

EXPERIMENTAL BRONCHOPNEUMONIA BY INTRA- BRONCHIAL INSUFFLATION.*

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PLATES 9 AND 10.

It is now generally assumed that the pathogenic organisms present in the lesions of the various forms of pneumonia form only one link in the chain of conditions which give rise to the development of these diseases. The natural or acquired specific resistance of the individual and of the species, the state of vitality of the individual and of the organ under consideration, are factors which, it is believed, determine the outcome of a bacterial invasion. This view was strengthened by the frequent failures in the attempts to produce pneumonias experimentally by using pure cultures of the corresponding microorganisms; and, in the case of bronchopneumonia, this view certainly seems quite plausible, since in most instances this form of pneumonia is associated with some other pathological condition. Recently, however, in forty-two out of forty-four dogs, Lamar and Meltzer¹ succeeded in producing lobar pneumonia with pure cultures of the pneumococcus. The injection of the cultures was made by a new method, that of intrabronchial insufflation. The two failures were due to some faulty step in carrying out the new technique. In the experiments of Lamar and Meltzer the animals were neither selected nor prepared in any manner, and the microorganism was, apparently, the essential factor in the causation of lobar pneumonia. The favorable outcome of these experiments suggested the advisability of applying the same method to the action of organisms usually found in association with bronchopneumonia.

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¹Lamar, R. V., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xv, 133.

EXPERIMENTAL PART.

We have carried out a series of intrabronchial insufflations with the streptococcus and the influenza bacillus. In its essentials the method consists in introducing a tube through the mouth, larynx, and trachea, deeply into a bronchus, and then injecting the culture through the tube. The details of this method are recorded in the paper of Lamar and Meltzer.²

Experiments with the Streptococcus.—The virulence of several strains of streptococci isolated from cases of empyema and bronchopneumonia was tested in white mice, and the strain finally chosen was obtained at autopsy from the heart's blood and lungs of a fatal case of bronchopneumonia. When inoculated intraperitoneally in doses of 0.5 of a cubic centimeter of a twenty-four hour bouillon culture, this strain caused in twenty-four hours the death of a mouse weighing fifteen grams. Cultures of this organism were injected intrabronchially in twenty dogs in quantities varying from five to thirty cubic centimeters. In some experiments the cultures were enriched ("angereichert") by centrifugalizing them and resuspending the organisms. In this way the quantity of culture injected was made to contain two to six times the number of cocci present in the uncentrifugalized broth.

Of the twenty animals experimented with, one died within twenty-four hours. This was the only case in which the pneumonic lesion contained, besides the streptococcus, the bacillus of distemper. The other nineteen dogs were killed at intervals varying from twenty hours to six days. All had typical pneumonic lesions in various stages of progression and resolution depending upon the time elapsing between the injection and the killing of the animal. These lesions contained the streptococcus in pure culture.

The clinical course in these experiments was mild and of short duration and did not differ greatly from that described by Lamar and Meltzer for the non-fatal cases in the experimental infection with the pneumococcus. A few hours after the intrabronchial insufflation the temperature of the animal rose in some instances to above 40.4° C., but it came down, as a rule, on the following day and the animal soon appeared practically normal.

²Lamar, R. V., and Meltzer, S. J., *loc. cit.*

Lesions.—In the dogs killed from twenty to twenty-four hours after injection, the areas of consolidation were limited usually to the right posterior lobe, but in addition they were sometimes scattered in the subcardiac and upper lobes of the same lung, and were occasionally found in the other lung. The extent of the consolidation varied with the size of the dose injected; following a small dose the lesion involved sometimes only a small and superficial area, while after a large dose as much as half the lobe might be consolidated. The lungs were pink in color, contrasting with the dark red of the consolidated areas which were distinctly lobular in distribution, aerated lobules being discernible between the solid ones. The section was moist, smooth, and mottled, passing gradually by an irregularly ragged line into the aerated lung substance of the rest of the lobe. The pleura was not inflamed in any case, though its luster was impaired in several instances. In no case was there a central pneumonia. The solid areas and the lung adjacent to them were always deeply congested. Edema was slight. Smears from the solid areas showed many streptococci. Cultures from these areas gave a fairly profuse growth. From the uninvolved lung and from the heart's blood there was no growth.

After forty-eight hours the areas of consolidation were larger than after twenty-four hours, thus showing that the lesion was a progressive one. The dogs killed at the end of two days showed an extensive area of consolidation in the right posterior lobe, as much as two thirds or three fourths of it being solid, and usually there were consolidated areas in the right middle, left posterior, and left upper lobes also. The adjacent bronchial lymph nodes were but slightly swollen, were red in color, and were moist on section.

On the third day resolution was going on and the lungs showed consolidated areas of narrow and superficial extent only, the periphery shading gradually through a more or less congested area into the aerated lung substance.

On the fourth and fifth days resolution had progressed so that only a dark discoloration was left to outline the areas originally consolidated. In only one case was a narrow strip of quite solid pneumonia found on the sixth day, and to this animal the very large dose of thirty cubic centimeters had been given.

The right lung was involved in nineteen of the twenty cases. The left lung was also involved in eight instances, and in one case only was the pneumonia limited to the left posterior lobe. In twelve cases the areas of consolidation were scattered in two or more lobes of the lungs, and in a general way it may be said that the larger the quantity of culture fluid injected, the more scattered and the more extensive were the lesions; and the more numerous the organisms, the more severe the lesion. The enriched ("angereichert") cultures produced a more diffuse and intense inflammation, but did not delay resolution, for on the fifth day one case which had received a culture enriched six times was completely resolved. Streptococci were abundant in the smears from the consolidated areas on the first and second days and grew well in cultures. On the third day they were no longer profuse, and no growth was obtained on the fourth day. In no instance did the heart's blood give a growth of streptococci.

Microscopic Examination.—On microscopic examination within twenty-four hours the picture varied according to the size of the dose injected. In the least severe case the animal received only five cubic centimeters of culture. In this instance the exudate consisted chiefly of large mononuclear cells (the desquamated epithelial cells of the alveolar walls), with comparatively few polymorphonuclear leucocytes, and very little fibrin. The capillaries in the alveolar walls were intensely congested, but no red blood cells were present in the alveolar contents. The cells lining the bronchial walls were intact and no inflammatory infiltration was noted in these walls. The most densely packed solid alveoli were grouped around the smallest bronchioles, and in the areas which were the most solid, polymorphonuclear leucocytes were relatively more numerous than in the less solid areas, which, at the periphery of the lesion, shaded into lobules whose alveoli contained only granular coagulated serum and a few epithelial cells. Cocci were present in the alveoli in large numbers, and also in the bronchi,—many of which contained masses of exudate, evidently sputum.

A larger dose, fifteen cubic centimeters, had called forth a far more solid lesion in which the leucocytes were decidedly more numerous, and in which the walls of the smaller bronchi were distinctly

infiltrated. A still larger enriched dose, thirty cubic centimeters, had produced a very marked purulent infiltration, not only of the walls of the alveoli and bronchioles, but of the connective tissue septa, and even of the adventitia of the blood-vessels. There was very little fibrin present. The cocci were numerous.

On the *second* day the relative severity of the lesion had increased greatly, both in quantity and in quality. Thus the leucocytic infiltration had extended so that all the structures in a solid lobule were uniformly involved,—the alveoli, bronchi, blood-vessels, and septa—and the section gave a general impression of pus. This infiltration was most marked in the dog to which the largest dose had been administered. The desquamated epithelial cells were present in the alveolar exudate, but the leucocytes outnumbered them. Only a very small amount of fibrin could be detected. Beyond the solid areas some alveoli contained red blood cells, and all the capillaries were congested, as well as the smaller bronchial vessels. Cocci were still numerous, but phagocytosis was not marked.

On the *third* day resolution was progressing so rapidly that an area of bronchopneumonia caused by a small dose (five cubic centimeters) showed scarcely any packed lobules. There were very numerous examples of macrophage activity and a granular mass was present between the cellular remnants. In the more severe cases distinctly solid alveoli remained about a bronchiole, while the periphery of the lobules was becoming emptied of their exudate by phagocytosis and cell degeneration. Cocci were still present at this time.

On the *fourth* day only a few solid alveoli remained close around the bronchioles, and on the *fifth* day none were seen. Only a granular mass with a few leucocytes and epithelial cells was left in the alveoli, but the septa were still edematous and infiltrated with leucocytes, as were the alveolar walls. On the *sixth* day only one case was examined. This animal had received a large dose (thirty cubic centimeters), and in its lungs some well preserved exudate was seen in the alveoli around a bronchus, while almost all of the area that had previously been solid had undergone resolution.

There was no evidence of organization in any of these cases.

It is well to bring out here the difference between the pneu-

monia produced in the experiments of Lamar and Meltzer with the pneumococcus, and the pneumonia produced in the present series of experiments with the streptococcus. (1) In the pneumococcus infections there was a mortality of at least 16 per cent.; in our experiments there was practically no mortality. (2) In the pneumococcus infections there was a pneumococcus septicemia in all the fatal cases; no bacteriemia occurred in our streptococcus cases. (3) In the pneumonia lesions from the pneumococcus, the consolidated part of the lung was always air-free and lobar in character, no matter how small or large the affected area was; in the pneumonia lesions produced by the streptococcus infection, no matter how large the area, or how intense the inflammatory process was, aerated lobules were always discernible between the solid one; *i. e.*, the streptococcus pneumonia was "lobular." (4) In the pneumococcus pneumonia there was always a definite pleurisy present even in all the non-fatal cases, while it was practically completely absent in the twenty cases of the streptococcus infection. (5) In the pneumococcus infection the cut surface of the consolidated areas was rather dry and granular; in the streptococcus infection it was very moist and smooth. (6) Fibrin was an important element in the exudate of the lesions brought about by the pneumococcus infection, while it played practically no part in the pneumonia consolidation in our experiments. (7) In the pneumococcus pneumonia the walls of the bronchioles, although filled with exudate, were free from infiltration with leucocytes; while in the streptococcus pneumonia the bronchioles were intensely infiltrated by the leucocytes, especially in the more severe lesions. (8) Resolution progressed much more rapidly in the streptococcus pneumonia than in the pneumonic lesions produced by the pneumococcus.

The most essential points are the differences in the nature of the consolidation and in the fibrin formation.

ILLUSTRATIVE PROTOCOLS.

I. *Dog 2*.—Fox terrier, male; weight, 4,350 gm.

April 26, 1911, 12:30 P. M. Under ether anesthesia, a 5 c.c. dose of a twenty hour plain broth streptococcus culture was injected intrabronchially. 5 P. M. Temperature, 39.9° C.

April 27, 10 A. M. Temperature, 39.4° C.

April 28, 11:30 A. M. Temperature, 39.2° C. The animal at this time did not appear sick, but was killed with chloroform.

Autopsy.—The upper lobe of the left lung was normal. The posterior lobe was congested. The right posterior lobe was larger than any other, was swollen, and in its posterior third contained solid areas which involved the base also. There were a few very small and superficial solid areas in the right middle lobe. The pleura was quite normal. The bronchial lymph nodes on the left side were normal. On the right side they were a little enlarged, red in color, and very moist on section. On section the consolidated lung was smooth, patchy, red, and very moist. A thin red fluid exuded readily from the cut surface. The trachea was normal; the mucosa of the bronchi was reddened, and contained frothy, blood-stained fluid. Smears from the solid area showed numerous streptococci. In cultures from the fluid area a growth of streptococci developed, but there was no growth in the cultures from the heart's blood and from the left upper lobe.

Sections from the consolidated area showed that the alveolar exudate consisted of some large mononuclear (epithelial) cells and of a larger number of polymorphonuclear leucocytes, many nuclei being fragmented. The alveolar walls were infiltrated with these cells, and so were the walls of the small bronchi, the lumen being plugged with a mass of exudate. No fibrin was present.

II. *Dog 16.*—Yellow male; weight 4,800 gm.

June 6, 1911, 3 P. M. Under ether anesthesia, 30 c.c. of a twenty-two hour plain broth culture of streptococci, enriched to double strength, were injected into a bronchus. 6 P. M. Temperature, 40.4° C.

June 7, 9:30 A. M. Temperature, 39.7° C. 11:30 A. M. Animal killed with chloroform.

Autopsy.—The right posterior lobe was half solid and dark red, contrasting with the pink, aerated anterior portion of the lobe. The right apex and the subcardiac lobe also contained a solid area, and the upper half of the left posterior lobe was solid. The pleura was quite normal. On section the consolidated lung was mottled dark and light red, the light red areas being the smaller; they were aerated, while the dark ones were airless. Congestion and edema were very marked. Frothy fluid exuded from the bronchi. The bronchial nodes on both sides were congested and swollen.

Smears from the consolidated areas showed many streptococci. Cultures from these portions grew well; the heart's blood was sterile. Sections from the consolidated portion gave a general impression of pus; that is, the alveoli were filled with polymorphonuclear leucocytes and some epithelial cells. The leucocytes were numerous in the walls of the alveoli and in the bronchial walls; the smallest bronchioles showed degeneration of their lining cells, and the alveoli nearest to them were very densely packed with exudate in which there were a few short fibrin threads. All the vessels were distended with blood, but there were very few red blood cells in the alveoli.

EXPERIMENTS WITH THE INFLUENZA BACILLUS.

The relative frequency with which the influenza bacillus is found in cases of pneumonia led us to choose this organism for the production of experimental bronchopneumonia in dogs. The influenza bacillus may be present in pure culture in a human pneumonic lung, in which case the lesion is always of the lobular type. It may also be associated with the pneumococcus in lobar pneumonia, but it is found most frequently together with the streptococcus, the staphylococcus, or the pneumococcus in various combinations in cases of lobular pneumonia of the peribronchial type. It seemed a matter of some interest, therefore, to know how the intrabronchial injections of influenza bacilli would affect the lungs of the animal experimented upon.

A strain of the bacillus isolated from a case of leptomeningitis and virulent for rabbits and monkeys was used. It was grown on rabbit blood agar in Blake bottles and washed off in salt solution. The growth of one to three bottles, suspended in five to thirty cubic centimeters of salt solution, was injected. Eleven dogs were experimented upon and none died. It must be remembered that the dog has been found to be very resistant to influenza bacillus infection.

Within five or six hours after the injection of the culture, the dog's temperature rose to about 39.8° C.; in two cases it reached 40.2° and 40.7° C. On the following morning it had dropped to 39° or thereabouts, and then to 38.2° or 36.5° on the third day. In one case it remained about 39° C. for three days. Cough was not noticed and the animals were not apparently ill. They were killed at intervals that varied between twenty-one hours and six days after the injection.

The earliest lesion studied was twenty-one hours old. In this instance a small dose had caused scattered areas of consolidation in all the lobes of both lungs, but only one area was larger than 0.25 of a centimeter in diameter. The largest area was in the right middle lobe and was three centimeters in length. The consolidated part was not deep, but was very solid, though distinctly "lobular" in character. All the solid areas were deep red in color, and there were many hemorrhages in the aerated lung beyond. The pleura was normal.

A more concentrated dose produced in the same time a far more striking lesion. In this case the entire right posterior lobe was solid and small consolidated areas were scattered in the subcardiac and upper lobes as well as in all the lobes of the left lung. These areas were distinctly solid, were dark red in color, hemorrhagic, and moist, but small areas of aerated lobules were still visible between the solid areas. Smears showed many bacilli which grew in cultures. The heart's blood gave no growth.

Forty-eight hours after the injection of the organisms the lesion was of the same character, solid and dark red, not sharply demarcated from the aerated lung around it. Hemorrhagic areas were present in the lung.

In three days resolution was going on, the solid areas were resilient, were smaller in extent, and not so firm. On the fourth day the condition was about the same, while on the sixth day the pneumonia had almost entirely disappeared. At this time, after a concentrated dose, some resolved areas and a solid mass occupying half of the right posterior lobe were seen.

Microscopic Examination.—At the end of the first day the lesion showed that the exudate consisted of epithelial cells peeled off from the alveolar walls, some red blood cells, and many polymorphonuclear leucocytes. The alveolar walls were clearly outlined by the distended capillaries, and were not infiltrated. The bronchial walls were quite normal, and the lumen contained exudate composed of red and white blood cells, granular debris, and many bacilli. Only a very small amount of fibrin was present in the alveolar exudate. In the less solid portions of the lesion, the alveoli contained much granular serum, a few red cells, and some epithelial cells. In the same period of time (twenty-four hours) a more concentrated dose caused a more marked leucocytic exudation. Many of the leucocytes with fragmented nuclei infiltrated the bronchial and alveolar walls. There was very little fibrin; many bacilli were present, and hemorrhages into the alveoli near the solid areas were very numerous.

At the end of the second day the exudate was even more markedly leucocytic in character, and a purulent infiltration was present in all the bronchial walls, interlobular septa, and the alveolar walls. All

the bronchi contained plugs of exudate and around the bronchi were densely solid alveoli. On the third day resolution had begun. The congestion had subsided, the phagocytic cells were numerous in the exudate, and all the leucocytes were undergoing fragmentation. Bacilli were still very numerous.

On the sixth day the lesion produced by a small dose was almost entirely resolved, some granular serum and cell debris remaining in some of the alveoli, while the purulent infiltration had subsided. A more concentrated dose had produced a lesion which, on the sixth day, showed resolving pneumonia, as well as large peribronchial areas of consolidation in which resolution had not even begun.

There was no pleural exudate in any one of these eleven cases. Cultures of influenza bacilli were obtained from the pneumonic areas on the first, second, and third days, but from the heart's blood no growth was obtained at any time.

In the influenzal bronchopneumonia, as in the streptococcal variety, the size of the dose of bacteria injected had a distinct influence on the pneumonic areas that developed. The most extensive and most severe lesions were produced by a larger and enriched dose, and in such a case resolution was also delayed as compared with that of a lesion caused by an injection containing fewer organisms. Hemorrhage and purulent infiltration are characteristic features of this variety of lobular pneumonia.

ILLUSTRATIVE PROTOCOLS.

I. *Dog 27*.—Black and white female; weight 3,700 gm.

June 6, 1911, 3 P. M. Under ether anesthesia, 10 c.c. of a suspension of influenza bacilli which had been grown in two bottles for twenty-four hours were injected intrabronchially. 6 P. M. Temperature, 40.2° C.

June 7, 9:30 A. M. Temperature, 39.6° C. 11:30 A. M. The dog was killed with chloroform.

Autopsy.—The posterior lobe of the right lung was swollen, large, solid, firm, and dark red. The entire lobe was involved. On section, this lobe was hemorrhagic, solid to the touch and moist, and revealed very few aerated lobules among the solid areas. The greater part of the lesion, however, was hemorrhagic rather than inflammatory. The pleura was normal. On the right upper lobe were scattered areas of consolidation. These were also present in the subcardiac and middle lobes. In the left lung there were solid areas in all the lobes. These solid areas were larger near the upper angle of the posterior lobe, and all of them were peribronchial and interspersed among the aerated lobules. The bronchial nodes were deeply congested and very moist.

Smears from the right posterior lobe showed many leucocytes and many Gram negative bacilli. The influenza bacillus grew in pure culture.

Microscopical Examination.—The alveoli were filled with peeled off epithelial cells, polymorphonuclear leucocytes, and some red blood cells; there was some granular fibrin in the exudate, but it was nowhere excessive. The smallest bronchi had lost their epithelial lining and there was leucocytic infiltration of their walls. The larger tubes, however, had normal walls, but contained aspirated masses of leucocytes and red blood cells. Empty alveoli were present next to solid lobules. Bacilli were very numerous in the alveoli and bronchi. Many alveoli at the periphery of the solid areas were filled with red blood cells, and many other alveoli contained serum only.

II. *Dog 29.*—Black male; weight 7,400 gm.

June 13, 1911, 11 A. M. Under ether anesthesia a 10 c.c. suspension of the twenty-four hour growth from three bottles of influenza bacilli was injected intrabronchially. 5 P. M. Temperature, 40.2° C.

June 14, 9:30 A. M. Temperature, 39.6° C.

June 15, 9:30 A. M. Temperature, 38.8° C.

June 16, 9:30 A. M. Temperature, 38.7° C.

June 17, 9:30 A. M. Temperature, 38.5° C.

June 19, 9:30 A. M. Temperature, 38.9° C. The animal was killed with chloroform.

Autopsy.—The only consolidation found was in the right lower lobe. This was about half solid, and beyond was a dark area which was evidently almost resolved. The consolidated portion was firm and smooth, and on section was very moist and of a greyish red color. The dark area was aerated and more congested than the rest of the lobe. All the remainder of both lungs was quite normal. The lymph nodes on the left side were normal. On the right side they were slightly swollen.

Smears and cultures were negative for influenza bacilli. Sections from the consolidated area showed that the alveoli were filled with epithelial cells and polymorphonuclear leucocytes. No fibrin was present. The capillaries in the walls were not distended, but leucocytes were present between the meshwork. All the bronchi, large and small, showed extensive degeneration of their lining cells and a leucocytic infiltration of their walls, this infiltration being continuous in places with the exudate in the surrounding alveoli. At the periphery of the consolidated area there was marked phagocytosis of the cell fragments by large mononuclear cells. In some cases whole red blood cells or leucocytes were contained in these macrophages. As the cells diminished in number a granular material occupied the alveoli, and there was a gradual transition from these areas to those of normal empty air cells. No bacilli were found in these sections.

The differences between the lesions produced by the injection of the pneumococcus into the bronchi and those caused by the influenza bacilli were as marked as were the differences between the lesions produced by the pneumococcus and those caused by the streptococcus. The fibrin was slightly larger in amount in the influenzal

pneumonia than in the streptococcal variety, but was by no means as marked a feature of the exudate as in the pneumococcus pneumonia. Hemorrhage into the alveoli was more marked in the influenza cases than in either the streptococcus or the pneumococcus lesions, and leucocytic infiltration was as severe. In gross appearance influenzal bronchopneumonia was distinctly patchy and lobular, not lobar in type, although the tendency was for a large portion of a lobe to be involved.

SUMMARY.

When intrabronchial insufflation of pure cultures of the streptococcus or of the influenza bacillus is properly carried out, it produces without fail a pneumonic lesion. This lesion is similar in its nature to the one known in human pathology as bronchopneumonia, and differs materially from the pneumonic lesion produced experimentally by the intrabronchial insufflation of pure cultures of the pneumococcus.

Considering the fact that none of the dogs used in the experiments with the pneumococcus and none of those used in the present investigation were selected or prepared in any way, the conclusion seems to be unavoidable that the proper invasion of the microorganism is the determining factor in the development of pneumonia, the condition of the animal being only a minor element in this regard.

Furthermore, since different organisms introduced in the same way and under conditions which are apparently the same produced distinctly different pneumonic lesions in animals of the same species, the further conclusion presents itself that the different types of pneumonia are produced by specifically different bacteria.

However, further investigation may show that the differences in the nature of the lesion are due rather to the degree of virulence of the causative microorganism than to differences in the species; that is, that different lesions may possibly be produced by organisms of the same species, provided they possess different degrees of virulence. Further experimentation may also show that the condition of the animal and of the affected organ which, in the onset and development of the pneumonic disease, is, perhaps, unimportant, may be the leading factor in determining the course and outcome of the disease.

EXPLANATION OF PLATES.

PLATE 9.

FIG. 1. Streptococcal bronchopneumonia of seventy-four hours' duration; dose 10 c.c.; twenty-four hour bouillon culture.

FIG. 2. Streptococcal bronchopneumonia of forty-eight hours' duration; dose 10 c.c.; enriched three times.

PLATE 10.

FIG. 3. Influenzal bronchopneumonia of twenty-four hours' duration; dose 10 c.c.; enriched twice.

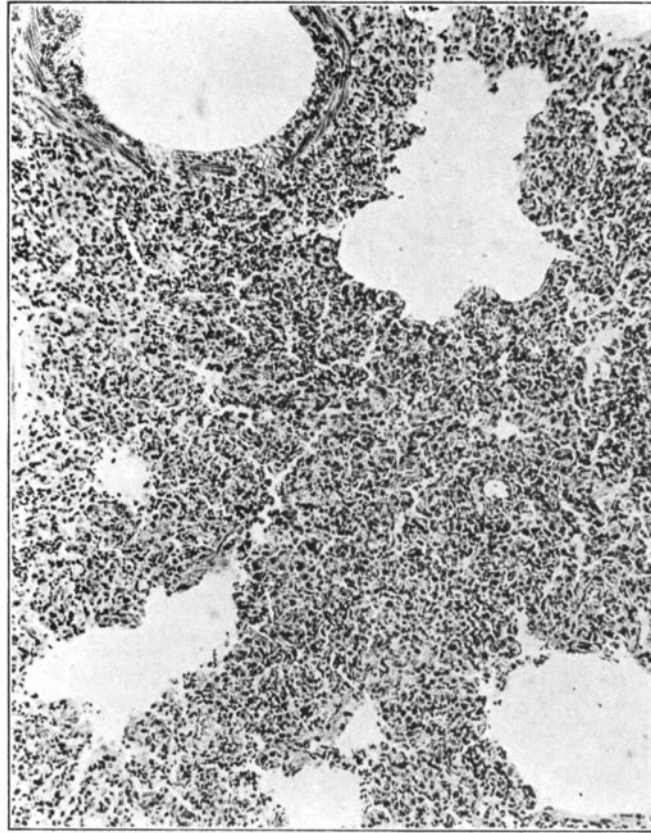


FIG. 1.

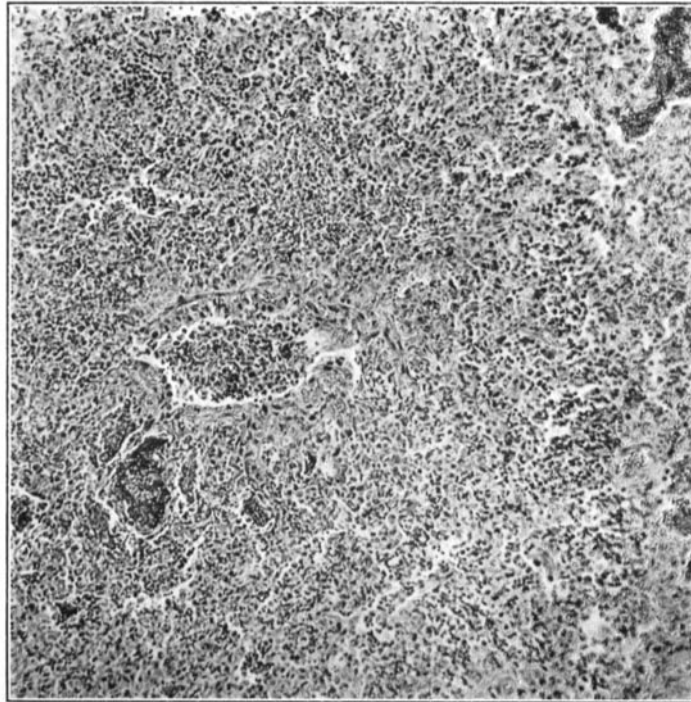


FIG. 2.

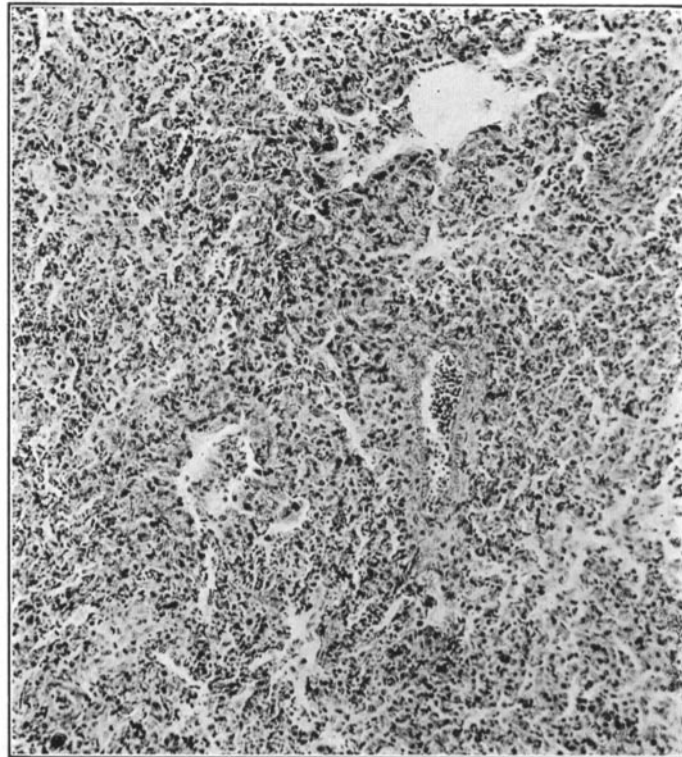


FIG. 3.