THE HISTOGENESIS OF CELLS IN EXPERIMENTAL PNEUMONIA IN THE DOG*

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The present study concerns itself principally with the origin of the cells and the sequence with which they appear in the exudate in experimentally produced pneumonia in the dog, which simulates closely both clinically and pathologically the disease occurring spontaneously in man. The general characteristics of the pathology and pathogenesis of experimental canine pneumonia have been described by Robertson and associates (1, 2) in previous reports from this laboratory. These earlier studies left open many questions, and with the development of new techniques, a further investigation of certain aspects of the evolution of the pneumonic lesion seemed to be indicated.

In addition to the study of the histogenesis of the cells of the inflammatory exudate, concomitant observations were made on the distribution of pneumococci, and other changes occurring during the different stages of the developing lesion. The present findings will be given in some detail not only for the sake of giving as complete a review as possible, but also because they have resulted in a reinterpretation of certain of the original observations. This will be brought out during the course of the discussion.

In order to study the evolution of the inflammatory process from its earliest inception to its termination, a series of animals were sacrificed at varying intervals of time after inoculation. The intervals were short in the early stages when the lesions were enlarging and were gradually lengthened as the disease progressed to its termination with recovery. Only specimens of lungs fixed immediately after death from sacrificed animals were used. Intrabronchial fixation and special staining techniques were employed to give the maximum cytological and histological detail. Several sections were taken from the consolidated lobes of each animal so that the cytological differences, if any, in various parts of the lesions could be studied in relation to the primary site of infection. When studying the cellular changes in the exudate and lung tissues, the more recent concepts of lung structure as well as the fundamental concept of cellular reactions in inflammation, in general, were kept in mind.

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Material and Methods

Dogs, Dosage, and Method of Inoculation.—Forty-seven apparently healthy dogs varying in weight from 8 to 14 kilos were used. The method employed in infecting the animals was devised by Terrell, Robertson, and Coggeshall (3). Briefly, it is as follows: After the dog was placed under morphine anesthesia and its larynx cocainized, pneumococci suspended in a 5 per cent starch-broth mixture were injected through a radio-opaque catheter (No. 9) introduced through the trachea into one of the lower lobe bronchi with the aid of a fluoroscope. The dosage varied from 0.01 to 0.05 cc. of an 18 hour broth culture of strain A_5 Type I pneumococcus suspended in 1 cc. of the starch-broth medium. With this method and dosage lobar pneumonia is produced in approximately 100 per cent of inoculations from which 90 per cent of the dogs recover (4). The clinical course of the disease was followed by daily observations in temperature, pulse, white blood cell count, and one or more roentgenograms until the animals were sacrificed or had recovered from the disease.

Single Infections.—Thirty-seven dogs were sacrificed at increasing intervals of time from 1 hour to 22 days after inoculation. Fourteen were killed at from 1 to 3 hour intervals within the first 24 hours, 10 at 6 hour intervals from 24 to 72 hours, and 13 from 4 to 22 days after the onset of their infections. All the dogs except one, which was killed during the first 48 hours, showed clinical evidence of active infections. Most of the dogs killed at later intervals had recovered on the 3rd or 4th day of the disease.

Reinfected Dogs.—Ten dogs were subjected to reinoculation in the same lobes from 3 to 4 weeks after the onset of their first infection. At the time of their second inoculation they had completely recovered from their previous disease and their lungs were clear by x-ray. They were sacrificed at 2, 4, 8, 12, 18, 22, 32, 41, 50, and 72 hours after inoculation. All the dogs except the 2 killed at 50 and 72 hours showed clinical evidence of active infection.

Method of Fixing Lungs.—The method of fixing the pneumonic lungs was the same as that used by the author (5) during his study of the pores of Kohn (6) in mammalian lungs, including those of the dog. Briefly, it was as follows: The animal was given intravenously a lethal dose (75 mg. per kilo of body weight) of pentobarbital sodium, which produced immediate death. The heart and lungs were quickly exposed, the aorta clamped, and the heart gently squeezed, after which a ligature was tied about the base of the heart to hold the blood in the congested pulmonary vascular system when the lungs were reexpanded with the fixing solution. Zenker-formol solution (9 parts of Zenker's fluid, without acetic acid, to one part neutral formalin) was used and introduced into the lungs through the trachea under low gravity pressure until the uninvolved lobes were reexpanded approximately to their normal inflated size. At the same time the fixing fluid also penetrated throughout the consolidated lobes. After ligating the trachea, the heart and lungs were removed *in toto* and immersed in the fixing solution for from 6 to 10 hours. The procedure from the time an animal was killed until its lungs were injected with the fixative took from 3 to 5 minutes.

Specimens for Microscopic Study.—After fixation several specimens were taken from the consolidated lobes of each animal. They were numbered and a record of the area from where they were taken was noted on diagrams of the lungs drawn in the

protocols. Control sections from uninvolved lobes were taken from each animal. Specimens from some animals were embedded in paraffin and those from others in nitrocellulose. All specimens were sectioned from 8 to 10 microns in thickness and placed serially on slides. Some of the sectioned tissues from each animal were stained with Maximow's (7) hematoxylin-eosin-azure II stain for cytological and histological details, others with Heidenhain's (7) azan stain for connective tissue, and others with Wallace's (8) modified Gram-Weigert stain for pneumococci, fibrin, and general histological detail.

Observations.—The observations on the clinical course of the disease in these series of dogs were similar to those noted by Terrell, Robertson, and Coggeshall (3), and Coggeshall and Robertson (9), during previous studies in the same animal and, therefore, will not be included in this report. The protocols of the individual animals will not be included because of lack of space. Spread of the infection occurred in 11 of the singly infected animals, but only the findings in the inoculated lobes will be presented. The data concerning the histogenesis of cells in the lungs of the reinfected animals will be given only in summary.

Pathogenesis of Lobar Consolidation

Macroscopic Observations.—The inflammatory process began in a small area at the site of inoculation subjacent to the pleura in the peripheral anterolateral region of the lobe. It enlarged in a contiguous and progressive manner so that approximately the whole lobe became consolidated within the first 24 hours after inoculation. The consolidated portions, regardless of size, of the lobes of the 14 animals killed within this time, were indurated, irregular in outline, and sharply demarcated from the uninvolved portions. The spreading margins of the lesions showed advancing borders of edematous and congested tissue, in which were small, red, inflammatory foci similar to the patchy beginning lesions at the sites of inoculation in the lungs of the animals killed between 1 and 4 hours. These foci blended to form the enlarging, uniformly consolidated areas. The anterior dependent portions of the lobes first became consolidated, then the inflammation spread posteriorly until the level of the hilum was reached. The last portion of the inoculated lobe to become consolidated, which occurred at about 36 hours, was the region about its superior posterior angle, which lies posterior to the main stem bronchus near the hilum. A fibrinous exudate was usually present on the pleural surfaces of the consolidated portions of the lungs of the animals killed between 6 and 48 hours after inoculation.

It was impossible in the gross to divide the individual lesions, either in the fresh or fixed state, into stages of congestion, red and gray hepatization, and resolution. The fixed, partially consolidated lobes, seen from their cut surfaces, of the animals killed during the first 24 hours after inoculation, varied in color from grayish, at their centers, to brownish-red, at their margins. As the consolidated areas enlarged, the grayish-red areas became correspondingly larger while the brownish-red areas were confined to a relatively narrow margin at their borders. The completely consolidated lobes of the animals killed from 36 to 96 hours after inoculation had approximately the same grayish-red appearance. Those from animals killed after this time gradually returned to their normal reddish color as resolution, which was usually complete from 8 to 10 days after inoculation, took place.

Microscopic Observations.—At 1 hour after inoculation, leucocytes had already begun to congest the capillaries and enter the air spaces at points along the septal walls at the site of inoculation. Only a few pneumococci could be seen suspended in the starch inoculum. After this time the inflammatory process increased in intensity and extent so that by 4 hours it had spread well beyond the site of inoculation. The focal areas had coalesced and the cellular exudate rather uniformly filled the air spaces, although the consolidated area occupied only a small portion of the lobe. Toward the margin of the lesions there were fewer cells in the exudate which consisted principally of serous fluid containing large numbers of pneumococci. Beyond the zone of edema-filled alveoli where the lung spaces contained apparently no fluid and the septal walls appeared normal under low power magnification, many pneumococci could be seen, under high magnification, lying in a thin layer of edema fluid on the alveolar surfaces and in the pores of Kohn.

The spreading borders of the enlarging areas of consolidation in the animals killed at later intervals during the first 36 hours of the disease had essentially the same microscopic appearance as did those of the 4 hour lesion. Serous exudate partially or completely filling the air spaces and containing large numbers of pneumococci was the characteristic feature (Figs. 1 to 3). The extent of the zone of edema and the number of organisms, however, varied in individual lesions. As the inflammatory process spread within the lobe, pneumococci diminished in numbers as cells increased in the exudate in the older areas of involvement. Thus in the consolidated lobe of the animal killed at 36 hours, many pneumococci were present in the air spaces of the recently involved portions of the lobes (Figs. 1 to 4) while none were seen in the exudate at or near the site of inoculation (Fig. 11). Phagocytosis of the pneumococci within the air spaces by the exudate cells, although it varied in individual lesions, appeared to be the chief means by which the exudate was cleared of organisms (Figs. 3 to 5). Phagocytosis of the intraalveolar organisms occurred in animals having severe septicemias. This is seen in the pneumonic exudate (Figs. 3 to 5, 11, and 12) of the animal which when killed at 36 hours had a bacteremia of a thousand colonies of Type I pneumococcus per cc. of blood. Pneumococci gradually disappeared from the exudate in the lungs of the animals killed after 36 hours. Pneumococci were seldom seen in the exudate in any part of the consolidated inoculated lobes of animals killed after 72 hours, although some animals were bacteremic and had suffered spread of their infections to other lobes by this time.

Pneumococci were never seen in the substance of the alveolar walls beyond the margins of, at, or within the developing lesions (Figs. 1 to 5, 11, and 12). At points where the leucocytes were leaving the capillaries, they were not seen in the substance of the septa (Figs. 6 and 7). The interstitial tissue of the pleura, blood vessels, and bronchi showed varying degrees of involvement which was never marked in the early lesions. In some areas the interstitial tissue was edematous, contained fibrin deposits, and was infiltrated with polymorphonuclear leucocytes, while other areas showed no reaction, yet the air spaces and bronchi in both regions showed the same degree of consolidation. In the early lesion organisms were seldom seen in the interstitial tissue showing an inflammatory reaction, and they were never seen in the congested lymphatics even though the adjoining air spaces contained large numbers. Large numbers of organisms, lying on the surfaces of epithelial lining cells, were pres-

ent in the bronchial exudate in the early lesions, but none were seen within or between these cells or invading the underlying connective tissue. Pneumococci were never seen in the lumina of the capillaries or blood vessels, although some of the animals were bacteremic at the time of death.

In spite of the intense inflammatory reaction which took place in the inoculated lobes, it resulted in little alteration of the general architecture of the lung parenchyma. No capillary or vascular thromboses were seen in any of the lesions. The small areas of lung which were traumatized by the starch inoculum healed by formation of a fibrotic scar. The remaining parts of the consolidated lobes underwent resolution. This began in focal areas usually near the sites of inoculation where the pneumonic process began. Beginning resolution was present in the lungs of the animals sacrificed at 72 hours and increased in extent, although in an irregular manner, in the inoculated lobes of those sacrificed after this time. It proceeded more rapidly in some animals than in others (Figs. 15 and 16), but was more or less complete in those sacrificed at 10 days or longer after the onset of the disease. Small focal areas of organization of the exudate which had collected in the alveolar ducts and respiratory bronchioles were noted in some of the recovered lungs (Figs. 17 and 18).

Histogenesis of Cells in the Exudate

During the course of the disease in these series of animals, quantitative and qualitative changes gradually took place in the cellular constituents of the pneumonic exudate. The exudate changed from one consisting principally of polymorphonuclear leucocytes to one of large mononuclear macrophages. The various kinds of cells will be discussed in the order of their appearance in the air spaces.

Pigment-Filled Mononuclear Macrophages.—Large, free, mononuclear cells containing dust pigment were present in small numbers in the air spaces of normal lungs of dogs. Such cells were also seen in the control sections from uninvolved lobes in this series of animals and also in the exudate in all the early lesions (Fig. 7). They ingested pneumococci, red blood cells, and polymorphonuclear leucocytes. In the older lesions these could not be distinguished from the many pigment-filled mononuclear macrophages which developed in response to the acute pneumococcic infection (Figs. 15 and 16).

Polymorphonuclear Leucocytes.—The polymorphonuclear leucocytes were the first cells to appear in large numbers in the air spaces in response to the implanted pneumococci. They entered the exudate principally from the alveolar capillaries and small blood vessels. In the 1 hour lesions some were already in the spaces while large numbers filled the capillaries and small blood vessels. They left the capillaries in groups forming asteroid configurations at many points along the septa (Figs. 6 and 7). The exudation of leucocytes soon followed the appearance of the infected edema fluid in the air spaces and thus was most pronounced in areas bordering the zone of edema. To a lesser extent, however, leucocytes could be seen entering the air spaces in sections taken from all parts of the consolidated areas during the first 24 hours of the disease when consolidation was taking place. Some of these cells passed directly

through the walls of the larger pulmonary, bronchial, and pleural vessels into the interstitial tissue, while others leaving the bronchial vessels passed directly into the bronchial exudate between the intact bronchial epithelial lining cells. The character of the exudate in the bronchi was similar to that in the finer air spaces. Regardless of their phagocytic activity, the polymorphonuclear leucocytes soon began to degenerate after entering the air spaces. Although they were the most numerous cell type in the exudate up to 48 hours, many at this time had pycnotic nuclei and had been taken up by the mononuclear macrophages (Fig. 13). In the 72 hour lesion the majority were undergoing degeneration. In the 4 day lesion where resolution had begun only a few non-degenerative forms were seen. In still older resolving lesions these gradually disappeared from the air spaces (Figs. 15 and 16).

Lymphocytes and Monocytes.—The lymphocytes and monocytes of the blood entered the exudate in the early stages of the disease along with the polymorphonuclear leucocytes. At first they were inconspicuous because of the predominance of the latter cells. In consecutively older lesions the hematogenous mononuclear exudate cells increased in numbers in the exudate and at the same time underwent morphological changes. These consisted of hypertrophy and transformation into large mononuclear cells which gradually assumed the appearance and phagocytic properties of typical macrophages. In the lesions of the animals killed within the first 8 hours after inoculation (Fig. 8), the majority of the small hematogenous mononuclear exudate cells were identical in appearance with the lymphocytes still within the lumina of the capillaries and blood vessels. A few resembled the monocytes of the blood. Once in the exudate they began to enlarge. Their nuclei (both lymphocytes and monocytes) became less compact, more vesicular, and assumed many shapes; and their cytoplasm increased in amount and became less basophilic. As the lymphocytes and monocytes first entering the exudate hypertrophied other small varieties continued to enter the air spaces, so that lesions of the animals killed between 8 and 36 hours after infection showed hematogenous mononuclear exudate cells of many sizes and shapes (Figs. 8 to 13). Many of these polyblastic exudate cells in the 12 to 24 hour lesions resembled monocytes of the blood, although the proportion of the latter cells to lymphocytes still within the blood had not increased.[1] Cells representing all transitional stages in the development of lymphocytes into monocytes and monocytes into small macrophages could be seen in the exudate. It was impossible to separate them into groups representing typical lymphocytes, monocytes, or macrophages (Figs. 9 to 12).

In the lesions of the animals killed from 15 to 36 hours after inoculation the number of large mononuclear cells showing phagocytic properties increased while the number of the smaller non-phagocytic varieties decreased (Figs. 9 to 11). By 36 hours the majority of the hematogenous mononuclear exudate cells had transformed themselves into macrophages, which had ingested red blood cells, pneumococci, and degenerating polymorphonuclear leucocytes (Fig. 11). In sections of the 36 hour lesion from areas which were more recently consolidated (Fig. 12), many of the mononuclear cells were of the smaller non-phagocytic variety, resembling the polyblastic mononuclear cells

[1] The cellular changes which took place in the peripheral blood and blood-forming organs will be given in a subsequent report.

in the exudate of the earlier lesions (Figs. 8 and 9). In the lesions of the animals killed from 48 to 72 hours after inoculation, the mononuclear macrophages became still larger and more numerous, but a few small non-phagocytic cells resembling lymphocytes and monocytes were present in the exudate until resolution was well advanced (Figs. 13 and 14). Degenerative forms of lymphocytes and monocytes were not seen in any of the lesions at any stage of the disease.

Septal Cells (Alveolar Epithelial Cells).—The term "septal cell" (10) refers to the cuboidal cells on the alveolar walls, which in the past have been called alveolar epithelium. In the early developing pneumonic consolidations, the septal cells were slow to react to the intraalveolar infection, and did not become detached (desquamated) from the alveolar surfaces when the exudation of serous fluid and leucocytes from the blood took place (Fig. 7). The first change noted in these cells was an increase in their size which became apparent in the 15 hour lesion (Fig. 9). In the 18, 21, 24, 30, and 36 hour lesions, the septal cells became progressively more prominent and appeared to be more numerous on the walls (Figs. 11 and 12). They hypertrophied in a uniform manner so that in any given lesion the septal cells were approximately the same size. They were large and conspicuous in older lesions until resolution was advanced (Figs. 15 and 16). As the septal cell increased in size, its nucleus remained oval and vesicular while its cytoplasm increased in quantity, lost its vacuoles, and became more basophilic. In lesions which were resolving, vacuoles again accumulated in their cytoplasms. The septal cells did not increase sufficiently in number during any stage of the disease to form a continuous covering over the alveolar surfaces, but appeared as isolated cells (Figs. 14 to 16). They were intimately associated with the connective tissue elements in the septa, for frequently their cytoplasm could be seen to extend into the interalveolar connective tissue stroma or across the septa to project into the adjoined alveolar spaces (Fig. 16).

It was difficult to determine how many of the septal cells became detached to contribute to the source of the alveolar macrophages, for many of the latter cells, derived from the hypertrophy of the hematogenous lymphocytes and monocytes, were present in the exudate before these local cells became conspicuous on the walls. Mitotic or amitotic figures, indicating proliferation, were rarely seen in the enlarged septal cells, and then only in 2½ day, 3 day, and some of the older lesions undergoing resolution. In the lesions showing occasional mitotic figures in septal cells, binucleated septal cells also were seen on the alveolar walls. Such binucleated cells were rarely seen in the exudate. Smaller developing septal cells were not seen in the alveolar walls to take the place of the larger ones should they desquamate without dividing, and there was rarely seen constriction of the cytoplasm at the attached portions of the large septal cells to suggest that they were going to free themselves and enter the exudate. The septal cells remained large and conspicuous on the walls throughout resolution although the macrophages in the exudate diminished in number (Fig. 16).

Although the hypertrophied septal cells did resemble the large, free phagocytic mononuclear cells in the exudate (Fig. 15), the former showed no phagocytic activity in any stage of the disease. In the early developing lesions pneumococci were frequently seen lying on the free surfaces of septal cells with none in their cytoplasms. In the later stages of the disease when the polymorphonuclear leucocytes and red blood cells were degenerating, none were seen in the septal cells, while large numbers were ingested by the free mononuclear cells in the exudate (Figs. 10, 11, and 13).

Fixed Histiocytes (Tissue Macrophages)

Mononuclear cells, other than the septal cells described above, were not observed to develop in or on the septal walls and enter the exudate in any stage of the disease. New histiocytes or the old pigment-filled ones in the interstitial tissue of the bronchial, blood-vessel, and pleural walls, were not seen to mobilize and enter the exudate. Mononuclear cells containing pigment in their cytoplasms increased in the interstitial tissue in the lesions undergoing resolution when the number of macrophages were decreasing in the exudate. These were similar morphologically to those in the alveolar spaces. Numerous smaller deeply basophilic mononuclear cells also accumulated in the interstitial tissue in the resolving lesions. The majority of these cells were of the lymphocyte and plasma cell varieties. Mitotic figures in these cells were not infrequently seen in the recovering lesions. Plasma cells were occasionally seen in the resolving lung exudate (Fig. 15).

Fibroblasts.—Fibroblasts in the interstitial tissue of the alveolar, pleural, vessel, or bronchial walls were not observed to give rise to cells which entered the exudate and functioned as macrophages.

Endothelial Cells.—There was no evidence that the cells lining the blood vessels, capillaries, and lymphatics of the lungs contributed either to the small or large mononuclear cells in the exudate in any stage of the disease. They remained flattened and intact when the blood leucocytes entering the air spaces passed through the vessel or capillary walls. The endothelial cells showed no phagocytic activity and were apparently not injured by the mechanical distortion produced by the migrating leucocytes, for thrombosis of the capillaries or larger vessels was not seen in the lesions of any of the animals.

Megakaryocytes.—Megakaryocytes, which were occasionally seen in the septal capillaries of the normal lungs, were also present in the capillaries in all the lesions. They appeared to be no more numerous in the pneumonic lungs. As far as could be determined, these cells did not enter the exudate during the course of the disease.

Epithelial Cells.—The epithelial cells lining the bronchial spaces, beyond the site of inoculation showed no necrosis or injury in any of the lesions as far as could be determined by the staining techniques employed. In some areas in the early lesions the epithelial lining of the bronchi became infiltrated with polymorphonuclear leucocytes, but there was no separation of the membrane from the bronchial walls. Proliferation and desquamation of individual epithelial cells were not seen. There was no phagocytosis of pneumococci by the epithelial cells, although in the early lesions large numbers of organisms were seen in the exudate covering their free surfaces.

Alveolar Phagocytes.—The mononuclear cells which showed phagocytic activity presented a wide variety of sizes and ingested constituents depending on the age of the lesions. In the early lesions the old large pigment-filled macrophages were the only mononuclear cells which contained ingested pneumococci and red blood cells, although numerous small hematogenous mononuclear cells were present in the exudate. In the 12 and 15 hour lesions some of these smaller cells had taken up an occasional pneumococcus and red blood cell (Fig. 9). As these cells enlarged, their phagocytic activity increased so that many phagocytic mononuclear cells of varying sizes soon appeared in the exudate (Fig. 10).

In the 36 hour lesion the majority of the mononuclear phagocytic cells at or near the site of inoculation were larger than those seen in the 24 hour lesion, and filled with red blood cells, polymorphonuclear leucocytes, and some pneumococci, showing various degrees of digestion (Fig. 11). They were still larger in the 48 hour lesion and contained large numbers of ingested red blood cells and degenerating polymorphonuclear leucocytes (Fig. 13). In the 3 to 4 day lesions the mononuclear phagocytes were somewhat larger than those seen in the 48 hour lesions and contained only fragments of ingested red blood cells and polymorphonuclear leucocytes (Fig. 14). In these lesions the red blood cells and pneumococci and the majority of the polymorphonuclear leucocytes had disappeared from the exudate. In the 5 and 6 day lesions the mononuclear macrophages were all of the large variety and contained fat vacuoles and greenish pigment in their cytoplasms (Fig. 15). They resembled in size the old pigment-filled mononuclear cells seen in the early lesions, and also the attached septal cells. Similar mononuclear cells were seen in diminishing numbers in the exudate in resolving lesions (Fig. 16).

In some areas in the pneumonic lungs, the macrophages, which left the alveoli during the course of resolution, collected in the alveolar ducts at their junction with the respiratory bronchioles. Here they apparently transformed themselves into fibroblasts, elaborated connective tissue fibers to form connective tissue plugs, which became vascularized as a result of the ingrowth of capillaries from points along the surrounding walls (Fig. 17). These connective tissue obstructions were composed essentially of large, pigment-filled, phagocytic cells and were seen in small areas in the lungs of most of the animals killed from 10 days to 3 weeks after recovery. The lung tissue peripheral to or surrounding these obstructions showed no organization or general architectural alteration (Fig. 18).

Reinfected Dogs

The gross and microscopic findings in the lungs of the 10 reinfected dogs were in general similar to those seen in the animals receiving only one infection. The disease appeared to be less severe and the pneumonic process developed more rapidly than in the singly infected animals. The observations on the pathogenesis of lobar consolidation, the kinds of cells, and the sequence with which they appeared in the exudate, were similar to those seen in the animals receiving single infections. The mononuclear macrophages developed rapidly in the exudate and were derived principally from the small hematogenous mononuclear exudate cells which appeared early in the disease, as was seen in the lesions of the singly infected animals (Figs. 19 to 22). The extent to which the local septal cells contributed to the alveolar phagocytes also could not accurately be determined in this series of reinfected animals. Some of the early lesions showed a moderate number of old, free pigment-filled mononuclear cells, but these were considered to be residual cells from the previous infection. The pigment-filled cells in the fibrous plugs which developed from the previous infection in some of the alveolar ducts did not appear to mobilize and enter the exudate during the course of the second infection.

DISCUSSION

The cellular responses to the pneumococcus in these series of pneumonic lungs of dogs were similar to those described by Maximow (11, 12), Bloom (13), Taliaferro and coworkers (14, 15), Kolouch (16), and others in inflammation in general. These investigators found that the blood leucocytes constituted the chief source of the cells which appeared in the field of inflammation, and that the macrophages, which eventually replaced the polymorphonuclear leucocytes, were derived principally from the hematogenous mononuclear cells (lymphocytes and monocytes) after they entered the exudate. The inflammatory process in the lungs differed from that of their observations only in that the cellular exudate entered spaces not normally occupied by cells. Because of this, as well as the thinness of the alveolar septa, and the relative scarcity of cells on and in them, the migration of leucocytes through the capillaries could be easily seen in the freshly, intratracheally fixed preparations. The manner in which the leucocytes left the capillaries, forming asteroid configurations along the septa, was similar to the way they enter the air spaces during pneumonia in man, as shown by Ribbert (17) and Loosli (18).

Before and while the exudation of leucocytes took place, little change could be seen in the local tissue cells in the alveolar walls, bronchi, blood vessels, and pleura. The septal cells did not undergo desquamation as is frequently considered to occur early in pneumonia in man (19–23) and in experimental lesions in animals (24–27). Because they were not reduced in numbers on the walls by detachment in the early stages, their enlargement and increase in numbers in the later stages cannot represent a reparative process—a regeneration of the denuded lining of the alveolar spaces—as is generally taught. The septal cells, although they enlarged, remained as isolated cells. The alveolar surfaces between them remained smooth and showed no thickening or alteration to suggest that they were covered by cytoplasmic expansions of the septal cells.

It was difficult to determine if the changes in the septal cells represented a macrophage reaction as Robertson, Coggeshall, and Terrell (1) and Gunn and Nungester (28) concluded. As Robertson and coworkers observed, there was also no evidence, in this study, of phagocytic activity by the septal cells in any of the lesions. Morphological similarity between the septal cells and free mononuclear cells in the older lesions, however, would not seem to be sufficient evidence to classify the former as macrophages. There was little evidence also to indicate that the swollen cells became detached when the number of macrophages were increasing in the exudate; for mitotic figures, indicating proliferation, were rarely seen in them and then only in the later stages when binucleated cells began to appear on the septa. Some of the septal cells probably did become free to function as macrophages, but desquamation occurred slowly, for the macrophages diminished in the exudate as resolution progressed, although during this time the septal cells remained large and conspicuous on the

walls. The swelling and proliferation of the septal cells and other cellular constituents of the walls could be interpreted as a result of mild toxic injury due to the soluble products liberated by the pneumococci growing in the exudate and alteration in their environment as a result of consolidation.

In the exudate of animals killed after 48 hours, the mononuclear cells were chiefly of the typical macrophage variety, and one could readily get the impression that they were derived from the local swollen septal cells. On the other hand, by following the changes in the alveolar exudate from the beginning of the pneumonic process and at close intervals throughout its course, it was clear that they arose, in a large part, from smaller non-phagocytic, basophilic mononuclear cells of hematogenous origin. In the early lesions, the latter cells were morphologically indistinguishable from the lymphocytes of the blood, from which they could be seen entering the exudate along with the polymorphonuclear leucocytes. A few resembled monocytes. The hematogenous mononuclear exudate cells continued to migrate from the blood vessels throughout the period of consolidation. After they entered the exudate, it soon became impossible to separate them into groups as typical lymphocytes and monocytes, for they began to enlarge, assume many sizes, shapes, and transform themselves into phagocytic cells. During this process they became morphologically typical of "polyblasts" described by Maximow (11, 12) and showed increasing degrees of phagocytosis. The hypertrophy and transformation of lymphocytes into monocytes and monocytes into macrophages were more or less complete by the time the local cells began to be conspicuous on the walls. As there was no evidence of degeneration of the non-granular leucocytes after they entered the exudate, a sufficient number were present in the early stages to account for the majority of the large phagocytic mononuclear cells seen in the older lesions which also showed a swollen septal cell reaction.

As far as could be determined, the small non-phagocytic cells which transformed themselves into macrophages did not arise in the local connective tissue of the septal, bronchial, blood vessel, or pleural walls (29, 30), or from the endothelial lining cells of the alveolar capillaries as Permar (31) concluded. Small basophilic mononuclear cells, which resembled lymphocytes and plasma cells, increased in the interstitial tissue in the recovering lesions as the result of migration from the blood followed by local proliferation. Likewise, there was no evidence that large phagocytic mononuclear cells first developed in the interstitial tissue and then entered the exudate. The large pigment-filled mononuclear cells seen in the early lesions were present in the air spaces before the pneumonic process began. These cells are referred to by some as newly desquamated epithelial cells. Similar cells which increased in numbers in the interstitial tissue during resolution were indistinguishable from those still within the air spaces and are considered to be cells which had previously matured in and subsequently migrated from the exudate.

Fox (32) recognized that small round cells in the early pneumonic exudate in the lungs of man underwent pigmentary changes and increased in size. Tchistovitch (33), studying the pneumonic process in dogs and rabbits, and Briscoe (34), during similar studies in guinea pigs, observed that the large mononuclear cells which became "powerful" phagocytes developed in the exudate from smaller non-phagocytic cells. Tchistovitch concluded that these cells were of hematogenous origin. Although Briscoe found it difficult to distinguish them from lymphocytes, he concluded that they arose from the alveolar epithelium. The present study of pneumonia in the dog is in agreement with their findings, concerning the development of macrophages from smaller non-phagocytic cells within the exudate. The gradual and progressive cellular changes which took place in the exudate indicate that the pneumonic process is a dynamic one. This is further shown by the fact that the microscopic findings could be more closely correlated with the age of the lesions than with their gross appearance or the clinical condition of the animals at the time of death. The inflammatory reaction in the case of pneumococcic pneumonia in man, most probably, is also a continuous dynamic process, although the usual description of the pathology does not give this conception. This is because the microscopic pathology is usually described only in relation to the long recognized gross stages of congestion, red, and gray hepatization, and resolution, with each stage being given a definite, independent, cytological picture.

In the current textbook discussions (21–23, 35, 36), and in the reports of the majority of those who have made special studies of the pathology of pneumonia in man (18, 30, 37, 38, 39), a wide variety of small non-phagocytic exudate cells of various sizes and shapes are described, assigned many names, and generally considered to be of hematogenous origin. Although it is recognized that these cells disappear from the exudate in the later stages of the disease, a cytogenic relationship between them and the larger mononuclear phagocytic cells is usually not considered. The latter cells are thought to be desquamated epithelial cells (20, 37) or histiocytes (29, 30, 40) which arise locally and enter the exudate at about the time resolution begins. In view of the close similarity between the canine and human lesions, an analogous study of the cells in the exudate of the pneumonic process in man would probably reveal the same sequence of changes (18) that has been observed in this study. The numerous "polyblastic" mononuclear cells which appear in the early stages would be found to represent transitional stages in the development of the hematogenous exudate cells into the larger phagocytic cells, and to be the chief sources of the latter. This would also explain their disappearance from the exudate in the older lesions, an observation that puzzled Pratt (37) but, in general, one that is not commented upon. Whatever the origin of the large mononuclear cells which eventually replace the polymorphonuclear leucocytes in the exudate, they become typical macrophages and serve an important function in overcoming the infection as shown by Robertson and Uhley (40).

Lymphatic, capillary, and vascular thromboses not infrequently described as part of the pathology of pneumococcic pneumonia in man (21, 37, 41, 42) were not observed in the freshly fixed lesions, although some of the animals were bacteremic when sacrificed. Megakaryocytes, similar to those described by Pratt in the lung capillaries of man, were also observed occasionally in the septal capillaries both in sections from consolidated and unconsolidated areas. There was no separation of the epithelium from the bronchial walls, and except for the small areas of trauma induced by the starch at the sites of inoculation, there was no necrosis or alteration in the general architecture of the lung parenchyma. The fibrous plugs occasionally observed in the alveolar ducts in the recovered lungs appeared to be the result of the transformation of the alveolar phagocytes into fibroblasts. The organization of the exudate only in the alveolar ducts and respiratory bronchioles without involving the surrounding lung parenchyma has not been, as far as can be ascertained, described in recovered pneumonic lungs in man.

The macroscopic observations on the development of pneumonic consolidation in these series of dogs are similar to those noted by Robertson, Coggeshall, and Terrell (1) during earlier studies of the disease in the same animal. However, microscopic examination revealed certain differences from their findings in respect to the distribution of pneumococci in the spreading lesion. While these authors describe the presence of pneumococci within the alveolar walls at an early stage of the inflammatory process, in the present study, organisms were not seen in the substance of the alveolar walls in areas within, at the margins of, or beyond the borders of the lesions. This discrepancy can be explained on the basis of the different techniques used in preparing the specimens for microscopic examination. Keeping the capillaries filled with the animal's blood and fixing the consolidated lungs intratracheally prevent collapse of the tissue, and one is able to visualize more clearly the alveolar walls and capillaries than when the specimens are simply immersed in the fixing solution. Large numbers of organisms were seen in the intratracheally fixed lungs, but they were invariably within the air spaces. Employing the special method of fixation used in this study, Robertson (43) has stated that his earlier interpretation that the pneumococci penetrated into the walls of the alveoli was incorrect.

In this study, pneumococcic pneumonia in the dog was from its earliest inception primarily an intraalveolar and intrabronchial infection. From the distribution of the pneumococci in the developing lesions it seemed most probable that they were spread throughout the air spaces as a result of the direct flow of the infected edema fluid from alveolus to alveolus through the pores of Kohn, and from bronchiole to bronchiole by aspiration during breathing and coughing. Gravity also appeared to play an important rôle in the direction of spread of the infection within the lobes. Injury to the lung tissues which resulted in exudation of the constituents of the blood into the air spaces was

brought about by the soluble toxic products liberated by the pneumococci growing within the exudate rather than by direct invasion of the septa by the organisms themselves. Spread of the organisms to the interstitial tissue was secondary to intraalveolar infection, and bore no relation to the extent or intensity of consolidation and, therefore, was not important in the mechanism by which consolidation was produced.

SUMMARY

The kinds of cells and the sequence in which they appeared in the inflammatory exudate were studied in a series of experimentally produced pneumonic lesions in dogs. There was a gradual and progressive change in the character of the exudate and the kinds of cells as the disease progressed. The microscopic findings could be more closely correlated with the age of the lesions than with their gross appearance or with the clinical condition of the animal at the time of death. The cells in the exudate came principally from the blood. The polymorphonuclear leucocytes were gradually replaced by larger phagocytic mononuclear cells. These were derived chiefly from the hypertrophy and transformation into larger phagocytic cells of the lymphocytes and monocytes of the blood after they entered the air spaces along with the polymorphonuclear leucocytes in the early stages of the disease. To follow the development of the hematogenous exudate cells into macrophages in the dog, the pneumonic process must be studied from its earliest inception and at close stages during the first 36 hours of the disease. The local septal cells contributed only in a minor way to the origin of the macrophages. Their principal reaction appeared to be one of enlargement without detachment from the alveolar walls. Consolidation of the lungs occurred as a result of the spread of the pneumococci through the air spaces by direct passage of the infected edema fluid from alveolus to alveolus through the pores of Kohn and from bronchiole to bronchiole from aspiration during breathing and coughing. The similarity of the histogenesis of the exudate cells in this series of experimentally induced pneumonic lesions in the dog and in those which occur spontaneously in man was discussed.

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EXPLANATION OF PLATES

Figs. 1 to 5 photographed from a section stained with Wallace's modification of the Gram-Weigert technique. Figs. 6 to 22 are photographed from sections stained with Maximow's hematoxylin-eosin-azure II stain.

PLATE 22

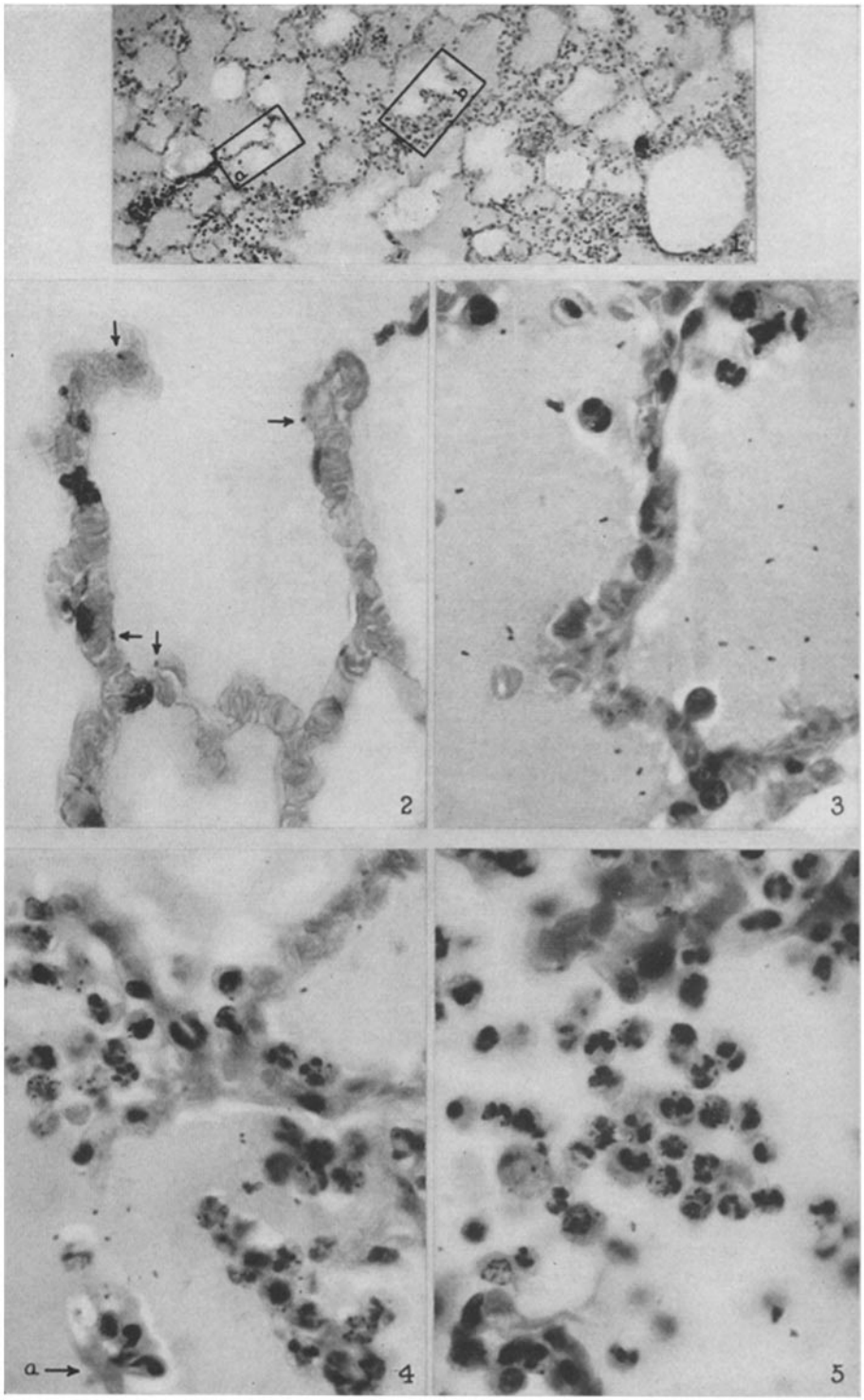
FIG. 1. Dog 1-65T killed 36 hours after left lower lobe inoculation. The lobe was completely consolidated except for a small area at the posterior superior angle near the hilum. The photograph is of an area at the spreading border of lesion several centimeters from the site of inoculation. The alveoli contain principally edema fluid with only focal exudation of cells. × 112.

FIG. 2. Dog 1-65T. Beyond zone of edema-filled alveoli, the septal walls have a normal appearance and show only a thin layer of serous exudate on their surfaces. Pneumococci (arrows) are present in the exudate but not in the substance of the septa. × 755.

FIG. 3. Dog 1-65T. Area *a* in Fig. 1 shows many pneumococci in edema-filled alveoli and none in the septal walls. × 755.

FIG. 4. Dog 1-65T. Area *b* in Fig. 1 shows edema fluid, pneumococci, and leucocytes in alveoli. Many pneumococci have been ingested by the leucocytes. *a*, leucocytes are entering the alveoli from septal capillary. × 757.

FIG. 5. Dog 1-65T. An older area of involvement just to the right of that shown in Fig. 1 nearer the site of inoculation shows no free pneumococci in the alveoli, but large numbers of leucocytes, filled with ingested organisms, are present. No pneumococci are seen in the substance of the alveolar walls. × 755.



(Loosli: Histogenesis of cells in pneumonia)

PLATE 23

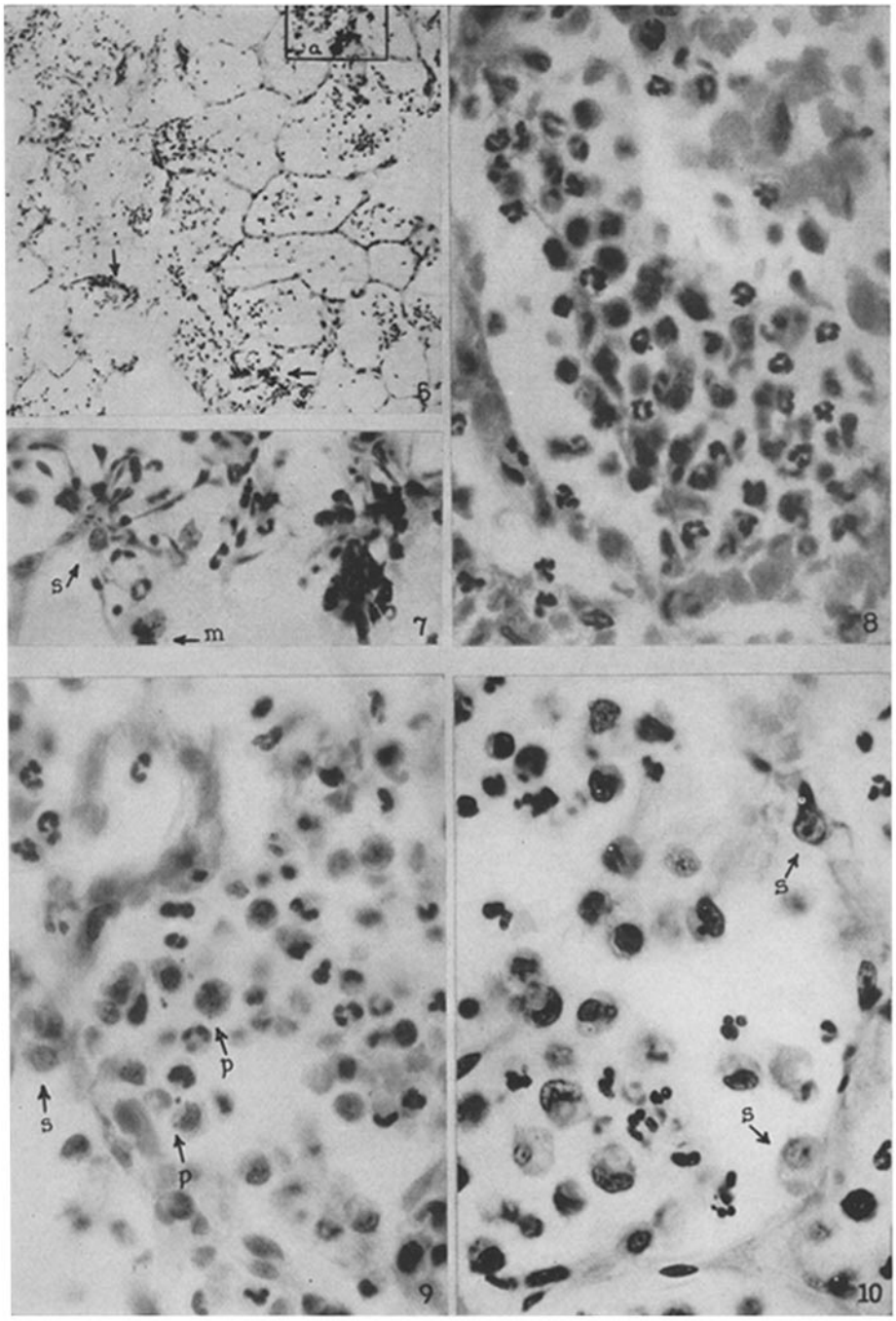
FIG. 6. Dog 64N killed 2½ hours after right lower lobe inoculation. Cellular exudation is beginning at the site of inoculation. Leucocytes are congesting (arrows) and leaving the capillaries at many points along the septa. × 112.

FIG. 7. Dog 64N. Area *a* of Fig. 6 shows the manner of migration of the leucocytes from the septal capillaries. One old pigment-filled macrophage (*m*) is seen. The septal cells (*s*) are not detached by the migrating leucocytes. × 560.

FIG. 8. Dog 1-52T killed 8 hours after right lower lobe inoculation. Area near the site of inoculation shows many small hematogenous mononuclear exudate cells resembling lymphocytes. Septal walls appear normal. × 755.

FIG. 9. Dog 77N killed 15 hours after right lower lobe inoculation. Area near site of inoculation shows beginning enlargement of the septal cells (*s*). The hematogenous mononuclear exudate cells are larger than those seen in the 8 hour lesion (Fig. 8). Some show ingested pneumococci (*p*). × 755.

FIG. 10. Dog 1-51T killed 24 hours after right lower lobe inoculation. Area near site of inoculation shows mononuclear exudate cells of many sizes and shapes. The majority are larger than those seen in the 15 hour (Fig. 9) lesion. All transitional stages between the small hematogenous mononuclear cells and the larger phagocytic ones can be seen. The septal cells (*s*) on the alveolar walls are larger than those seen at 15 hours (Fig. 9). × 755.



(Loosli: Histogenesis of cells in pneumonia)

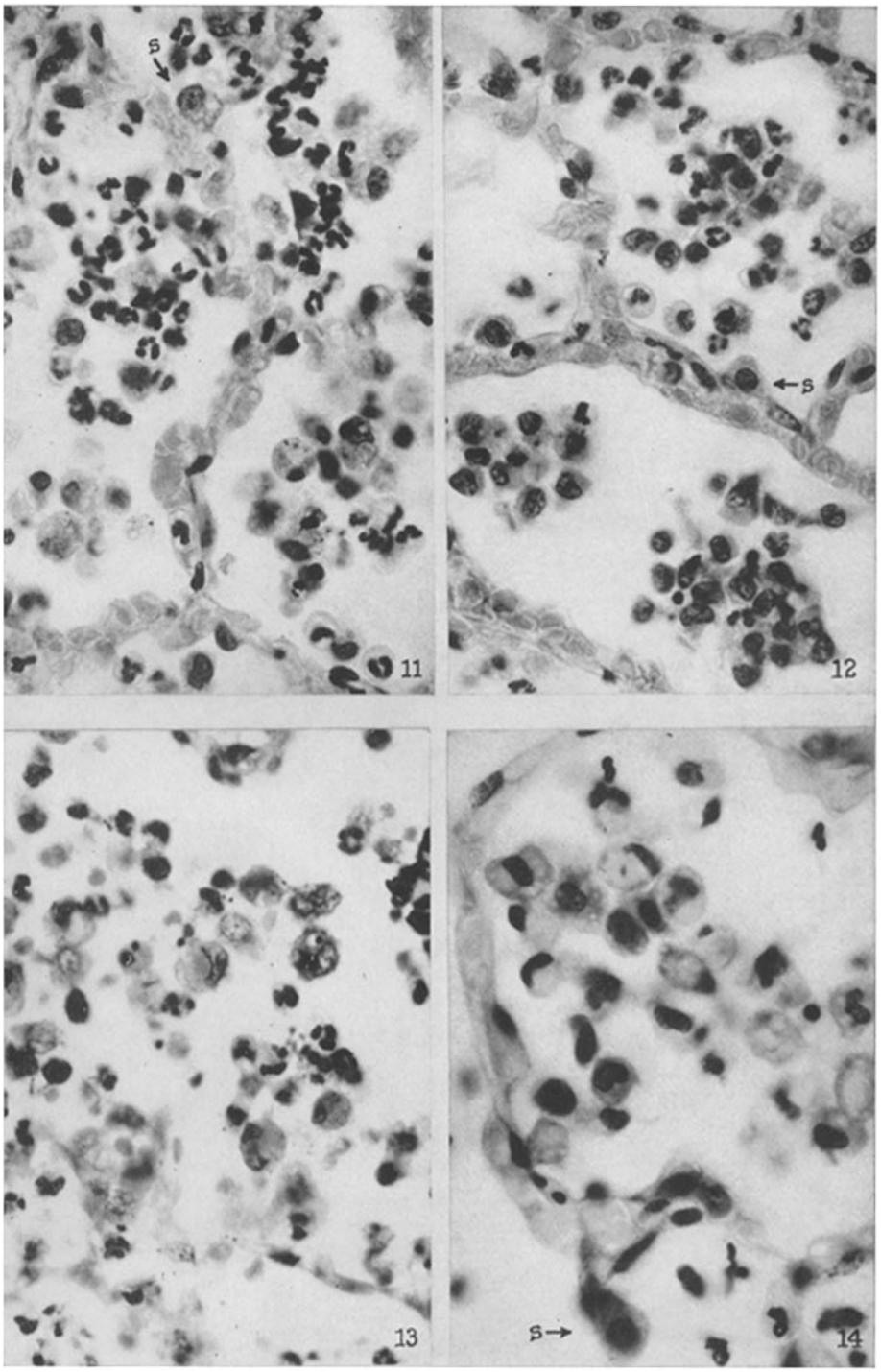
PLATE 24

FIG. 11. Dog 1-65T killed 36 hours after left lower lobe inoculation. Area near site of inoculation shows still more large phagocytic mononuclear cells than were present at 24 hours (Fig. 10). A few smaller mononuclear cells showing transitional stages in their development into macrophages are present. The septal cells (*s*) are larger but no more numerous than they (*s*) are at the margin of the lesion (Fig. 12). No pneumococci are seen. × 675.

FIG. 12. Dog 1-65T. A more recently involved area near the margin of the lesion, near that in Fig. 5, shows numerous small deeply basophilic hematogenous mononuclear exudate cells of varying sizes and shapes. The larger ones are phagocytic. Many of these cells resemble the lymphocytes and monocytes still within the blood vessels, as well as the mononuclear cells in the exudates of the earlier lesions (Figs. 8 to 10). No large phagocytic mononuclear cells such as those seen near the site of inoculation are present. × 675.

FIG. 13. Dog 1-70T killed 48 hours after left lower lobe inoculation. The exudate near the site of inoculation consists principally of large actively phagocytic mononuclear cells. Only a few smaller mononuclear cells are present. The polymorphonuclear leucocytes are degenerating. × 755.

FIG. 14. Dog 73R killed 72 hours after right lower lobe inoculation. The exudate near the site of inoculation consists essentially of still larger mononuclear macrophages. The alveolar walls appear normal except for the presence of isolated hypertrophied septal cells (*s*). × 755.



(Loosli: Histogenesis of cells in pneumonia)

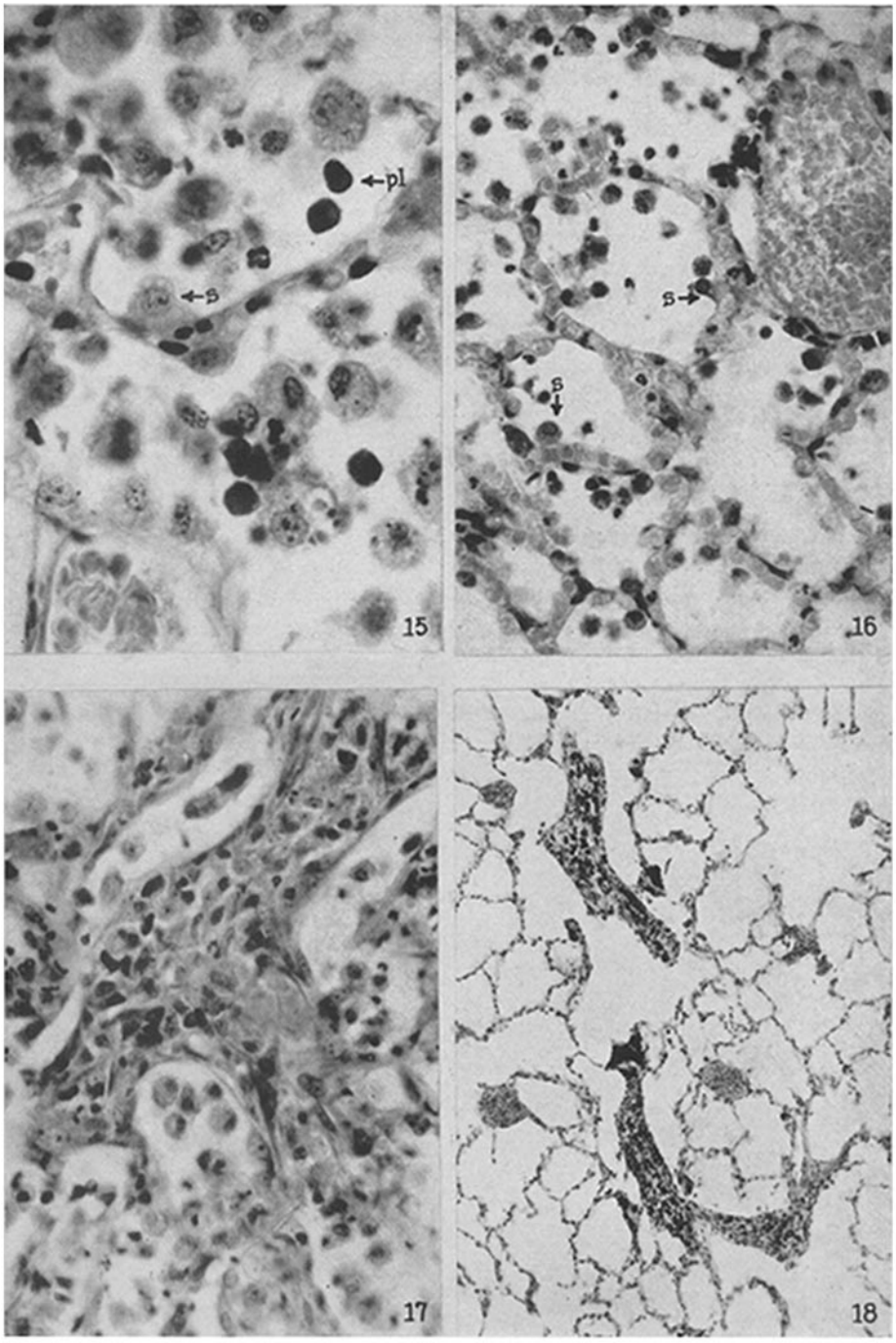
PLATE 25

FIG. 15. Dog 91N killed 6 days after right lower lobe inoculation. Resolution has not yet begun in this area. The majority of the large mononuclear macrophages contain pigment in their cytoplasm, and now resemble the pigment-filled macrophages which were occasionally seen in normal lungs. The septal cells (*s*) are markedly swollen and resemble the free macrophages in size but contain no pigment in their cytoplasm. A few plasma cells (*pl*) are present. × 675.

FIG. 16. Dog 79R killed 5 days after right lower lobe inoculation. Resolution is advanced. Pigment-filled, mononuclear macrophages have decreased in numbers in the exudate. The septal cells (*s*), however, are still large and conspicuous on the septal walls. × 375.

FIG. 17. Dog 28P killed 10 days after right lower lobe inoculation. Area shows a beginning organization of the macrophages which have collected in an alveolar duct. × 375.

FIG. 18. Dog 35P killed 22 days after right lower lobe inoculation. Resolution is complete, but obstructions in the alveolar ducts formed by organized collections of macrophages still persist. They are attached to the walls by small strands of connective tissue. The surrounding lung tissue appears normal. × 112.



(Loosli: Histogenesis of cells in pneumonia)

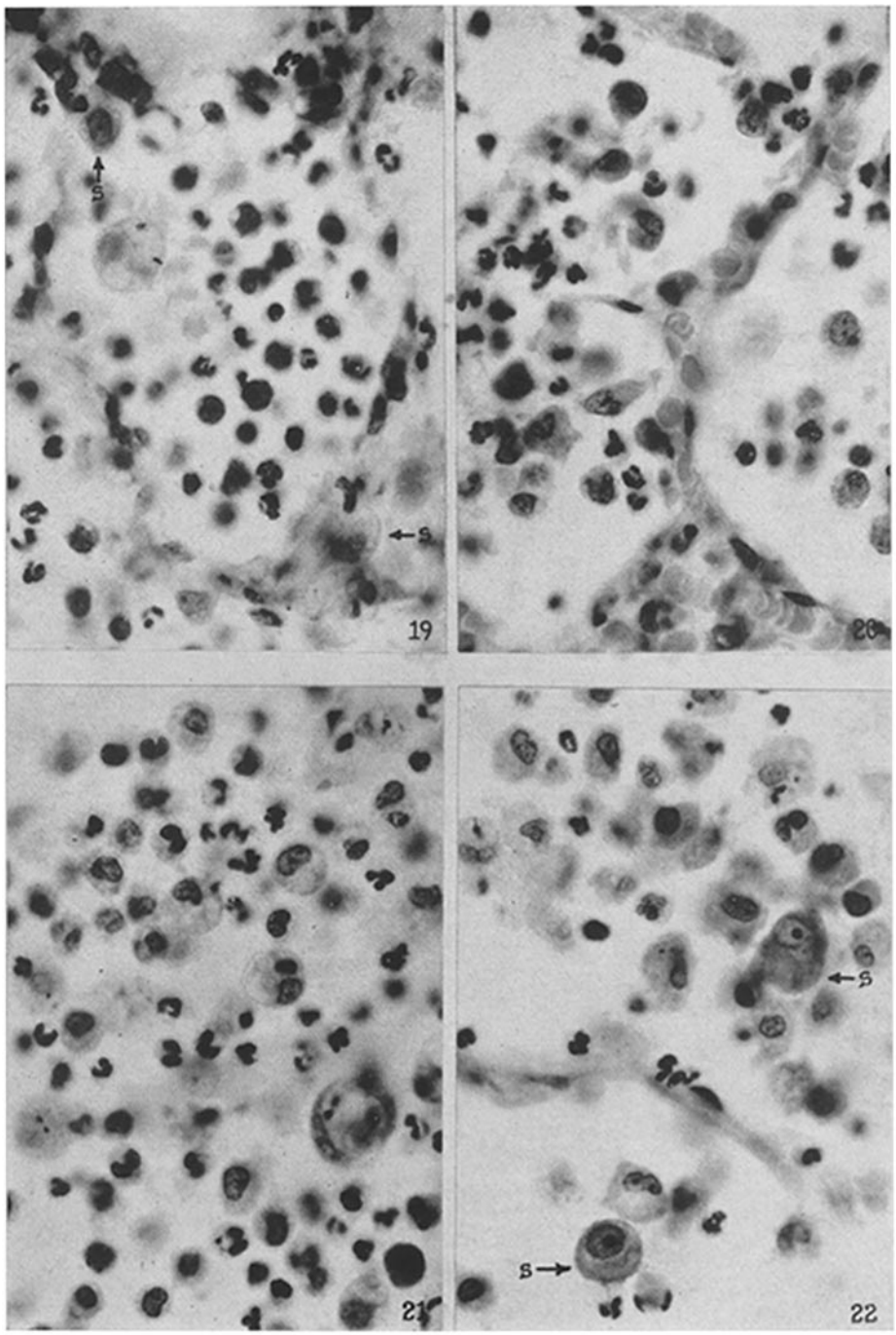
PLATE 26

FIG. 19. Dog 94N killed 12 hours after the second right lower lobe inoculation. The septal cells (*s*) are large, but the mononuclear exudate cells are small and many resemble lymphocytes. They are similar to the cells seen in the 8 hour (Fig. 8) lesion of the dog receiving a single infection. × 750.

FIG. 20. Dog 87N killed 22 hours after the second right lower lobe inoculation. Mononuclear exudate cells are larger than those in the 15 hour (Fig. 9) lesion. × 750.

FIG. 21. Dog 75R killed 32 hours after the second right lower lobe inoculation. The mononuclear exudate cells are of many sizes. The majority are larger than those seen in the 22 hour (Fig. 20) lesion. The larger ones are actively phagocytic. × 750.

FIG. 22. Dog 86R killed 50 hours after the second right lower lobe inoculation. The mononuclear exudate cells are all of the large phagocytic variety. The two large deeply staining cells are recently detached septal cells (*s*). × 750.



(Loosli: Histogenesis of cells in pneumonia)