than from the primary lesions which by the end of 48 hours were fairly dry.

Left Upper Lobe Lesions.—In dogs with primary infections of the right lower lobe, secondary lesions were produced in the left upper lobe by injecting exudate into the trachea or left main bronchus with the dog on its left side and tilted head up. Four of the animals treated in this way are shown in Text-fig. 3. The resulting secondary lesion in dog 1-08 V is especially significant since at the time of autopsy the primary lesion in the right lower lobe was already in a state of recovery as shown by sterile cultures. Part of the relatively large quantity of exudate injected into dog 48 V probably flowed into the right upper lobe when the animal was returned to its normal position as this lesion appeared to be of the same age as that in the left upper. Serial x-rays of one of these dogs, 1-69 V, are shown in Figs. 7 to 9.

Experiments of this nature were carried out in 32 dogs. In only one instance did a secondary lesion fail to occur following the implantation of fluid pneumonic exudate into the bronchi or trachea. This failure could be accounted for by an unsatisfactory technical procedure. The induced secondary lesions were not always confined to the lobe in which it was desired to implant the exudate. Multiple secondary lesions were much more common when relatively large quantities of exudate, 0.5 to 1 cc., were employed, suggesting an overflow to other lobes. However, the increased severity of the disease produced by added pulmonary involvement may at times favor spontaneous spread from the initial lesion. This possibility was suggested by the findings in several instances. With new lobe involvement there was frequently a rise in temperature and sometimes the appearance of bacteremia. In two instances secondary lesions were pro-



TEXT-FIG. 3. Production of secondary lobar lesions in dogs with primary lesions 24 to 48 hours of age.

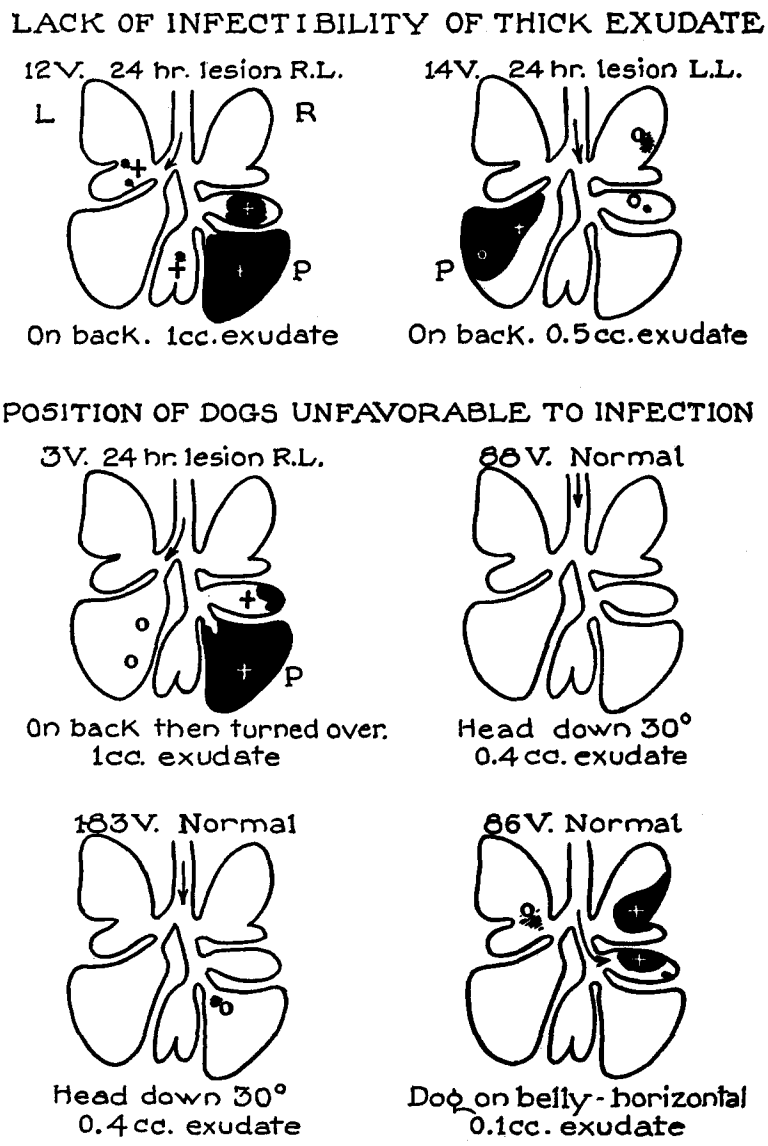
duced 4 days after the initiation of the experimental disease. The primary lesions in both these dogs were in a state of resolution and yielded sterile cultures.

Controls on Fluidity of Exudate and Position Unfavorable to the Effect of Gravity

In order to determine whether the viscosity of the infected exudate was as important a factor in the production of secondary lesions as the foregoing experiments would indicate, two dogs were injected with a thick viscous exudate—one which did not drop readily from the aspirating pipette. This exudate contained a large number of neutrophilic leucocytes and most of the pneumococci were intracellular. However, many microorganisms were extracellular as seen by the plated dilution of the material which yielded 400 millions per cc. These two animals, one with a primary lesion in the right, and the other in the left lower lobe, exhibited no secondary lesions by x-ray 24 hours after the exudate injection and at autopsy the only evidence of infection in the injected lobes was several minute areas of infiltration (Text-fig. 4). In one dog pneumococci were cultured from these areas; in the other they were sterile. The consolidation of the right middle lobe found in dog 12 V probably represents an extension from the right lower.

Positions unfavorable to the flow of the exudate into the bronchi were then tested. 1 cc. of fluid exudate was injected into the left main bronchus of dog 3 V, placed on its back in a position which would permit the exudate to flow into the left lower lobe. Immediately following injection the dog was taken off the board and allowed to stand up. X-ray 24 hours later showed the left lower lobe clear and at autopsy only the primary lesion in the right lower lobe and a small area of consolidation of the right middle were found. Cultures of the left lower lobe were sterile. Exudate was then injected into the trachea as shown on Text-fig. 4 in two normal dogs, 88 V and 1-83 V, placed on their belly with the head tilted down at an angle of 30°. Neither dog showed any signs of infection or any abnormality of the lungs at autopsy.

These results raised the question as to how quickly exudate would penetrate the bronchial tree to a locus where change in position would no longer prevent infection. Accordingly, 0.1 cc. of exudate was dropped directly into the opening of the right middle lobe bronchus of a normal dog placed horizontally on its belly. In this position the bronchus to the middle lobe extends ventrally. 1 minute later the dog was turned over on its back and maintained in that position for 40 minutes. 24 hours later consolidation of approximately two-thirds of the right middle lobe and one-third of the right upper lobe was found. This suggests that the exudate flows rapidly into the terminal airways and when it reaches that locus, infection



TEXT-FIG. 4. Controls on fluidity of exudate and position unfavorable to effect of gravity.

occurs despite change in position which would favor drainage from the lobe bronchi.

Microscopic Pathology.—The histological appearance of the artificially induced secondary lesions was in every respect similar to that of metastatic lesions occurring spon-

taneously and primary lesions of the same age produced by the starch inoculum (2). Certain of the primary lesions of dogs sacrificed at 72 hours showed evidence of beginning resolution while the induced secondary involvement exhibited an active spreading process. This condition of clearing and spread occurring simultaneously in different lobes of the lung has been observed occasionally during the natural course of the experimental disease (3).

DISCUSSION

We believe that the results of these experiments provide a satisfactory explanation of the mechanism by which interlobar spread of the pneumonic process takes place in the experimental animal. If our interpretation is correct, metastatic lesions occur only when the primary lesion is sufficiently edematous to permit escape of fluid exudate through its main bronchus into that of another lobe or lobes, the direction of flow being determined largely by gravity.

The possibility of pneumococci passing from one lobe to another by any route other than that of the bronchial passages seems to be excluded by the anatomy of the lung. Very occasionally two lobes are connected by small bridges of respiratory tissue. This finding, however, is unusual. In the great majority of dogs each lobe is a discrete respiratory unit. A retrograde lymph flow has been invoked to account for the spread of a pneumonic process from the hilum to the periphery of a lobe but this seems unlikely and has no experimental support. For pneumococci to reach another lobe by way of a postulated reversed lymph current it would be necessary for them to penetrate the wall of the trachea or bronchi at the hilum. We have been unable to find any suggestion of such an interstitial spread and extensive microscopical study has failed to reveal any evidence of the primary invasion of the more peripheral parenchymatous tissues.[2] Moreover, if this were the route of spread one would expect to find pneumococci in the unconsolidated tissue of dogs with single lobe lesions since the tracheas of these animals contain large numbers of organisms. The blood stream as a means of transport seems to be excluded since the dogs reported in this study had sterile blood cultures. Furthermore, it has not been possible to produce lobar pneumonia in the dog by the intravenous injection of even very large quantities of pneumococcus culture (5).

It should be pointed out that in these experiments on the injection of pneumonic exudate, the dogs were under the influence of morphine. In this respect conditions were not the same as those under which metastatic lesions occur spontaneously. Furthermore, other influences of which we

[2] Loosli's observations on the pathogenesis of the experimentally produced primary lesion are in accord with these findings (4).

have no knowledge may enter into the mechanism of spontaneous spread. Such factors as alterations in the pneumococcidal power of the blood and changes in the number of leucocytes may well play a rôle since diminution in either the humoral or cellular elements occurs much more frequently in the animals with multilobar than in those with unilobar lesions.

The question arises as to what relationship these findings in the canine disease bear to the problem of interlobar spread in the human being. An objection to making any but very limited comparisons may be put forward on the ground that most of the experimental animals were sacrificed within 48 to 72 hours, while spread in human lobar pneumonia usually occurs after this stage in the disease. In this connection it should be pointed out that the whole evolution of the pneumonic process occurs much more rapidly in the dog and furthermore, it is possible in the experimental disease by adjusting the infecting dose to predict with considerable certainty whether or not metastatic lesions will occur (6). Again we have observed in a large number of animals infected with a dose producing a monolobar lesion only, that maximum involvement occurs in the great majority of instances by the end of 24 hours.

Possibly the best evidence that the same mechanism of interlobar spread operates in the human patient as, we believe, occurs in the experimental animal, lies in one conspicuous difference exhibited by the two diseases in the sequence of progressive lobar involvement. Whereas spread to the opposite lower lobe occurs commonly in human pneumonia (7) and constitutes the most frequent bilobar involvement it is rarely seen in the dog until after all the lobes on the side of the initial lesion have become involved. The semi-reclining position of the pneumonia patient would favor the gravitation of infected edema fluid into the lower lobe bronchi, while the prone position of the dog would have the reverse effect. Certain anatomical differences between the lungs of man and dog, aside from the position of the bronchi, make any exact comparison of the sequence of interlobar spread impossible. Furthermore, in contrast to the dog, the interlobar fissures of the human being are often incomplete and sometimes entirely lacking, so that adjacent lobes may be contiguous if not in part continuous. While the interlobar septa appear to constitute a barrier to extension of the inflammatory process, we do not know whether this is always effective.

Numerous questions concerning the precise mechanism by which the injected edema fluid acts to initiate new foci of infection await further elucidation.

SUMMARY

On the basis of earlier findings, which suggested that interlobar spread in experimental canine pneumonia was due to the flow of infected edematous exudate from the initially involved lobe to other parts of the lung by way of the air passages, an attempt was made to induce secondary lesions by means of the intrabronchial and intratracheal injection of fluid pneumonic exudate. Such exudate was aspirated from the lungs of dogs with rapidly evolving lesions and injected through a bronchoscope into other animals showing well localized monolobar involvement which is seldom associated with spread to other parts of the lung. The deposition of relatively small amounts of fluid exudate in the larger air passages 24 to 48 hours after the onset of the disease was followed regularly by the occurrence of secondary lesions provided the dog was maintained in a position favoring the flow of fluid into the depths of the lung. Lesions in the various lobes could be produced at will by arranging the position of the animals so that the injected fluid would be carried by gravity into the most dependent bronchial opening nearest the point at which the exudate was deposited. Pneumonia was produced by this means as readily in normal dogs as in those with infection already present. If, however, the dog was placed in a position unfavorable to the flow of fluid into the bronchi, infection did not occur. Likewise the injection of viscid pneumonic exudate, with the animal tilted at an angle most favorable for entrance into the lobe bronchi, did not result in pulmonary involvement. These artificially induced secondary lesions resembled in every way both macroscopically and microscopically those occurring spontaneously in the course of the experimental disease.

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EXPLANATION OF PLATES

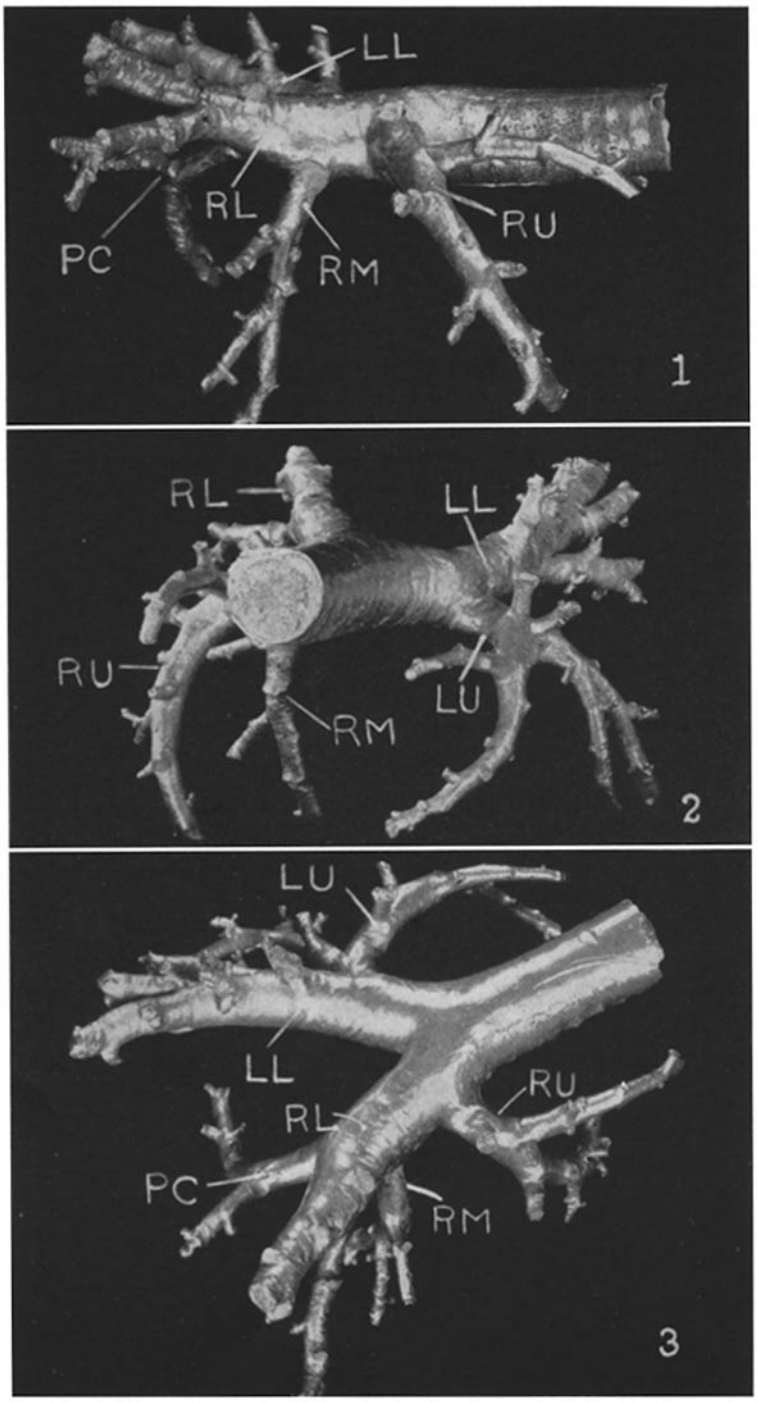
PLATE 7

FIG. 1. Wood's metal cast of bronchial tree of dog, made by Dr. C. G. Loosli. The excised lung was suspended from the trachea and inflated with air to approximately its normal expanded size. The air was allowed to flow through the lung for 24 hours by which time it was dry and hard. Wood's metal at a temperature of 76°C. was then poured into the trachea. The air-containing tissue was removed as far as possible by dissection and complete removal of the bronchial walls and remaining tissue secured by immersion in cleaning fluid. View from right side in approximately the standing position of the dog. The left upper lobe bronchus was deleted from the photograph.

FIG. 2. View of cast from the left anterior aspect to show the point at which the left upper lobe bronchus leaves the left main stem.

FIG. 3. Showing the position of the bronchi as seen from the back when the dog is placed on its right side with the head elevated approximately 20°. With the animal in this position, fluid exudate deposited in the trachea tends to flow first into the right upper lobe bronchus.

The different lobes of the dog's lung are indicated as follows: R. U. = right upper; R. M. = right middle; R. L. = right lower; L. U. = left upper; L. L. = left lower; P. C. = postcardiac.



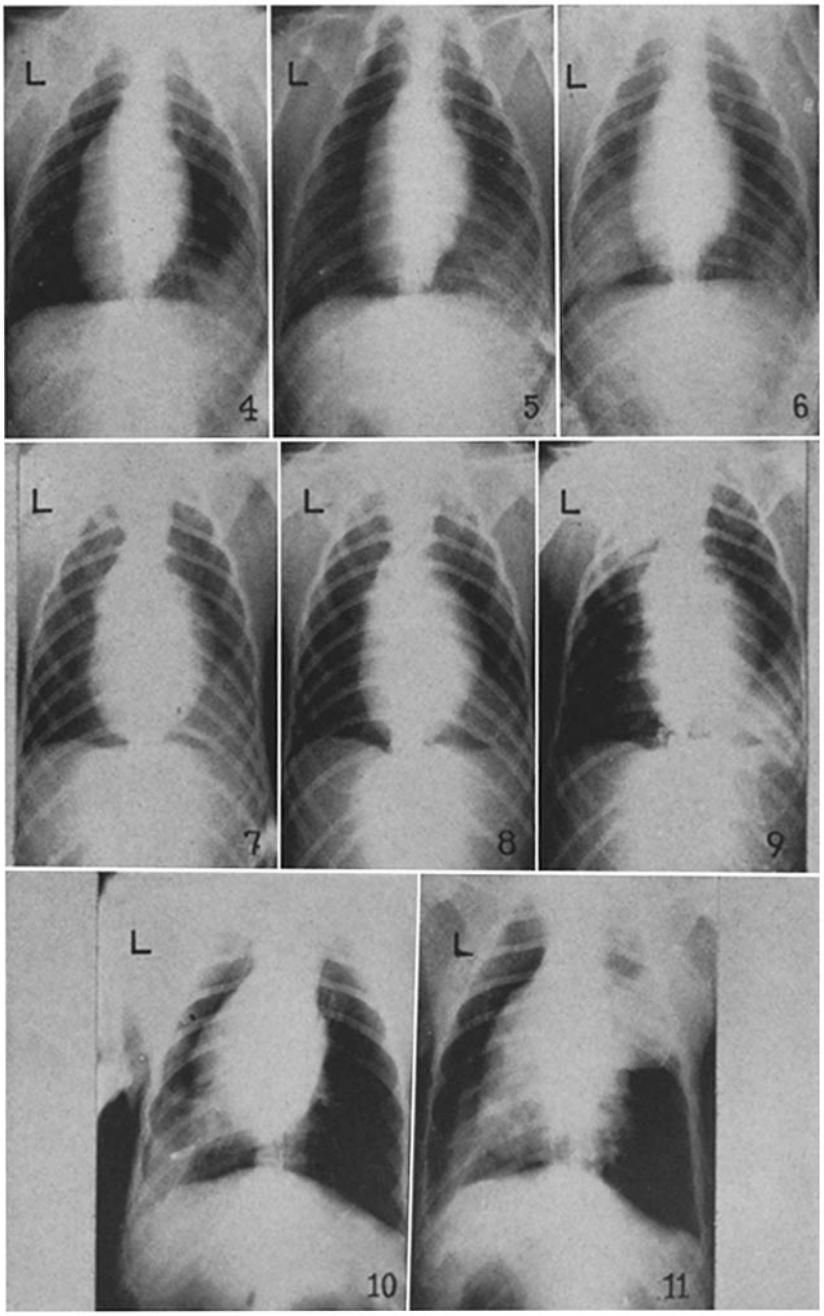
(Robertson and Hamburger: Pathogenesis of pneumococcus pneumonia. II)

PLATE 8

FIGS. 4 to 6. Serial x-rays of dog 56 V infected with 0.0001 cc. pneumococcus Type I culture in the right lower lobe. Fig. 4 shows consolidation of most of the right lower lobe at the end of 24 hours. By 48 hours (Fig. 5) the whole lobe had become involved. At this time the dog was placed on its back, head elevated 30°, and 0.4 cc. of fluid pneumonic exudate was deposited in the left lower lobe bronchus. X-ray at 72 hours (Fig. 6) shows consolidation of the left lower lobe and clearing of the primary lesion. The autopsy findings are shown in Text-fig. 2.

FIGS. 7 to 9. Serial x-rays of dog 1-69 V infected with 0.00001 cc. pneumococcus Type I culture in the right lower lobe. X-ray at 24 hours (Fig. 7) shows consolidation of the right lower lobe. A second x-ray at 48 hours (Fig. 8) shows the lesion essentially unchanged. At this time the dog was placed on its left side, head elevated about 15°, and 0.25 cc. of fluid pneumonic exudate deposited in the trachea about 2 inches above the carina. 24 hours later x-ray revealed a secondary lesion of the upper part of the left upper lobe in addition to the right lower lobe primary lesion. See Text-fig. 3 for autopsy findings.

FIGS. 10 and 11. Serial x-rays of dog 22 V infected with 0.001 cc. pneumococcus Type I culture in the left lower lobe. At 24 hours x-ray (Fig. 10) revealed consolidation of the left lower lobe. At this time the dog was placed on its right side, head up 30°, and 1 cc. fluid pneumonic exudate deposited in the right main bronchus above the opening of the right upper lobe bronchus. X-ray 24 hours subsequently (Fig. 11) revealed consolidation of the upper half of the right lung field. The initial lesion in the left lower lobe was beginning to clear. The findings at autopsy are shown in Text-fig. 2.



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