STUDIES ON THE PATHOGENESIS OF EXPERIMENTAL PNEUMOCOCCUS PNEUMONIA IN THE DOG

I. Secondary Pulmonary Lesions. Relationship of Bronchial Obstruction and Distribution of Pneumococci to Their Inception

By MORTON HAMBURGER, M.D., and O. H. ROBERTSON, M.D.

(From the Department of Medicine and the Douglas Smith Foundation for Medical Research, The University of Chicago, Chicago)

Plate 6

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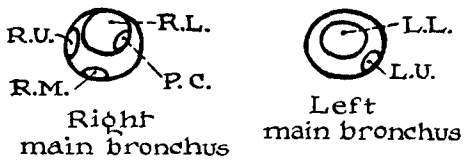
The present investigation represents an attempt to determine some of the conditions within the lung which favor the inception of pneumococcus pneumonia. While it is not unlikely that changes, both general and local, play a rôle as so called predisposing factors, such experimental and clinical observations as are available suggest that the local changes are the more important of the two. The problem was approached by way of a study of the origin of secondary lesions occurring spontaneously during the course of experimental canine pneumonia since it seemed possible that the local conditions responsible for the production of a new lobar lesion might be of the same general nature as those concerned in the initiation of the primary infection.

Little is known of the mechanism by which interlobar spread takes place, either in human lobar pneumonia or in the experimental disease in the dog. The view generally held and an eminently reasonable one, is that new lesions arise as a result of the aspiration of infected exudate or sputum from the primary lesion but this conception fails to account for the manner in which the tenacious viscid material reaches the smaller bronchi of another lobe and initiates infection. New light has been thrown on this subject by Loeschcke (1), who observed that the younger spreading lesions, in the lungs of patients dying of lobar pneumonia, were characterized by a peripheral zone of pneumococcus-laden edema fluid. He suggested that aspiration of this fluid exudate might represent one means by which pneumonia spreads from lobe to lobe. In our study (2) of the evolution of the lesion of experimental pneumococcus pneumonia in the dog, the margin of the growing lesion from a very early stage was observed to consist of edema-filled alveoli containing pneumococci. The amount of edema in the lesion was found to depend largely on the size of the infecting dose. Lesions produced by doses of culture which result in a high mortality are much more edematous than those produced by smaller inocula from which the animals usually recover. Not infrequently the bronchi from such wet lobes, when observed through the bronchoscope, are seen to be filled with

foamy edema fluid. In this respect the amount of edema in the initial lesion is related to the incidence of spread since metastatic lesions occur much more frequently in animals infected with the larger dosage.

In support of the concept that edema fluid may play the principal rôle in the transport of pneumococci from one lobe to another by way of the air passages, is the observed sequence of interlobar spread in canine pneumonia as related to the anatomical arrangement of the bronchi and the prone position of the dog. The direction of interlobar spread in the dog is fairly regular. When infection is initiated in the right lower lobe with a dose which ordinarily leads to multilobar lesions, the second lobe to become involved is the right middle. Next in order are the right upper and the postcardiac lobes. Reference to the photograph of the Wood's metal cast of the bronchial tree (at the end of Paper II of this study) shows that the right and left main bronchi lie in the same horizontal plane as the trachea. The two lower lobe bronchi are inclined at a slightly upward, *i.e.* dorsal angle. The bronchus to the middle lobe leaves the floor of the right main bronchus and extends ventrally and laterally. That to the upper lobe opens into the lateral wall of the right main stem 1 to 2 cm. cephalad of the right middle



TEXT-FIG. 1. Diagrammatic view through bronchoscope showing the openings of the several lobe bronchi into the right and left main stem bronchi. Dog in standing position. P.C. = postcardiac. R.L. = right lower, etc.

bronchus and extends laterally and ventrally. The openings of the bronchi are shown in the view through the bronchoscope depicted in Text-fig. 1. The bronchus to the postcardiac lobes branches off the median wall of the right lower lobe bronchus at a level considerably above that of the middle lobe and at about the level of the opening of the upper lobe bronchus. It extends medially and somewhat ventrally.

With the initial lesion in the left lower lobe the second lobe most often uninvolved is the left upper. The bronchus to this lobe opens into the floor on the lateral side of the left main stem[1] (Text-fig. 1). When spread to the opposite side occurs it is almost always in the upper or middle lobes. This usually takes place after all the lobes on one side have become involved but occasionally the second lobar lesion may be on the opposite side. The opposite lower lobe is almost always the last to become consolidated.

The anatomical arrangement of the bronchi, together with the fact that the dog with pneumonia lies in his cage most of the time with the head and upper thorax on a somewhat lower level than the rest of the body, affords easy opportunity for pneumococcus-laden edema fluid to flow into the middle or upper lobes. However, we do not know

[1] In some animals the fissure in the lower part of the left upper lobe is so deep as to produce what appears to be a left middle lobe but the tissues of the two portions are continuous at the hilum. The bronchus to this semi-detached part is a branch of the upper lobe main stem.

to what extent such intrabronchial flow of infected exudate fluid actually occurs. Is this material continually penetrating to the depths of the uninvolved lobes in all cases of pneumonia to a greater or lesser degree but in animals with non-spreading lesions being quickly eliminated, or in such instances does the exudate (probably small in amount) fail to reach the terminal airways? Furthermore we have no information as to whether the intrabronchial implantation of infected edema fluid is capable of producing secondary lesions without the aid of other predisposing factors.

The following experiments were undertaken with the purpose of elucidating the manner in which interlobar spread takes place.

Methods

Production of Pneumonia.—Young, healthy dogs were infected with a highly virulent strain of pneumococcus Type I (A_5) suspended in a 6 per cent arrowroot starch-broth mixture and injected into a terminal bronchus through a radio-opaque catheter according to the method previously described (3). The temperature and pulse of each dog were taken at least twice before infection, and the white blood cells counted. Dogs showing fever, tachycardia, or leucocytosis were rejected. Temperature of 102.9°F., pulse rate of 130, and white blood count of 25,000 were considered the upper limits of normal. Temperature and pulse were recorded at the end of 24 hours, and, in survival experiments, twice daily. Roentgenograms of the chest were taken in all cases at the end of 24 hours, and repeated when indicated in survival experiments.

For the purpose of producing bronchial obstructions sterile starch, such as used in the initiation of infection, or viscid mucin suspension was injected into a terminal bronchus. The animal mucin was prepared according to the method of Nungester and Jourdonais (4).

In order to produce single lobe lesions a dose was selected, 0.01 cc. or 0.02 cc. of culture suspended in 1 cc. starch-broth mixture, which has been found to induce pneumonia regularly, but with unilobar lesions only in the majority of dogs (5). A few animals develop more extensive lesions following this dose. In the second part of the study much smaller infecting doses were employed. Multilobar lesions were produced with the same amount of pneumococcus culture (0.02 cc.) suspended in 6 cc. starch-broth mixture, which was then deposited in two or three sites (2 or 3 cc. in each) in a single lobe. Animals infected with this dose ordinarily show, at the end of 24 hours, lesions in more than one lobe and frequently metastatic lesions on the opposite side (5).

Method of Sacrificing Animals.—The animals were sacrificed by the rapid intravenous injection of pentobarbital, about 600 mg. being used. They die quickly, without struggle, within a few seconds after injection of the drug.

In the performance of autopsies, the trachea was clamped in the neck before the thorax was opened. The lungs were then examined and cultured in the inflated state. Cultures made under these conditions were less liable to contamination from other lobes or other lesions in the same lobe than when the lungs were collapsed. Cultures were always made of the heart's blood, and of all except the very smallest lesions. The method of culture of normal lung tissue is described later.

Microscopic Sections.—Sections were fixed in Zenker-acetic acid (5 per cent), and stained with Maximow's hematoxylin-eosin-azure II, as well as with Wallace's modification of Gram-Weigert's stain (6).

I. Attempts at Artificial Production of Secondary Lesions

It was thought that if, during the height of pneumonia, small amounts of edema fluid reached lobes in which nevertheless metastatic lesions did not develop, it might be possible to trap such material in the lobe, prevent its elimination, and induce infection there.

(*a*) *Injection of Starch into a Normal Lobe at the Height of Pneumonia.*—24 hours after the initiation of infection, 2 cc. or 3 cc. of sterile starch were deposited in the terminal bronchus of the opposite lower lobe of seven

TABLE I

Effects of Injection of 2 to 3 Cc. Sterile Starch into Opposite (Normal) Lower Lobe of Dogs with 24 Hour Unilobar Pneumococcus Lesion

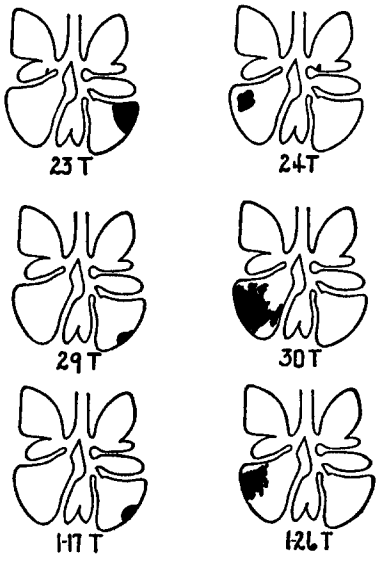
Dog No.	Amount of starch injected	Extent of lesion in starch-injected lobe 24 hrs. later		Culture of lesions	Outcome
		X-ray	Autopsy		
	cc.				
82 S	3	No lesion		—	No further change. Recovered 3 days
83 S	3	? very small lesion	—	—	Died 12 days with contaminating infection
60 S	3	⅓ lobe	—	—	No further change. Recovered 4 days
1-99 T	2	⅓ lobe	⅓ lobe	Initial les. = pn. Starch les. = 0	
2-10 T	2	Very small lesion	Lesion 3 × 3 cm.	Initial les. = pn. Starch les. = 0	
2-59 T	2	No lesion	Lesion 2 × 3 cm.	Initial les. = pn. Starch les. = 0	
59 S	2	? very small lesion	—	Consol. lobe = pn. Starch les. = 0	Killed 5th day. Starch lesion = 1 cm. diameter

dogs with single lower lobe lesions. Three of the dogs were sacrificed 24 hours after the injection of the starch. The results of such an experiment in seven dogs are shown in Table I. Two animals developed a lesion suggestive of lobar consolidation but involving not more than ⅓ of the lobe, whereas the other five showed either a very small patch or no lesion at all in the lobe where the starch had been deposited. Four of these animals were allowed to recover, but a comparison of the roentgenograms with those of uninfected dogs injected with sterile starch indicates that the lesions probably represent simply a reaction of the lung to the starch, and not a pneumococcus pneumonia. In the three dogs sacrificed, the starch lesions

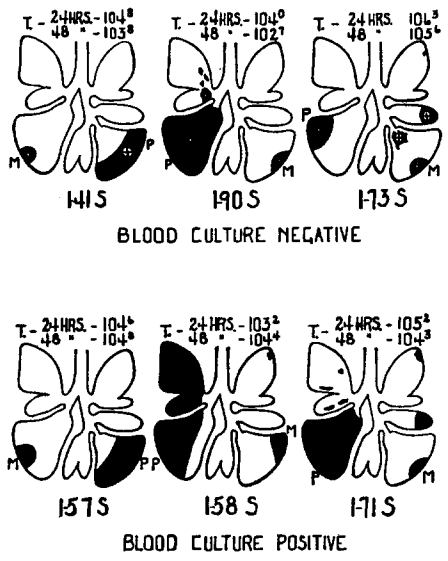
were sterile, and were similar to those seen in normal dogs injected with sterile starch alone (Text-fig. 2).

Effect of Sterile Starch on the Lung.—

The effect of the injection of 2 cc. or 3 cc. of sterile starch into a lower lobe of six normal dogs was then investigated. None of the animals developed fever. Two dogs developed leucocytosis. The dogs were sacrificed at the end of 24 hours. Gross consolidation of varying extent was



TEXT-FIG. 2



TEXT-FIG. 3

TEXT-FIG. 2. Pulmonary lesions produced in normal dogs by the intrabronchial injection of sterile starch.

TEXT-FIG. 3. Effect of injection of mucin into a normal lobe at the height of pneumonia.

P = primary lesion. M = mucin-injected area. + = pneumococci recovered on culture. 0 = no pneumococci recovered on culture.

found in every dog (Text-fig. 2). The consolidated areas (marked in black on the diagrams) were firm and dark red. The amount of edema present varied somewhat, though the lesions tended to be rather dry. Two animals (24 T, 23 T) showed very small patches of discoloration in the opposite upper lobe. The lesions were sterile in five dogs, but Gram-negative bacilli were recovered from the lesions of 23 T. The heart's blood was sterile in all six dogs.

Microscopic examination revealed an acute pneumonitis, in every instance, the predominant cell of which was the polymorphonuclear leuco-

cyte. However, macrophages had begun to appear in fair numbers and were active in engulfing fragments of fibrin and erythrocytes. The amount of fibrin in these lesions was variable, and not characteristic. The most striking characteristic of the starch lesion was the amount of hemorrhage, which was usually in excess of that found in pneumococcus lesions. The amount of edema was moderate.

(*b*) *Injection of Mucin into a Normal Lobe at the Height of Pneumonia.*—Since mucin has been used successfully as a suspending medium (4) and plugging material (5) in the production of experimental pneumococcus pneumonia, has a viscosity greater than the starch paste, and may per-

TABLE II

Effects of Injection of 3 Cc. Sterile Mucin into Opposite (Normal) Lower Lobe of Dogs with 24 Hour Unilobar Pneumococcus Lesion

Dog No.	Extent of x-ray lesion in mucin-injected lobe 24 hrs. later	Outcome
93 S	Very small lesion 1–2 cm. diameter	No further increase in size of mucin lesion. Dog died hemolytic streptococcus infection on 7th day
1-30 S	Very small lesion 1–2 cm. diameter	No further increase in size of mucin lesion. Dog recovered 4 days
1-34 S	Small lesion 2 cm. diameter	No further increase in size of mucin lesion. Dog recovered 6 days
91 S	⅓ lobe	Developed bacteremia at 48 hrs. and showed spread to opposite upper lobe. Mucin lesion became lobar consolidation at 96 hrs. Recovered 6 days
81 S	Whole lobe	No increase in size of lesions after 48 hrs. Recovered 4 days
86 S	Whole lobe	No increase of lesions after 48 hrs. Recovered 3 days

haps enhance the virulence of small numbers of pneumococci,[2] it was considered possible that this substance might be more effective than starch in the artificial initiation of metastatic lesions. Accordingly 3 cc. of sterile mucin were injected into normal lower lobes of each of twelve dogs, 24 hours after infection with the same size dose used in the starch experiments, *i.e.* one which ordinarily results in a monolobar lesion.

The results in the first six animals (not sacrificed) are shown in Table II. Two of them at 24 hours showed roentgenographically, lesions indicative of lobar consolidation in the "mucin lobe," though the clinical course of these animals did not differ essentially from that of other dogs infected with

[2] Nungester, Jourdonais, and Wolf (7) believe that mucin enhances the virulence of many kinds of bacteria.

the same dose but without the additional injection of mucin on the opposite side. Their blood cultures remained sterile. The x-rays of three of the remaining four animals showed small patches at the site of mucin injection, compatible with the shadows cast by lesions due to the injection of a similar amount of sterile mucin in normal dogs. The sixth dog (91 S) developed a moderate bacteremia, exhibited spread to the opposite upper lobe (left), and the shadow in the mucin-injected lobe gradually increased to involve the whole lobe by the 4th day, although the consolidation was never intense. This animal recovered.

The other six dogs were sacrificed 24 hours after the injection of mucin (Text-fig. 3). In all these animals the x-ray showed only a small shadow at the site of mucin injection and at autopsy, also, the lesion was restricted to a patch of consolidation not exceeding 3 × 4 × 4 cm. Though the dose chosen usually produces simple unilobar lesions, four of these six animals showed spontaneous spread or bacteremia or both. It is noteworthy that despite these complications, lobar spread was not produced in the lobe where mucin was deposited. The patches were no larger than those produced by the same amount of mucin in normal dogs. It was not possible to draw conclusions from lung cultures of bacteremic dogs, but it will be observed that in the three dogs whose blood cultures were negative, the mucin lesions were sterile. One of these animals (1-90 S) had cleared its lungs of pneumococci by the end of 48 hours, though at the time the attempt was made to produce spread, its temperature was 104°F.

Injection of Mucin into a Normal Lobe Immediately before Infection.—At the time the previous experiments were carried out, it was considered important to observe the effect of mucin injected into a normal lobe just before infection of the opposite lower lobe, instead of at the height of pneumonia. Each of the five dogs thus treated showed only a small shadow at the site of mucin injection when roentgenograms were taken at the end of 24 hours, but a good lobar lesion on the other side, where the usual inoculum of pneumococci in starch had been placed. All the animals made uneventful recoveries without evidence of metastatic lesions or bacteremia.

The microscopic appearance of the mucin lesions differed considerably from those produced by a similar amount of sterile starch. 2 cc. of starch usually led to more hemorrhage, edema, and damage to the alveolar walls than did 3 cc. of mucin, and 3 cc. of starch was occasionally followed, in 24 hours, by actual necrosis of lung tissue. This was not seen in any of the mucin lesions.

These experiments show that it is not possible to produce secondary lesions regularly by the injection of sterile plugging or traumatizing substances into the uninvolved lobes at the height of lobar pneumonia. In only three of 19 dogs was there x-ray evidence of such interlobar spread. Even in dogs showing spontaneous spread, metastatic lesions did not develop at the site of injection of starch or mucin. Moreover, it must be remembered that the very technique of depositing these substances in the lung involves traversing the already contaminated trachea with the catheter. In some of the experiments the catheter was first actually introduced into the bronchus leading to the infected lobe, but even then, a sufficient number of organisms was presumably not carried over to the opposite lobe to lead to pneumonia. It is significant, also, that in four dogs with bacteremia, pneumonia did not develop at the site of mucin injection.

II. Distribution of Pneumococci in Lungs of Dogs with and without Spread

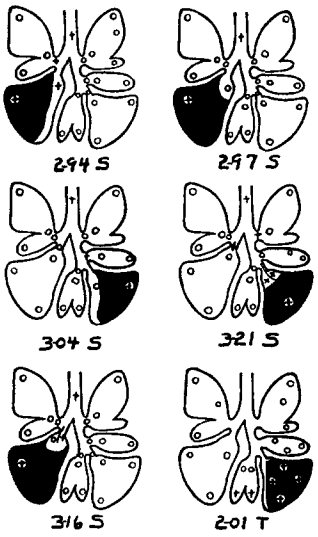
The failure to produce spread with any degree of regularity by the introduction of foreign substances into an uninvolved lobe led us to investigate the distribution of pneumococci in various parts of the lung during the height of pneumonia. The animals in this group fall into two categories: those in which the lesion was restricted to one lobe, and those in which spread had occurred spontaneously. As described earlier, different sized inocula were employed to produce monolobar and multilobar involvement. Only dogs with negative blood cultures are reported.

Methods.—The dogs were sacrificed 24 hours after infection. Two methods of lung culture were employed. In most of the dogs, the thorax was opened under aseptic conditions, following clamping of the trachea in the neck. With the lungs *in situ*, small pieces of tissue, weighing 0.08 to 0.45 gm., were removed by scissors from each of three widely separated areas of each lobe, but in every instance, from the peripheral portion, close to the pleural surface. The tissue was immediately placed in ordinary culture tubes containing about 4 cc. serum-dextrose broth, and incubated at 37°C. Microscopic examination of similar samples showed them to contain, in addition to the alveolar spaces, a few respiratory bronchioles and rarely a small terminal bronchiole.

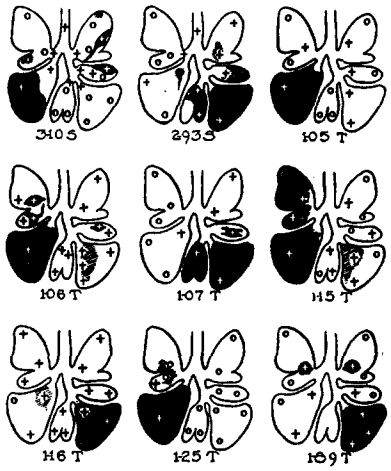
In some of the dogs, the lungs were removed from the body as soon as the trachea was clamped and the heart's blood cultured, and suspended in the inflated condition from a ring stand. Cultures were then made by inserting a finely drawn pipette into the lung substance a few millimeters beneath the pleura. A small amount of material was thus obtained and immediately transferred to a culture tube containing serum-dextrose broth. A separate sterile capillary pipette was used for each culture. In removing lung tissue for culture, the scissors and forceps were flamed after the removal of each sample. In later experiments, separate sterile scissors and forceps were used for the excision of each bit of tissue.

In some of these animals, cultures were also taken from the main bronchus to each

lobe. The technique was simple. The exterior of the trachea was painted with iodine, the lumen exposed with a sterile pair of scissors, and a culture taken from this area. In order to obtain an uncontaminated culture from the lumen of a bronchus, a small sterile glass tube was placed in its mouth, and a wire loop passed through the tube about a centimeter or two down the bronchus itself, where a culture of material from the wall was made. In most instances, a small amount of visible but colorless material was obtained from the bronchi of the uninfected lobes. Care was taken not to introduce material from the trachea.



TEXT-FIG. 4



TEXT-FIG. 5

TEXT-FIG. 4. The distribution of pneumococci in the lungs of dogs with unilobar lesions. + = positive cultures. 0 = negative cultures.

TEXT-FIG. 5. Distribution of pneumococci in the lungs of dogs showing multiple lesions.

Solid black represents even consolidation. Hatched line areas indicate patchy irregular involvement.

(*a*) *Dogs with Unilobar Lesions.*—

The results in the six dogs of this experiment are shown in Text-fig. 4. Pneumococci were recovered from the lesion, but not from any other portions of the lung. The only exception was dog 2-01 T, the periphery of whose normal postcardiac lobe yielded pneumococci. Cultures of the walls of the normal lobe bronchi were sterile in many instances.

In three of the dogs, the cultures from normal lobes were made with sterile capillary pipettes: in three, fragments of tissue were cultured from each lobe, and in one of the three, from each of two or three areas of every lobe. The cut surfaces of the consolidated lobes in all six animals were moist but in no instance wet.

(*b*) *Dogs Showing Multiple Lesions.*—

In striking contrast to the dogs with unilobar lesions is the series of nine animals showing spread to other lobes at the end of 24 hours (Text-fig. 5). Most of these dogs had been infected with doses calculated to lead to spread, but two animals in this series (1-25 T, 2-93 S) developed small or moderate sized metastases from a dose that usually causes pneumonia limited to one lobe. Dog 1-05 T is included because, though the only grossly consolidated tissue was that in the inoculated lobe, some of the unconsolidated lung appeared edematous and proved to be the seat of microscopic lesions. The cut surfaces of primary lesions and many of the metastatic ones were very wet; often dripping edema fluid.

Gross involvement of the lung of a dog always leads to discoloration that immediately differentiates it from the salmon-pink color of normal tissue. In this group of experiments, pneumococci were recovered not only from the tissue infected in the gross, but also from tissue apparently normal in other parts of the lung. This applied equally well to normal looking tissue in other parts of the same lobe, in other lobes on the same side, and to lobes on the opposite side. Not only were the cut surfaces of the cultured samples themselves normal in the gross, but the contiguous cut surfaces, from which the microscopic sections were taken, were also normal in appearance.

The number of positive cultures obtained from tissue normal in the gross varied. In dog 1-25 T, for example, only one such positive culture was obtained, whereas in dog 1-16 T, pneumococci were recovered from every sample of apparently normal tissue cultured. The average number of positive cultures from apparently normal tissue was 4.5 per dog.

Microscopic Pathology.—The microscopic examination of these metastatic lesions exhibited pneumonia in every stage of its development. Most, but not all, of the tissue normal in the gross from which pneumococci were cultured was microscopically normal and pneumococci were not present in sufficient numbers to be seen. In a few sections, pneumococci were present. They were found lying free in edema fluid within the alveoli and along the alveolar walls. By focusing one could determine that the organisms lay outside the septa. None were observed to be within the capillaries. Their extramural position was clearly marked in certain alveoli where a thin layer of pneumococcus-containing edema fluid was adherent to the alveolar wall. Except for possible dilatation of the capillaries in these latter regions, there was no evidence of inflammation. Polymorphonuclear leucocytes were not present, though a rare alveolar macrophage had taken up one or more pneumococci. No pneumococci were seen in walls of bronchi or in lymphatics.

In certain areas small, compact masses of deeply staining exudate containing pneumococci and a few white and red blood cells were seen in the smaller bronchioles, the respiratory bronchioles, alveolar ducts, and in places extending into the alveoli, although

the surrounding tissue was normal (Figs. 1 and 2). Much larger masses of this same material were occasionally observed to be present in medium sized bronchi.

In areas where the inflammatory reaction had begun pneumococci were present in considerable numbers. Many alveoli, although filled with edema, contained no cellular exudate; in some the polymorphonuclears had begun to appear. By the time cellular exudate of any extent was detectable microscopically, the lung was no longer normal in the gross. In none of these early lesions was there evidence of interstitial inflammation and with very rare exceptions pneumococci were not seen in the walls of the bronchi or blood vessels. It was only in certain sections exhibiting great numbers of pneumococci that an occasional microorganism was detected in the perivascular and peribronchial lymphatics.

DISCUSSION

Aside from providing a probable explanation for our failure to induce metastatic lesions by plugging the terminal bronchi in normal lobes of dogs infected with doses that produce only monolobar involvement, the foregoing experiments contribute considerable information on the rôle played by edema fluid in the dispersal of pneumococci from the initial focus of infection. The widespread distribution of pneumococci found throughout the lungs of dogs with multilobar involvement in contrast to the sterility of the uninvolved lung tissue of the animals with single lobe lesions could be accounted for by the relative wetness of the initial lesions in the two series of dogs. The bronchoscopic observation of foamy exudate in the bronchi of living animals with spreading lesions indicates that a considerable amount of edematous fluid is being discharged from such areas of infection. Similar observations in dogs with well localized single lobe lesions have revealed at most, flecks of thick tenacious exudate in the bronchus of the involved lobe. From what we know of the rapidity with which fluids introduced into the trachea of normal animals penetrate to the depths of the lung (8, 9), it appears probable that the greater the fluidity of the pneumonic exudate the less effective is its elimination through the trachea.

The finding of infected cell-poor exudate in the terminal airways of the normal lung tissue in dogs exhibiting metastatic lesions elsewhere, provides direct evidence for the manner in which pneumococci are transferred from the initial lesion to other parts of the lung. How frequently such deposition of infected exudate results in a new focus of infection, we do not know, but our microscopical sections reveal all stages of the beginning inflammatory process from the presence in the alveoli of compact masses of pneumococcus-containing exudate without evidence of tissue reaction, to the characteristic development of the initial pulmonary lesion. The first apparent change from the normal consists in the distribution of pneumo-

cocci along the surface of the alveolar wall where they can be seen sometimes to lie in a thin layer of edema fluid adherent to the walls but more often no fluid is visible. Whether this thin fluid layer represents the beginning of the inflammatory reaction we cannot say but at any rate, it seems likely that the pneumococci are carried by this means along the alveolar surface and through the pores of Cohn into adjacent air sacs as was clearly demonstrated by Loosli (10) in his study of the early primary lesion. Very shortly thereafter an exudation of edema fluid into the air spaces occurs, at first non-cellular, but followed soon by the appearance of leucocytes, principally neutrophiles. These observations on the nature of the initial response of the lung tissue to the presence of pneumococci are in harmony with Rhoads and Goodner's (11) finding of an early outpouring of edema fluid in dermal pneumococcus infection in rabbits, and are such as one might expect in view of demonstration by Sutliff and Friedemann (12) of an edema-producing substance in filtrates of young pneumococcus cultures.

The subsequent changes in the lesion consisting of a gradual migration of leucocytes and erythrocytes into the locus of infection are in every way similar to those which we have described elsewhere (2) as occurring at the spreading margin of lesions initiated with starch-suspended pneumococci.

The experiments in which starch and mucin were employed to plug the terminal bronchi of normal lobes in dogs with well localized single lobe lesions, throw relatively little light on the rôle played by obstruction in the inception of pneumonia. They do suggest, however, that local interference with the normal eliminatory mechanism may not be the principal factor in determining whether or not infection takes place. The numbers of pneumococci deposited in the normal sterile lung by the contamination of the catheter in its passage through the trachea were probably very small. However, it seems not unlikely that microorganisms may well have been present in the injected lobe in certain of the experimental animals exhibiting spread to other lobes at the time of the test but which nevertheless did not develop a lesion at the site of the obstructing mass. While the deposition of starch or mucin in an uninvolved lobe was relatively seldom followed by a pneumonic process in this region, the presence of such injected mucin in the lung of dogs with pneumonia was associated with an increased incidence of bacteremia and secondary lobe lesions elsewhere, a phenomenon not observed in the starch-injected dogs. This finding would lend some support to the conclusion of Nungester, Jourdonais, and Wolf (7) that mucin enhances the pathogenicity of the pneumococcus and it may possibly be related to the recent observation of Kempf and Nungester (13), namely that intravenously injected pneumococci localized

in the lungs of mice which had received a previous intrabronchial inoculation of sterile mucin. On the other hand, mucin injected into the opposite lower lobe of the dog at the time of infection had no such effect nor have we observed any constant difference in the mortality rate of dogs infected with starch-suspended pneumococci and those in whom infection was produced by the injection of pneumococci suspended in gelatin-Locke's solution followed by 2 to 3 cc. of mucin.

Data concerning the numbers of pneumococci necessary to induce pulmonary infection in the dog, their optimum locus of distribution, and the respective rôles played by obstruction and local irritation will be presented in subsequent studies.

The second communication, immediately following, represents a further investigation of the rôle played by pneumonic exudate in the production of interlobar spread.

SUMMARY

A study has been made of the mode of origin of the secondary lesions occurring spontaneously during the course of experimental pneumococcus pneumonia in the dog. It was observed that the primary lesions of dogs exhibiting interlobar spread contained much more edema fluid than did those in which the inflammatory process remained confined to a single lobe. Furthermore, the sequence of spread from lobe to lobe in relation to the anatomical arrangement of the bronchi and the prone position of the animal was such as to suggest that secondary lobe involvement arose as a result of edematous pneumonic exudate flowing into the more dependent bronchial openings.

Experiments were undertaken to determine whether pneumococci are constantly being distributed throughout the lung in the experimental disease in varying degree yet produce secondary foci of consolidation only if the microorganisms reach the terminal airways and are retained there through some interference with the normal eliminatory mechanism. Attempts to produce secondary lesions in dogs with non-spreading single lobe involvement, by means of plugging a terminal bronchus of a normal lobe with starch paste or mucin were largely unsuccessful. In only three out of 19 instances did a lesion develop at the site of obstruction. An investigation was then made of the distribution of pneumococci in the lungs of dogs at the height of the pneumonia. In dogs with single lobe lesions pneumococci were recovered from the lesion itself but not from any other part of the peripheral lung tissue, whereas in animals showing spread to other lobes pneumococci were found to be distributed widely throughout the

lung in both the apparently normal and the involved lobes. Some of the microscopic sections of the uninvolved parts of the lungs of dogs with metastatic lesions revealed small masses of pneumococcus-containing exudate in the smaller bronchi and terminal airways of otherwise normal tissue. This finding, in conjunction with the detection of beginning inflammatory changes in other areas normal in the gross, would seem to provide direct evidence of the manner in which pneumococci are transferred from the initial lesion to other lobes and highly presumptive evidence for the mode of origin of the secondary lesions.

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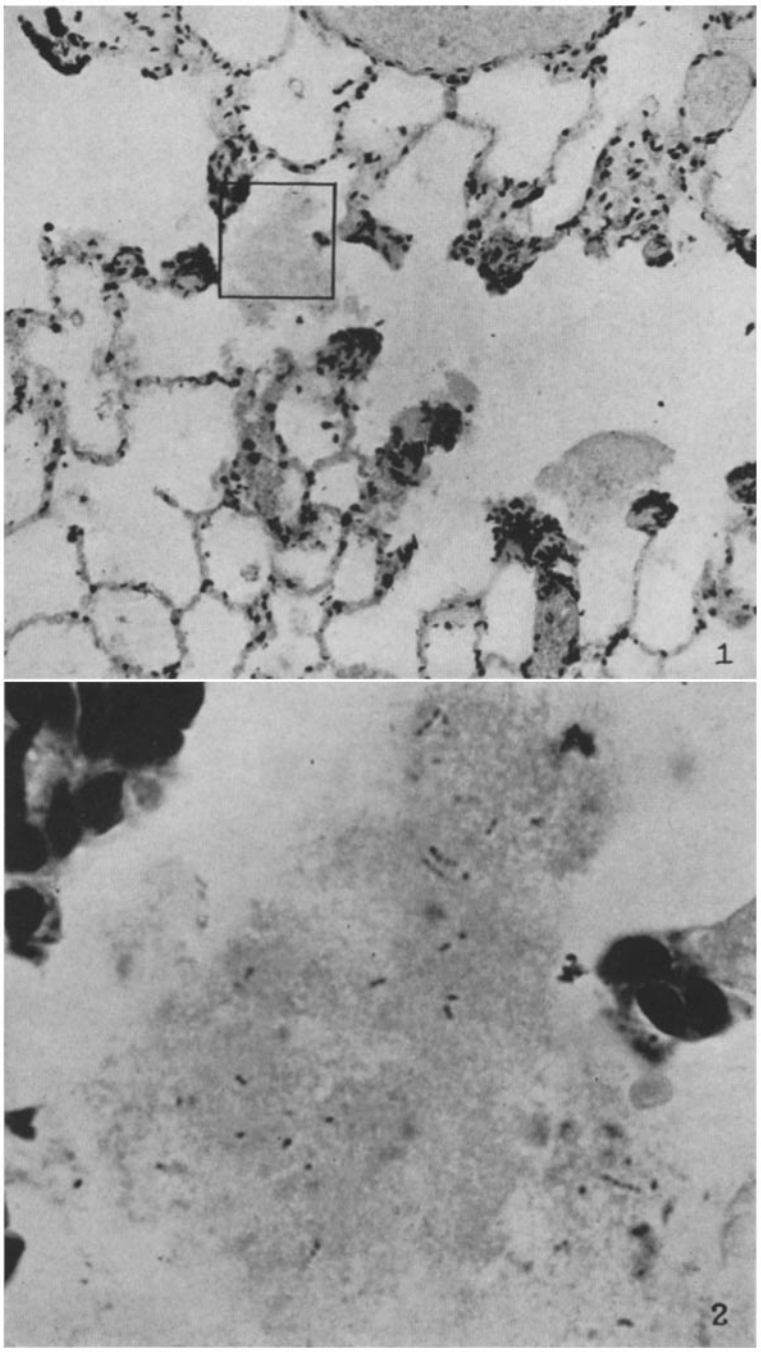
EXPLANATION OF PLATE 6

Tissues were fixed in Zenker-formol solution, embedded in paraffin, and stained by Maximow's hematoxylin-eosin-azure II method.

FIG. 1. Photomicrograph of uninvolved lobe of dog exhibiting secondary lesions elsewhere in lung. Section through respiratory bronchiole which contains several masses of exudate one of which is marked by a square. The surrounding lung tissue is normal in appearance. × 200.

FIG. 2. Oil immersion view of exudate mass in Fig. 1 marked by square. Shows many pneumococci and only a very occasional leucocyte and red blood cell. × 1300.



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