# A STUDY OF THE PATHOLOGY OF THE CHICK EMBRYO INFECTED WITH A TRANSMISSIBLE AGENT ISOLATED FROM SHEEP LUNGS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Alexander M. Ramirez

1950

This is to certify that the

thesis entitled

A Study of the Pathology of the Chick Embryo Infected with a Transmissible Agent Isolated from Sheep Lungs.

presented by

Alexander M. Ramirez

has been accepted towards fulfillment of the requirements for

M.S. degree in Animal Pathology

Frank Thorp Jr.
Major professor

Date March 14, 1950

O-169

# A STUDY OF THE PATHOLOGY OF THE CHICK EMBRYO INFECTED WITH A TRANSMISSIBLE AGENT ISOLATED FROM SHEEP LUNGS

by

Alexander M. Ramirez

**A Thesis**

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Animal Pathology

1950

THESIS

TABLE OF CONTENTS

ACKNOWLEDGMENT

I wish to express my sincere and respectful appreciation to Dr. Frank Thorp, Jr. who gave constant guidance in this work. The scientific basis he gave will always be remembered with gratitude.

Thanks also are given to Miss Sylvia Laine for the valuable aid in technicological training and assistance in preparation of this manuscript. Appreciation is extended to Miss Esther Smith and Mr. M. L. Gray for taking the photographs, to Dr. Tsuling Chow for teaching me the technique of working with chick embryos and to Dr. R. D. Barner and Dr. H. W. Dunne for helping with the micropathology and editing the manuscript.

In addition, I wish to thank Dr. Russell A. Runnells for his suggestions in examination of the slides prepared for this thesis.

# INTRODUCTION

Pneumonia commonly occurs among all species of domestic animals. Since most pneumonias are caused by bacteria and viruses, it is readily understood that the inflammatory exudate of one sick animal can easily contaminate the feed and water of all the healthy animals in the herd or flock. This as well as contact with each other accounts for the presence of several cases of pneumonia among a group of animals during a short period of time.

An analysis of mortality among lambs showed that the deaths resulting from pneumonia tended to be the highest of all the diseases with which they are affected. At the Michigan Agriculture Experiment Station, Venkatachalam et al (1949) made a survey of lamb mortality occuring the first 56 days of life. Pneumonia accounted for 8.7% of the total number of dead lambs. There were several of these lambs from which no bacteria were cultured, so a virus etiology was suspected. A considerable number of pneumotrophic viruses have been isolated from both man and animals, but relatively little has been done in the study of the etiology of sheep pneumonia.

The purpose of this investigation was two fold: to determine whether or not a transmissible agent other than bacteria was present and if so, to determine if it would grow in the developing chick embryo and produce lesions.

# REVIEW OF LITERATURE

The first report of pneumonia in sheep caused by an unknown agent was made by Friedberger and Frohner (1910). These investigators reported an enzootic catarrhal pneumonia of lambs in which the main symptoms and lesions were fever, pneumonia and dullness of the lungs. "Postmortem revealed lobular catarrhal-desquamative foci of inflammation", sometimes accompanied by caseous necrotic areas. In some cases pleuresy and purulent fibrinous pericarditis were observed.

Mitchell (1915) described the pathological and clinical findings in "Jagziekte" or Chronic Catarrhal Pneumonia of Sheep. Besides the classical lesions, he mentioned the presence of small grayish translucent nodules on the surface of the lungs. The cut sections of the lungs also showed nodules. In long standing cases larger nodules were observed. When these nodules were cut a mucoid exudate and blood oozed to the surface of the section.

The most important microscopic changes were found in the sections of the nodules, which were spherical or oval in shape. The center of the nodules consisted of two types of cells, those with large nuclei resembling plasma cells and a second type which resembled fibroblasts. This central zone was surrounded by a narrow zone of fibroblasts arranged in concentric layers and gave the appear-

ance of encapsulating the nodule. In some sections proliferation of the bronchial epithelium occurred.

From observation and experiments, the author concluded that this type of pneumonia in sheep was caused by a virus.

Cowdry (1925) conducted controlled experiments to determine if there was any difference in the susceptibility of South African and American sheep to pneumonia. He reported the occurrence of a specific cattarrhal pneumonia in South African sheep which was called Jagziekte. Large areas of the lungs were edematous and inflamed. The difference between American and South African sheep pneumonia seemed to be significant. In about 33 per cent of the South African animals, the interalveolar tissue was definitely thickened beyond the range of variation noted in the lungs of American sheep. The thickenings occurred in localized areas which were several millimeters in diameter. The thickening was caused by the engorgement of the alveolar capillaries and by the accumulation of macrophages and lymphocytes. Many of the macrophages passed into the alveolar lumina where they assumed the appearance of typical epithelioid cells. It was found that these infiltrative and exudative changes were primary to the proliferation of epithelium which always arose in parts of the lungs thus modified. The author was of the opinion that the disease was contagious. He observed that it was trans-

missible to healthy sheep on the introduction of an animal known to have this type of pneumonia. He further observed that most probably the causative agent was a virus associated with a gram negative bacillus and a gram positive diplococcus.

Henderson (1929) described a contagious Pleuropneumonia of goats. A coccobacillus could be found in infective blood during the early stages of the disease, but this microorganism was not established as the etiological agent.

Dungal (1931) made a study of contagious pneumonia of sheep in Iceland. The cause of this disease was found to be a bacterium resembling the Pasteurella group. Its relationship to the Pasteurella group was discussed.

Meissner and Koser (1931) (Germany) reported that about 50 per cent of the lambs suffered from pneumonia. From these cases several organisms were isolated, such as B. bipolaris ovisepticum (43%), B. coli (23%) and anaerobes. From the pneumonic lungs that did not show bacteria, the cause of death was ascribed to dietetic errors.

Walker (1931) reported that contagious pleuropneumonia of goats in Kenya could be experimentally reproduced by injecting the filtered pleural exudate into the trachea of normal goats.

Delpy (1932) described the presence of Epizootic

Pneumonteritis in sheep of Persia, which differed from the other diseases of sheep. This disease was insidious in nature, and usually no symptoms were present until a few hours before the animal died. The disease was confusing since it occurred in three different forms; a pneumonic type, an intestinal type and a type having the classical lesions of hemorrhagic septicemia. Cultures from the blood of sick animals showed the presence of a Pasteurella and an unidentified gram-negative bacillus, closely related to the coli group. A bacterin was prepared from these two organisms which gave protection after injection into sheep.

Stylianopoulos (1933) reported pleuropneumonia of goats in Greece, which appeared similar to that described for sheep from Asia Minor. The disease caused a heavy mortality which varied from 65 to 98 per cent, being more severe during the winter. The etiological factor was thought to be a virus that did not survive long in vitro. The virus was present in the infected lung and in the pleural exudate. Experimental infection could be accomplished by pulmonary passage. The incubation period varied from three to six days.

Doukaloff (1935 (Russia) reported that infectious pleuropneumonia of goats caused considerable loss. Artificial infection was performed by inoculating liver or spleen emulsions intratracheally or subcutaneously with or without

filtration, proving that the cause was a filterable virus.

Kiseleff (1935) (Russia) transmitted pleuropneumonia of goats by injecting animals with infected liver, lymph nodes, spleen, lungs, heart and kidneys. The author concluded that the causative agent was a virus. However, the data given in support of this statement was insufficient. Attempts were made to prepare a vaccine using edematous fluid taken from the site of subcutaneous inoculations of experimentally infected goats, but the results obtained were inconclusive.

Gilbert (1937) reported infectious pleuropneumonia of goats in Palestine. The incubation period was less than 48 hours. The causative agent was a virus, shown to be communicable to sheep which were less susceptible than goats. The disease was not fatal unless the animals were exposed to severe cold weather or if they were in poor condition. The mortality in goats was from 60 to 80 per cent while sheep mortality ranged from 40 to 50 per cent.

Blakemore and Bosworth (1938) described Jagziekte of sheep in England, in which the most characteristic symptom was the tendency of fluid to run from the nostrils when the head was inclined. This fluid was of a clear serous character. The gross lesions were found to be confined to the lungs and smaller bronchi. The lungs were increased in size and showed extensive areas of consolidation. A striking feature of these lesions was that they were

water-logged and when squeezed much fluid was expressed. They were grayish in color. The ventral parts of the lungs were involved and the cut surface also revealed small focci scattered throughout the apparently normal tissue. The microscopic changes were identical to the ones described by Cowdry (1925).

Baumann (1938) stated that infectious pleuropneumonia in goats had been recognized in Algiers, Germany, Italy and Greece and the etiological agent was said to be a filterable virus although a variety of bacteria, predominantly Pasteurellae, were isolated in advanced cases.

Montgomerie, Bosworth and Glover (1938) described a enzootic form of pneumonia in sheep that occurred in North Wales where this disease showed a fairly regular seasonal incidence. The macroscopic and microscopic characteristics as well as the bacteriological picture were described. In most of the cases, a Pasteurella-like organism was isolated but in few instances bacteria were not found. It was suggested that an outbreak might be precipitated by sudden environmental changes such as inclement weather.

Langham et al (1942) reported on a study of the pathology and bacteriology of the lungs of nine cattle, twelve sheep and ten pigs which were affected with bronchopneumonia. Lesions were confined mostly to the apical and

cardiac lobes of the lungs and the disease appeared to start as an inflammatory process of the bronchioles. Pasteurella oviseptica was isolated from the lungs of eight of the twelve sheep.

Bawa (1945) reported contagious Pleuropneumonia of goats, a specific caprine disease in India, which was responsible for a mortality varying from 60 to 100 per cent. The symptoms, course of disease, pathology and epizootiology were similar to the description given by Longley (1940). The etiological agent appeared to be a filterable virus which was not transmissible to sheep.

Morcos, et al (1946) described contagious Ovine Pneumonia, the cause of which was found to be a filterable virus. On experimental inoculation this virus was pathogenic for rabbits but not for guinea pigs, white rats, mice or pigeons. Secondary organisms were isolated which played an important part in complicating the specific disease. A formalized vaccine was prepared from liver and spleen tissue which protected sheep against the infection.

# MATERIALS AND METHODS

The agent isolated in this study was obtained from a female Suffolk lamb which showed the following lesions at necropsy: bilateral pneumonia with emphysema, congestion and red hepatization. There was a dull appearance and marked interstitial edema of both lungs. The interlobular septa were thickened in the consolidated areas. The bronchi contained abundant dirty grayish exudate and the mucosa was congested. Similar changes were found in the trachea. Sections of the consolidated areas and the congested zone were removed and fixed in Zenker's solution.

The microscopic picture of the lungs showed a great degree of congestion, edema and perivascular cuffing with lymphocytes and macrophages (Fig. 1). A serous fibrinous exudate was present on the thickened pleura. Below the pleura a few giant cells were seen. Cultures made from the lung of this sheep were negative for bacteria. The tissue was stored for a month in a deepfreezer (-25° C.) prior to its use in this study.

Three grams of lung tissue were ground in a mortar and pestle and mixed with three mls of nutrient broth. This suspension was treated with penicillin and streptomycin to avoid the possibility of contaminants (Rose, Pearce and Molloy, 1946). One thousand units of penicillin and 0.1 mg of streptomycin were used for each gram of

tissue. After the first two passages of the lung material in the chick embryo the use of antibiotics was discontinued.

Smith (1935) and Burnet (1936) showed that some strains of Influenza virus grew on the chorioallantoic membrane of chick embryos. Attempts were made to cultivate a transmissible agent on the chorioallantoic membrane of the developing chick embryo.

Supply of eggs --- Fertile eggs from healthy white Leghorn hens were used inthis study and were purchased from the Poultry Department at Michigan State College.

Preliminary Incubation --- One to three day old eggs were incubated for 12 days, at a temperature of 99° F. and a humidity of 86 per cent. The incubator used was of an electric type manufactured by the Chicago Scientific Company*. The eggs were rotated twice a day during the incubation period. It was observed that the chorioallantoic surface was increased when the humidity was low and decreased when high.

Candling of the eggs --- This was done in a dark room with a microscope lamp.

The eggs were candled after three days of incubation to eliminate the infertile ones and the dead embryos. The candling was repeated on the twelfth day and the location

---

*Chicago, Illinois.

of the original air sac was marked. An area devoid of large blood vessels in the chorioallantoic membrane, where the artificial air space was to be made, was also marked on the shell.

Tincture of iodine was applied to the area where the egg shell was to be drilled. The eggs were opened with a stationary electric motor driver drill and disc.* The shell was cut with care to avoid injury to the underlying shell membrane and chorioallantoic membrane. The methods chosen were the ones used by Beveridge and Burnet (1946), Dunham (1942 and Alexander (1938). An oval opening 3 mm. by 2 mm. was made, by removing part of the shell with a side to side movement of the rotating disc. The surface of the egg over the air space was drilled to make a hole sufficiently deep to perforate the shell.

The inoculation of the chorioallantoic membrane was made according to methods of Burnet (1946). Twelve day old embryos were used. The eggs were inoculated within three quarters of an hour after removal from the incubator to avoid adhesion of the chorioallantoic membrane to the shell membrane. A slit was made in the shell membrane avoiding injury to the chorioallantoic membrane. The separation of the chorioallantoic membrane was made by the production of the artificial air space. This was done by gentle suction with a rubber bulb that was applied to the

---

*Chicago Wheel & Mfg. Co., Chicago, Illinois.

groove over the original air space which caused the chorioallantoic membrane to drop from the shell membrane. The inocula were checked for sterility before each inoculation. Two tenths ml of the inoculum was placed upon the chorioallantoic membrane with a tuberculin syringe and an 18 gauge needle. A drop of melted paraffin was used to seal the opening in the shell. Every third egg served as a control and was inoculated with 0.2 ml of nutrient broth. After inoculation, the eggs were placed in special racks. Care was taken not to disturb the original position of the air space. These racks were then placed in the incubator and the eggs were candled twice a day.

Some of the embryos were examined each day following inoculation up to and including the eighth day. To examine them, the surface of each egg was brushed with iodine and the paraffin removed by flaming with a Bunsen bruner. The egg shell was broken with sterile forceps and a very small opening made so that a platinum loop could be passed over the chorioallantoic membrane. Bacterial contamination was checked by agitating the loop in nutrient broth and incubating at $37^{0}$ F. for 72 hours. The eggs were then placed on a suitably moulded pad of cotton moistened with Phemerol. The shell was broken away to the level of the dropped chorioallantoic membrane. The chorioallantoic membranes were clipped away at the boundary of the artificial air space in order to remove the entire inoculated area. The chorioallantoic membranes which showed lesions on the

surface were saved for passage. The membranes were cut with a sterile scissors and forceps. These membranes were placed into screw top vials and kept in the deepfreezer.

To prepare the chorioallantoicmembranes for egg passage, 3 or 4 grams of membrane were added to 3 or 4 mls of broth and the mixture ground in the Waring Blendor.

This procedure was used to carry the transmissible agent through nineteen egg passages.

Before tissues were removed for histological study, Helly's solution was poured into the eggs while they were kept in the original positions on the rack. They were disturbed as little as possible during the following hour of fixation to avoid wrinkling and distortion of the membranes. Then the partially fixed tissues were removed from the eggs and further fixed in Helly's solution for five or six hours.

After fixation in Helly's solution (Mallory, 1938) the tissues were treated as follows:

| | | |
|---|---|---|
| Washed in water | 24 hrs. | |
| Alcohol 80% | 12 hrs. | |
| Alcohol 95% | 6-8 hrs. | |
| Alcohol 100% | 1-3 hrs. | |
| Cedar Wood Oil | 36 hrs. | |
| Paraffin (2 changes) | 24 hrs. | M.P. 49-50° C. |
| Imbedded in Paraffin | | M.P. 56° C. |

Since some of the tissues were thin the length of time the tissues were kept in alcohols varied from Mallory's (1938) original method.

Before passing to cedar wood oil, the organs were removed and sections of 5 to 7 mm cut for infiltration and embedding. Tissue sections 5-7 micra in thickness were cut. The following organs were studied: chorioallantoic membrane, lungs, heart, liver, spleen, kidney and brain. Several sections of each organ were examined for lesions.

Sections were stained with Harris' Hematoxylin and Eosin (Mallory, 1938) for general histological study. Mac Callum's (1919) modification of Goodpasture's method for staining gram positive and gram negative bacteria in tissue sections also was used.

# RESULTS

Macroscopic Lesions --- The eggs were candled and examined daily after inoculation. After three or four days incubation the infected embryos showed the following symptoms and lesions.

1. Sluggish movements to the extent that in some cases the embryo appeared dead.
2. The large blood vessels of the chorioallantoic membranes were less prominent than those of the control eggs.
3. Upon opening the shells the infected embryos, in most cases, were living, but upon exposure to the air and lower temperature death occurred much sooner than in the controls.
4. The infected embryos were small in some cases being about half that of the controls. (Fig. 2)
5. In infected embryos the abdominal wall failed to enclose the visceral organs.
6. The lesions were most prominent in those cases in which the embryos had succumbed to the infectious agent.
7. The mortality rate could not be determined since most of the eggs were opened for pathological examination at various times. However when death occurred it was approximately on the seventh day following inoculation.
8. The chorioallantoic membrane was congested and showed

hemorrhages varying in size from petechiae to ecchymoses. The membrane was thickened, gelatinous and edematous. Areas of opacity or "pocks" were observed. (Fig. 3) These areas were either homogenous or granular in appearance. On these patches, particularly on those lying along the large blood vessels, the tissue had proliferated to form yellowish white nodules. (Fig. 4) These nodules varied in size from one to two millimeters in diameter and showed a dark grayish necrotic spot.

9. Consistent changes were present in the lungs. It was difficult to remove the lungs from the body cavity due to the presence of a serofibrinous exudate between the lungs and ribs. The lungs appeared to be slightly increased in size, were swollen, and when cut appeared more flaccid than those of the controls.

10. The lesions observed in the spleen and brain were not constant. Some of the spleens appeared to be increased in size. The medulla oblongata appeared to be swollen, edematous and exhibited petechial hemorrhages.

Microscopic Lesions --- The chorioallantoic membrane showed a thickening of the ectodermal layer and in some instances the cells were necrotic. (Fig. 9) In the mesenchymal layer, formations of epithelial pearls appeared which often

resembled the tumor cells of squamous cell carcinoma and in other instances "Hassall's" corpuscles of the thymus. (Fig. 6) The epithelial pearls appeared as irregular bodies distributed at intervals between engorged blood vessels. These were located in different zones of the mesenchymal layer and most of them were situated deep in the mesoderm. The larger pearls showed the greatest degree of degeneration and the nuclei exhibited pyknosis, karyorrhexis and karyolysis. The nuclei located in the center of the epithelial nests were round, while those near the surface tended to be oval or elongated and parallel to the periphery. (Fig. 7) The chromatin of the nuclei in some instances was located at the periphery. Balloon degeneration of the epithelial cells was noticed. (Fig. 8) Concentric proliferation of connective tissue was observed around these epithelial pearls. (Fig. 7) In the older lesions the mature fibroblasts occupied the periphery of the pearls while the more immature fibroblasts were located in the zone next to the old concentrically arranged connective tissue. Other epithelial nests showed complete necrosis and sometimes only a hyalinized zone remained. (Fig. 7) There was usually a collection of eosinophilic polymorphonuclear leucocytes in the mesoderm near these pearls. Vascular dilatation of the blood vessels, areas of hemorrhage, proliferation of connective tissue and in some, infiltration of heterophiles surrounding the mesenchymal blood vessels were observed. It was significant that all the areas in which the epithelial

pearls were found corresponded to the "pock" lesions located on the surface of the chorioallantoic membranes. The changes were in sharp contrast with that shown by the normal chorioallantoic membrane (Fig. 5).

In the early passages of the virus, the embryos showed pneumonia which was characterized by the presence of serous exudate in the bronchi, followed by a thickening of the alveolar walls. (Fig. 11) The capillaries showed congestion, "perivascular cuffing" by leucocytes. (Fig. 13) Below the pleura and along its entire length hemorrhage was often seen. Edema was present in the interstitial tissue and after each passage of the agent this lesion was more pronounced. (Fig. 12) Another feature noticed in early stages was the presence of a heavy fibrinous layer of exudate over the thickened pleura. Lymphocytes were present in this fibrinous exudate. (Fig. 15) Around the alveolar openings, hyalinization of the muscle fibers was noticed. (Fig. 14) Many of the bronchi showed an increase in thickness of the lamina propia. There was probably epithelial proliferation since some of the cells seemed to be incarcerated in the form of "nests" by the connective tissue of the lamina propia. (Fig. 16) These changes appear in significance when compared to the lungs of normal chick embryos. (Fig. 10)

All of these features were observed in the nineteen passages of the agent through the chick embryo.

# DISCUSSION

The isolation of a transmissible agent from pneumonic lungs of sheep, capable of growing on the chorioallantoic membrane of the developing chick embryo had not been reported. Since the agent was pneumotropic, its pathogenic capacities for the chick embryo were compared to those of known agents that caused respiratory infections in goats and fowls.

The most characteristic lesion on the developing chorioallantoic membrane was the proliferative process which occurred, at first in the form of epithelial pearls, and later degeneration characterized by necrosis and hyalinization. Epithelial pearls had been observed by Longley (1946) in the mesenchymal layer, after inoculating the chorioallantoic membrane with pleuro-pneumonia organisms obtained from goats. During this investigation the tissue was cultured for pleuropneumonia organisms. However, no evidence of these forms were found in the cultures nor in the tissue sections.

Tang et al (1936) observed that pleuropneumonia organisms when inoculated on the chorioallantoic membrane of chick embryos, failed to produce lesions in the internal organs. In this investigation lesions were observed in the lungs after chorioallantoic inoculation of the transmissible agent.

Brandly, Thorp and Prickett (1948) studied the response of chick embryos to tissues of chickens affected with the avian leukosis complex and to tissues of normal birds. They reported that sections of chorioallantoic membrane showed keratinisation, vacuolization and "pilling up" of the ectodermal cells. There was a general thickening of the mesoderm in which the developing cells resembled the granulocytic series. Tumor formation with necrotic foci were seen at times. Epithelial pearls were frequently observed in the mesenchyme. Leucocytic cells when present, were usually in small nests, perivascular in position.

Woodruff and Goodpasture (1931) studied fowl-pox infection of the chorioallantoic membranes and reported that lesions were characterized by a marked hyperplasia of the ectodermal layer and by a thickening of the mesenchymal layer. Hyperplasia of entodermal cells was also noticed together with the presence of inclusion bodies. In the present investigation no proliferation of the entodermal layer was seen nor were inclusion bodies observed. Histologically, the lesions appeared to represent primarily the response of the ectodermal epithelium to the virus with secondary changes in the other layers.

The "curled" position of embryos as reported by Fabricant (1949) and Loomis (1949) following inoculation with bronchitis virus was not observed in this investigation.

The pathological changes in embryos were noted after the third passage of the transmissible agent isolated from sheep lungs.

The only marked lesions in the internal organs were seen in the lungs. The spleen was increased in size in some cases (splenomegalia) but no microscopic changes were observed.

When the chicks were old enough to hatch, they appeared to be too weak to break the shells and most of those that did hatch, lived only a short time.

The infected embryos were much smaller than the normal ones. This indicated that apparently there was interference with the metabolic processes. The chick embryo passage of a non bacterial transmissible agent and the accompanying lesions suggested a pneumotropic type of virus.

# SUMMARY AND CONCLUSIONS

1. A non bacterial transmissible agent was isolated from pneumonic lungs of sheep. This agent was maintained on the chorioallantoic membrane of developing chick embryos through 19 serial passages.

2. By passage of this agent in chick embryos pneumonia was produced in the embryonic lung.

3. A constant and characteristic macroscopic change was the presence of "pocks" on the chorioallantoic membrane.

4. The most persistent and distinctive microscopic lesions of the chorioallantoic membrane were the "epithelial pearls" formed by proliferation of the ectodermal cells in the mesenchyme. Necrosis of the ectodermal layer and necrosis and hyalinization of the epithelial pearls were also observed.

5. An apparent interference of growth processes in the chick embryos was observed.

6. The agent isolated in this study suggested a pneumotropic type of virus.

# BIBLIOGRAPHY

Alexander, R. A.
Studies on the neurotropic virus of horse sickness. VI Propagation in the developing chick embryo. Onderstepoort Jour. Vet. Sci. & An. Ind. 11:9-19, 1938.

Brandly, C. A., Frank Thorp, Jr. and C. O. Prickett,
Response of chicken embryos to tissues of chickens affected with the Avian Leukosis Complex and to tissues of normal birds. Poultry Sci. 28:486-498, 1949.

Baumann, R.
Pathological anatomy of contagious pleuropneumonia of goats. Vet. Rec. 50:1510, 1938.

Bawa, H. S.
Contagious pleuropneumonia of goats with special reference to immunization. Indian Jour. Vet. Sci. & Anim. Husb. 16:1-10, 1945.

Beveridge, W. C. B. and F. M. Burnet,
The cultivation of viruses and Rickettsiae in the chick embryo. His Majesty's Stationary Office. London. Special Reports series No. 256, 16, 1946.

Blakemore, F. and T. J. Bosworth
The occurrence of Jagziekte in England. Vet. Rec. 53:35-36, 1941.

Burnet, F. M.
Influenza virus on the developing egg. Brit. Jour. Exp. Path. 17:282-293, 1936.

Cowdry, E. V.
Studies on the etiology of Jagziekte. Jour. Exp. Med. 42:323-346, 1925.

———
Laboratory technique in Biology and Medicine. ed. 3. The Williams & Wilkins Co., Baltimore. 117-118, 1948.

Delaplane, J. P. and H. O. Stuart
Studies of infectious bronchitis. Rhode Island Agric. Exper. Sta. Bull. 273, 1939.

Delaplane, J. P. and H. O. Stuart
The modification of infectious bronchitis virus of chickens as the result of propagation in embryonated chicken eggs. Rhode Island Agric. Exper. Sta. Bull. 284, 1941.

Delpy, L.
Epizootic of pneumoenteritis in sheep in Persia. Rev. Gen. Med. Vet. 41:398-407, Abstracted from Vet. Bull. 3:319, 1933.

Doukaloff, I. A.
Infectious pleuropneumonia in goats. Soviet Vet. No. 9, pp. 55-61, Abstracted from Vet. Bull. 7:70, 1937.

Dungal, N.
Contagious pneumonia in sheep. Jour. Comp. Path. & Therap. 44:126-143, 1931.

Dunham, W. B.
Egg inoculator and shell membrane teaser for virus culture. Sci. 95:609, 1942.

Friedberger and Frohner's
Text book of Veterinary Pathology. Hurst and Blackett, London. 2:74, 1910.

Gilbert, S. J.
Infectious pleuropneumonia of goats in Palestine: Communicability to sheep. Jour. Comp. Path. & Therap. 50:33-38, 1937.

Henderson, W. H.
Annual Report of the Veterinary Department for the year 1929. Lagos Govt. Printer. 84. Abstracted from Vet. Bull. 1:328-329, 1931.

Kiseleff, E. V.
Vaccination against pleuropneumonia of goats. Lagos Govt. Printer. 84. Abstracted from Vet. Bull. 7:70, 1931.

Langham, R. F., Frank Thorp, Jr., R. T. Ingle, and L. B. Sholl
Some observations on the pathology of pneumonia in the food-producing animals. Amer. Jour. Vet. Res. 3:139-145, 1942.

Longley, E. O.
Contagious pleuropneumonia of goats. Indian Jour. Vet. Sci. 10:127-197, 1940.

Loomis, L. N.
Pathology of the chick embryo infected with infectious bronchitis virus. Thesis, School of Graduate Studies, Michigan State College, 1949.

MacCallum, William G.
The pathology of the pneumonia in the United States Army camps during the winter of 1917-1918. Monograph No. 10. Rockefeller Institute of Medical Research, New York, 47, 1919.

Mallory, Frank Burr
Pathological technique. W. B. Saunders, Philadelphia. 1938.

Miessner, H. and A. Koser
The 7th and 8th Reports of the imperial center (Germany) for combating diseases of breeding animals for the period 1st April 1929 to 31st March 1931. Deuts tierarztl Wesch. 39:693-697 1931. Abstracted from Vet. Bull. 2:530-532, 1931.

Mitchell, D. T.
Investigation into Jagziekte or chronic pneumonia of sheep. South Africa Dept. Agr. Dir. Vet. Res. Reports 3-4, 585, 1915.

Montgomerie, R. E., T. J. Bosworth and R. E. Glover
Enzootic pneumonia in sheep. Jour. Comp. Path. and Therap. 51:87, 1938.

Morcos, Z., O. A. Zaki and R. Zaki
Contagious ovine pneumonia caused by a filterable virus. Vet. Med. 41:202-204, 1946.

Rose, H. M., E. Pearce and E. Molloy
Effects of penicillin and streptomycin on bacterial contamination of chick embryo inoculated with unfiltered sputums. Proc. Soc. Exp. Bio. and Med. 62:124-127, 1946.

Smith, W.
Cultivation of the virus of influenza. Brit. Jour. Exp. Path. 16:508, 1935.

Stylianopoulus, M.
Pleuropneumonia of goats in Greece. Rev. Gen. Med. Vet. 42:401-416. 1933. Abstracted from Vet. Bull. 4:379-380, 1934.

Tang, F. F., H. Wei and J. Edgar
Further investigations on the causal agent of bovine pleuropneumonia. Jour. of Path. & Bact. 42:45-51, 1936.

Venkatachalam, G., R. H. Nelson, Frank Thorp, Jr., R. W. Luecke and M. L. Gray
Causes and certain factors affecting lamb mortality. Jour. Anim. Sci. 8:392-397, 1949.

Walker, J.
Contagious Pleuropneumonia of goats in Kenya. Ann. Rept. Dept. Agr., 1931. Abstracted from Vet. Med. 41:202-204, 1946.

Woodruff, A. M. and E. W. Goodpasture
The susceptibility of the chorioallantoic membrane of chick embryos to infection with the fowl-pox virus. Amer. Jour. Path. 7:209, 1931.

Fig. 1. The histopathology of the original lung from which the transmissible agent was isolated. H&E. 170 X.

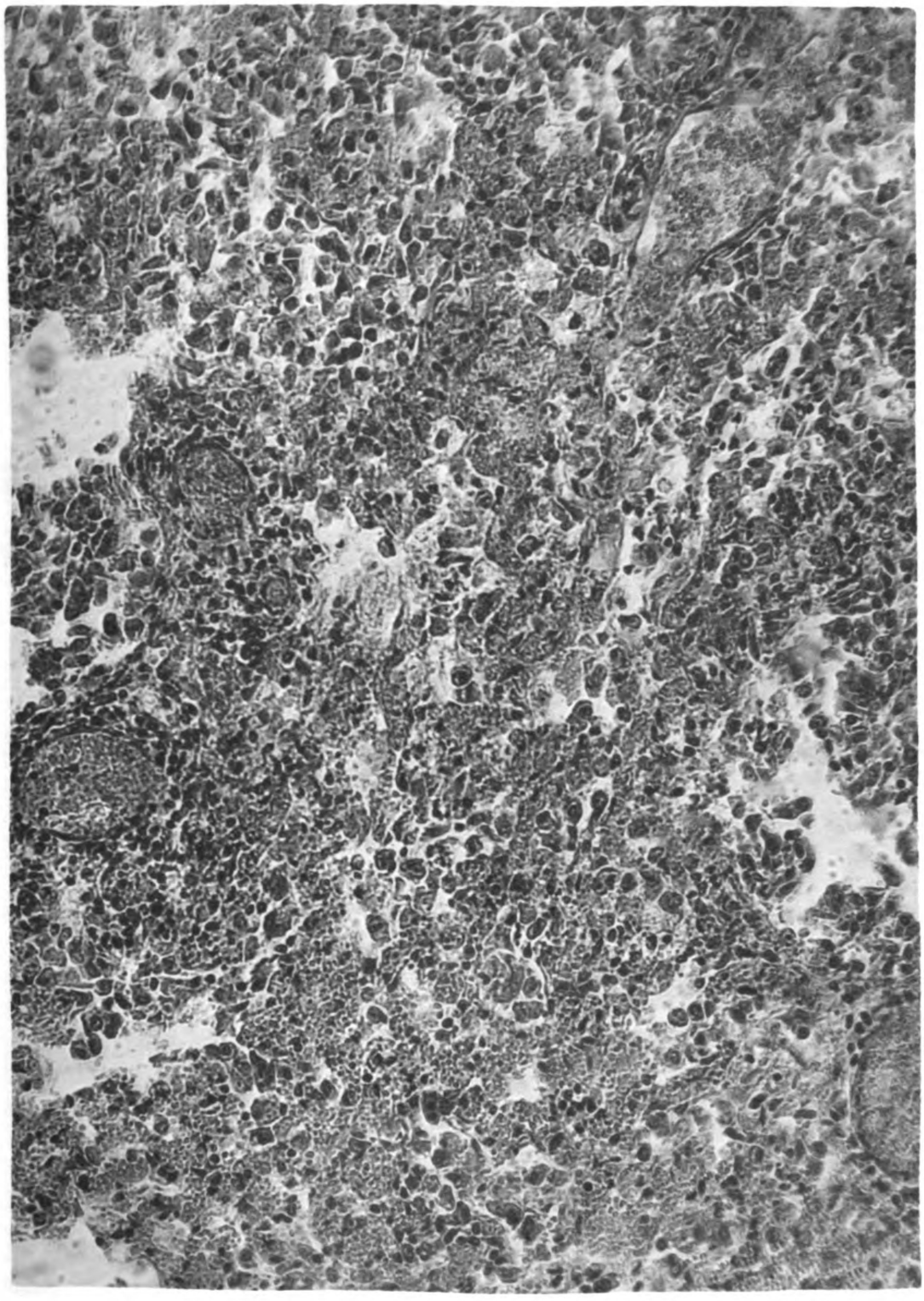

Fig. 1

Fig. 2. Two embryos each 19 days old. The control shown at the left is twice the size of the infected embryo.

Fig. 2.

Fig. 3. Chorioallantoic membrane showing edema, hemorrhage and the presence of opaque areas. 15 X. Ansco color print.

Fig. 4. Chorioallantoic membrane showing a large yellowish white nodule with hemorrhage. Note the engorged blood vessels. 8 X. Ansco color print.

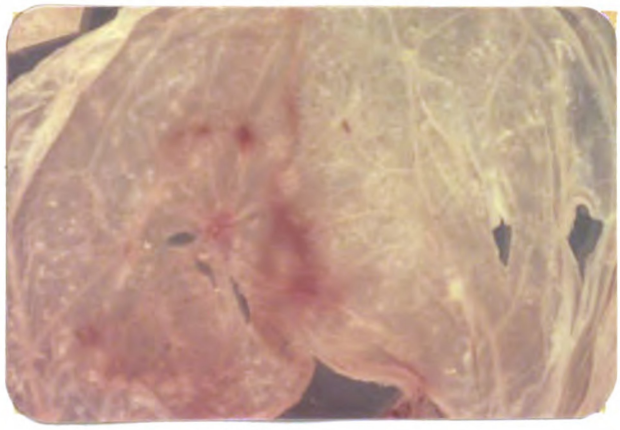

Fig. 3

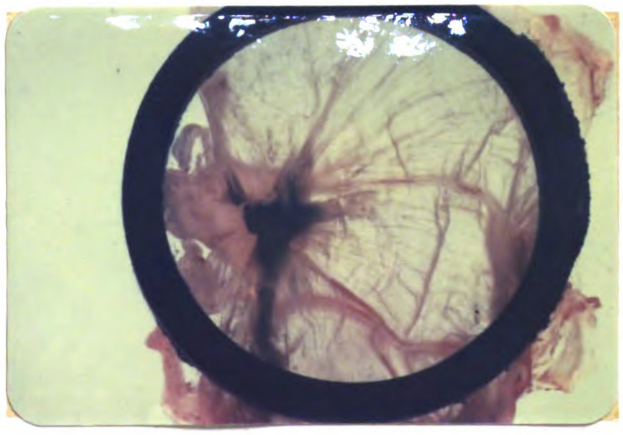

Fig. 4

Fig. 5. A normal chorioallantoic membrane of an 18 day old embryo. H&E. 70 X.

Fig. 5

Fig. 6. Chorioallantoic membrane showing the epithelial pearls, thickness of the mesodermal layer, congestion of capillaries and edema with extensive leucocytic infiltration. H&E. 116 X.

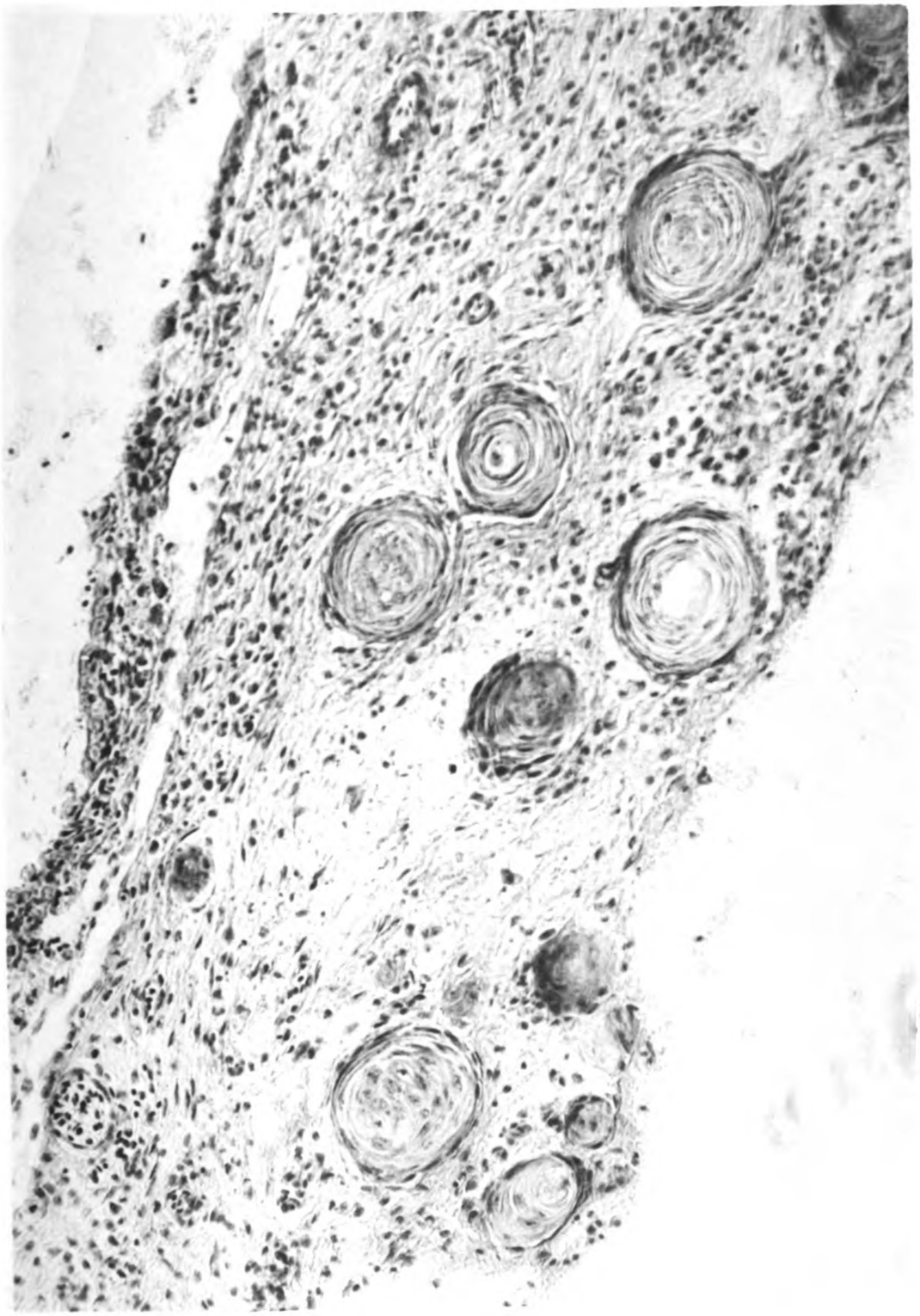

Fig. 6

Fig. 7. Chorioallantoic membrane showing epithelial nests with cells in various stages of degeneration, connective tissue around the nests and congestion of capillaries. H&E. 170 X.

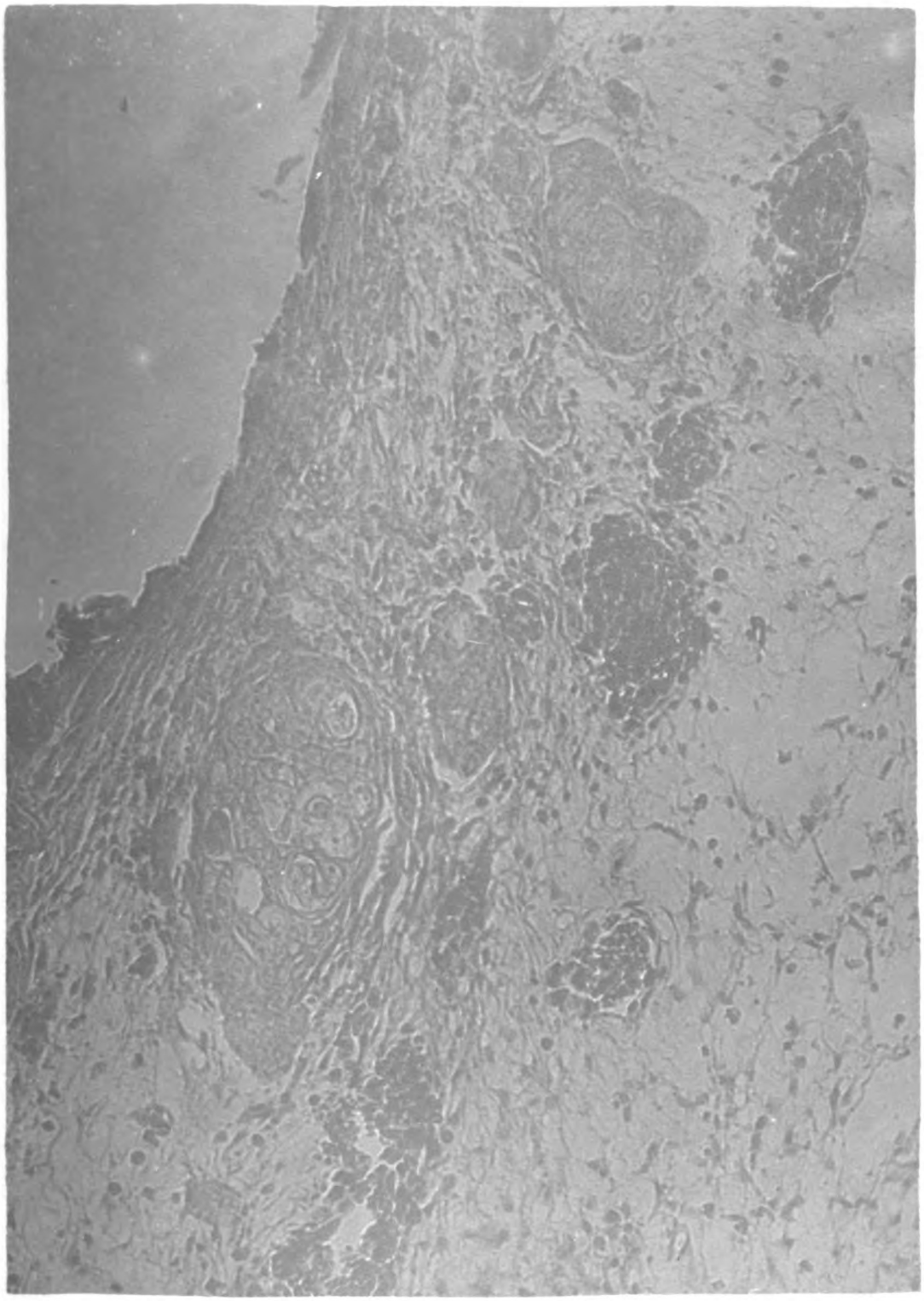

Fig. 7

Fig. 8. Chorioallantoic membrane showing a more severe degeneration of the epithelial nests. Balloon degeneration and hyalinization of the epithelial cells. H&E. 170 X.

Fig. 8

Fig. 9. Chorioallantoic membrane with necrosis of ectodermal layer and epithelial pearls; also extensive leucocytic infiltration. H&E. 70 X.

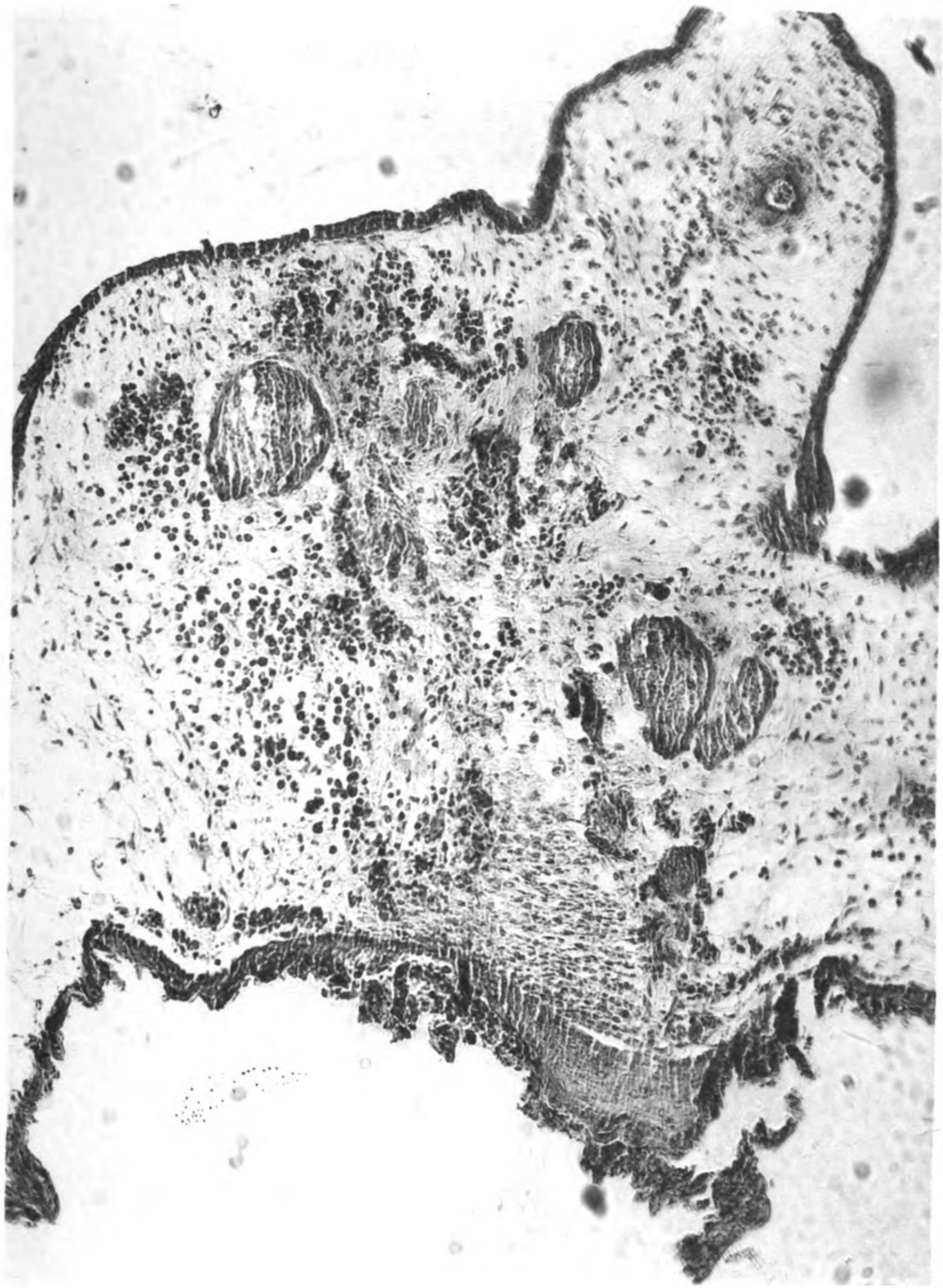

Fig. 9

Fig. 10. Normal lung of an 18 day old embryo. H&E. 100 X.

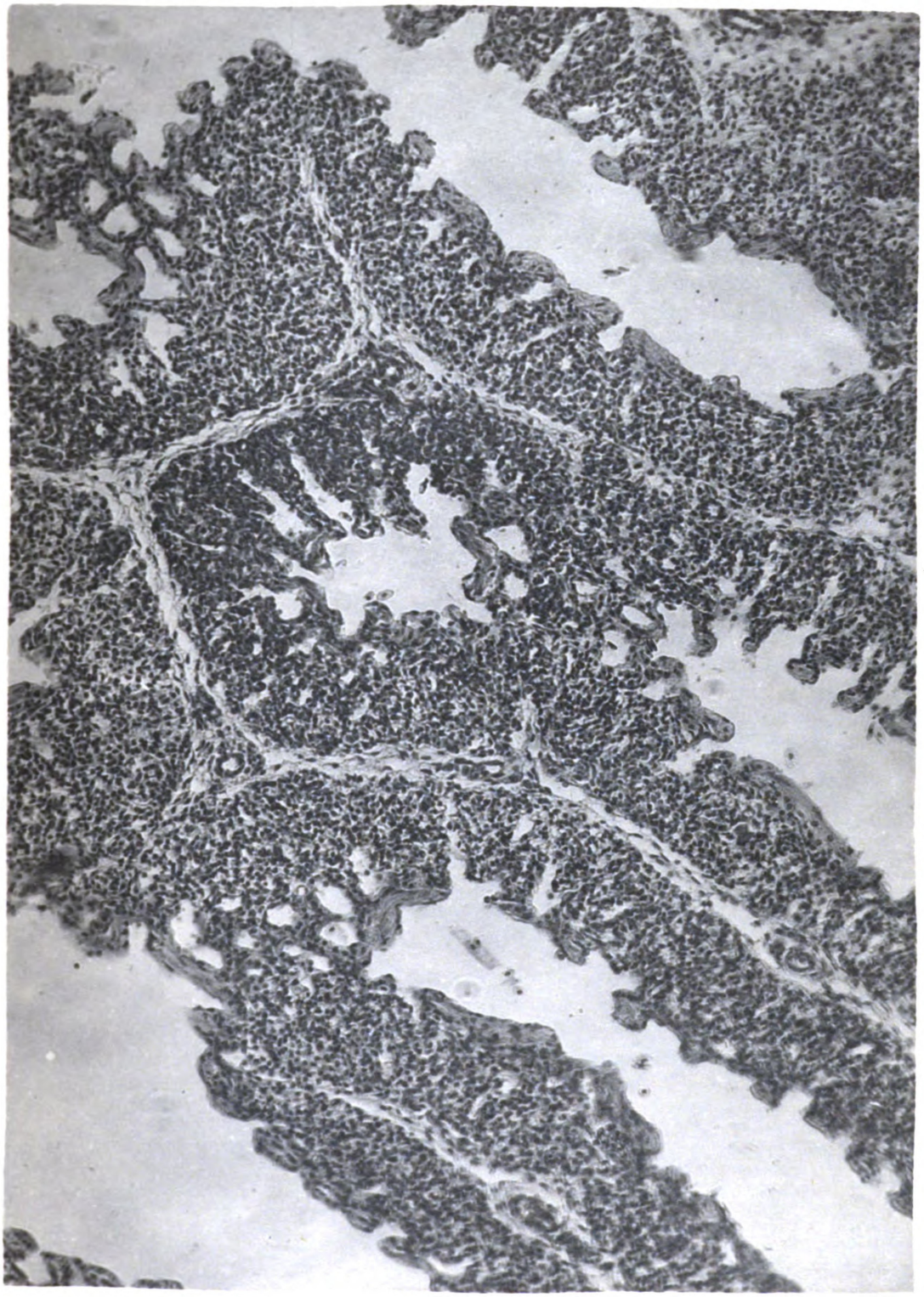

Fig. 10

Fig. 11. Infected lung showing alveoli filled with serous exudate and a few red blood cells. Remaining lung tissue shows edema. H&E. 100 X.

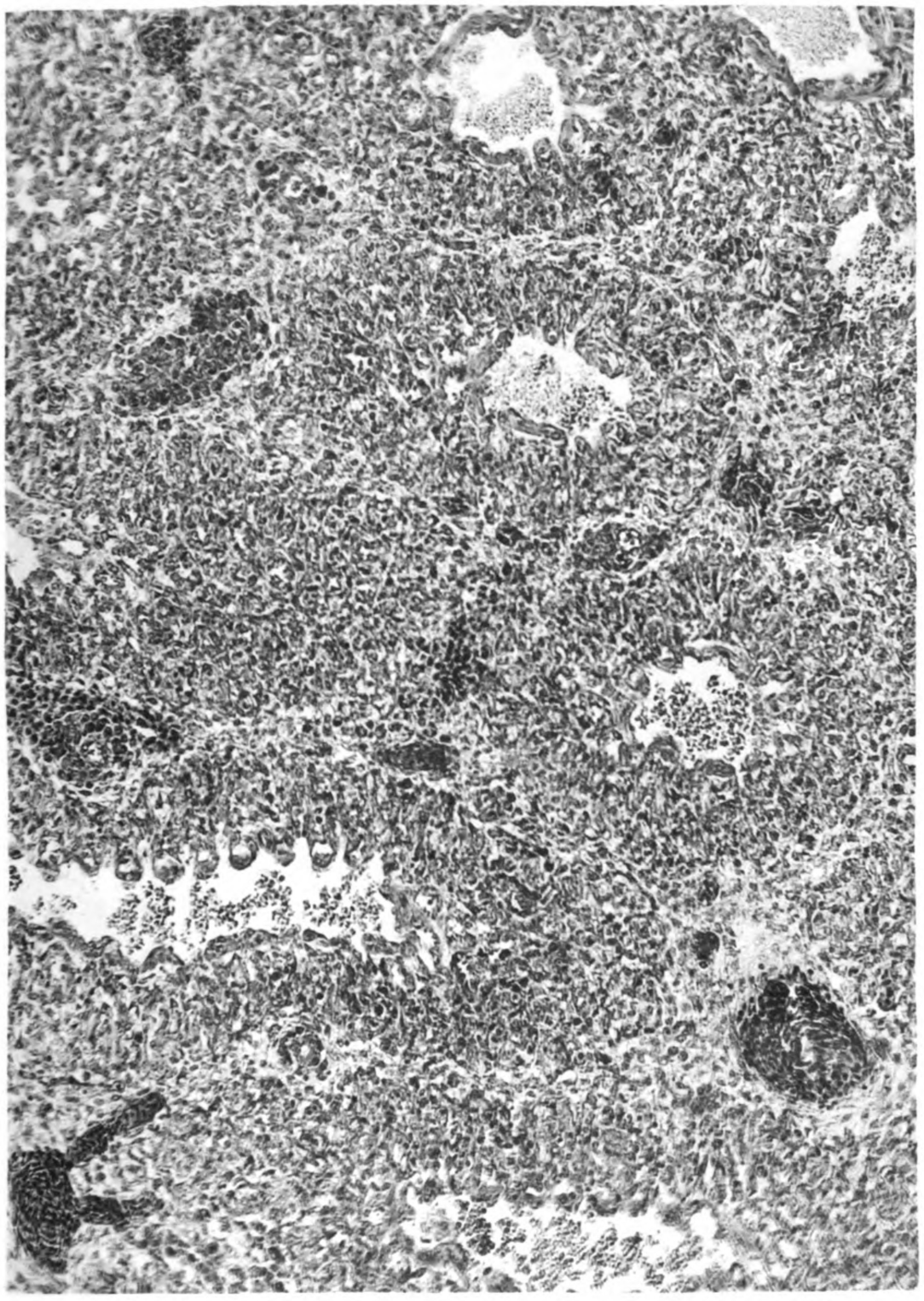

Fig. 11

Fig. 12. Infected lung showing serous exudate, involving the entire organ. The alveolar walls are thickened and capillaries congested. H&E. 100 X.

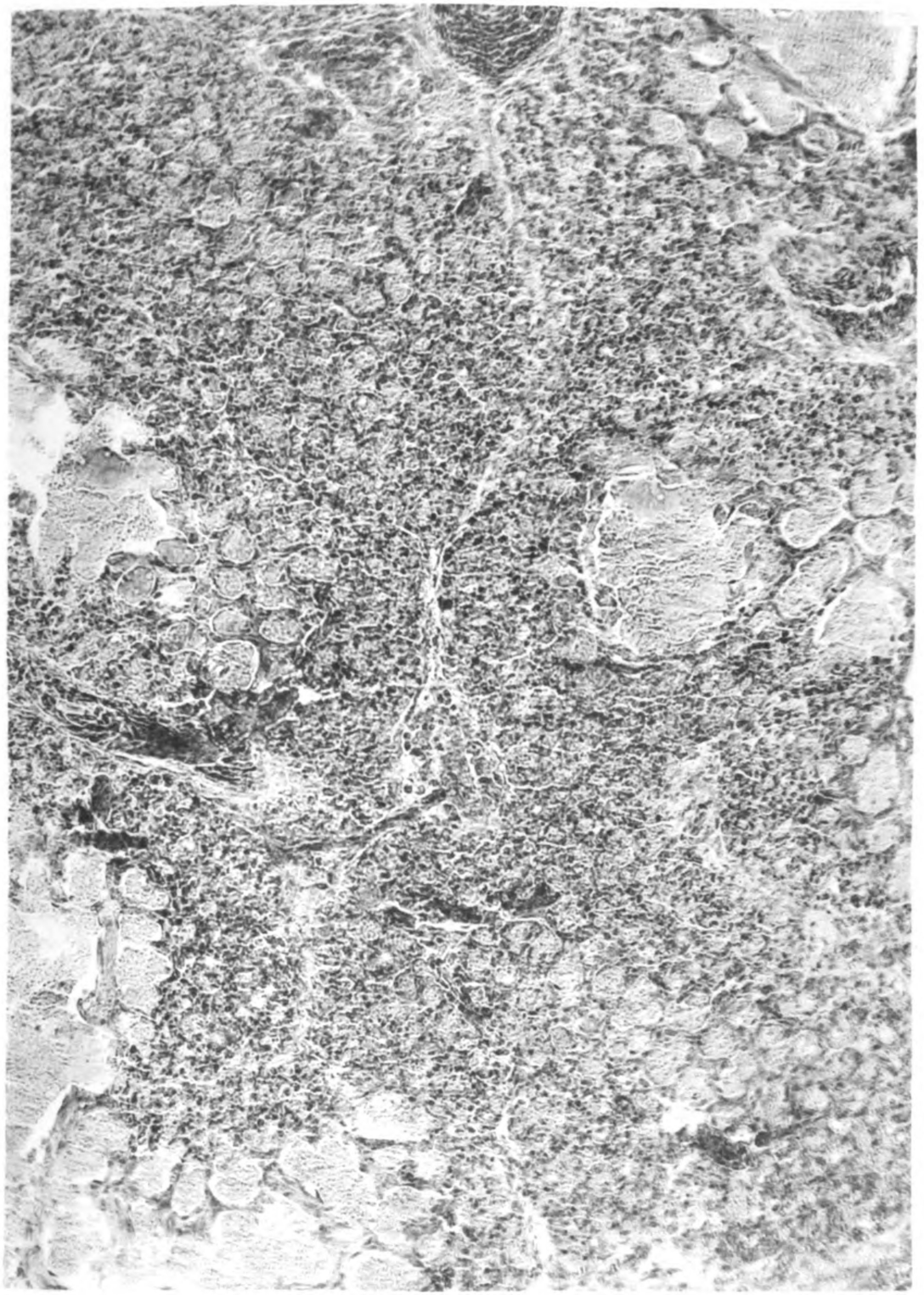

Fig. 12

Fig. 13. A blood vessel of the lung showing congestion and "perivascular cuffing" by leucocytes. H&E. 380 X.

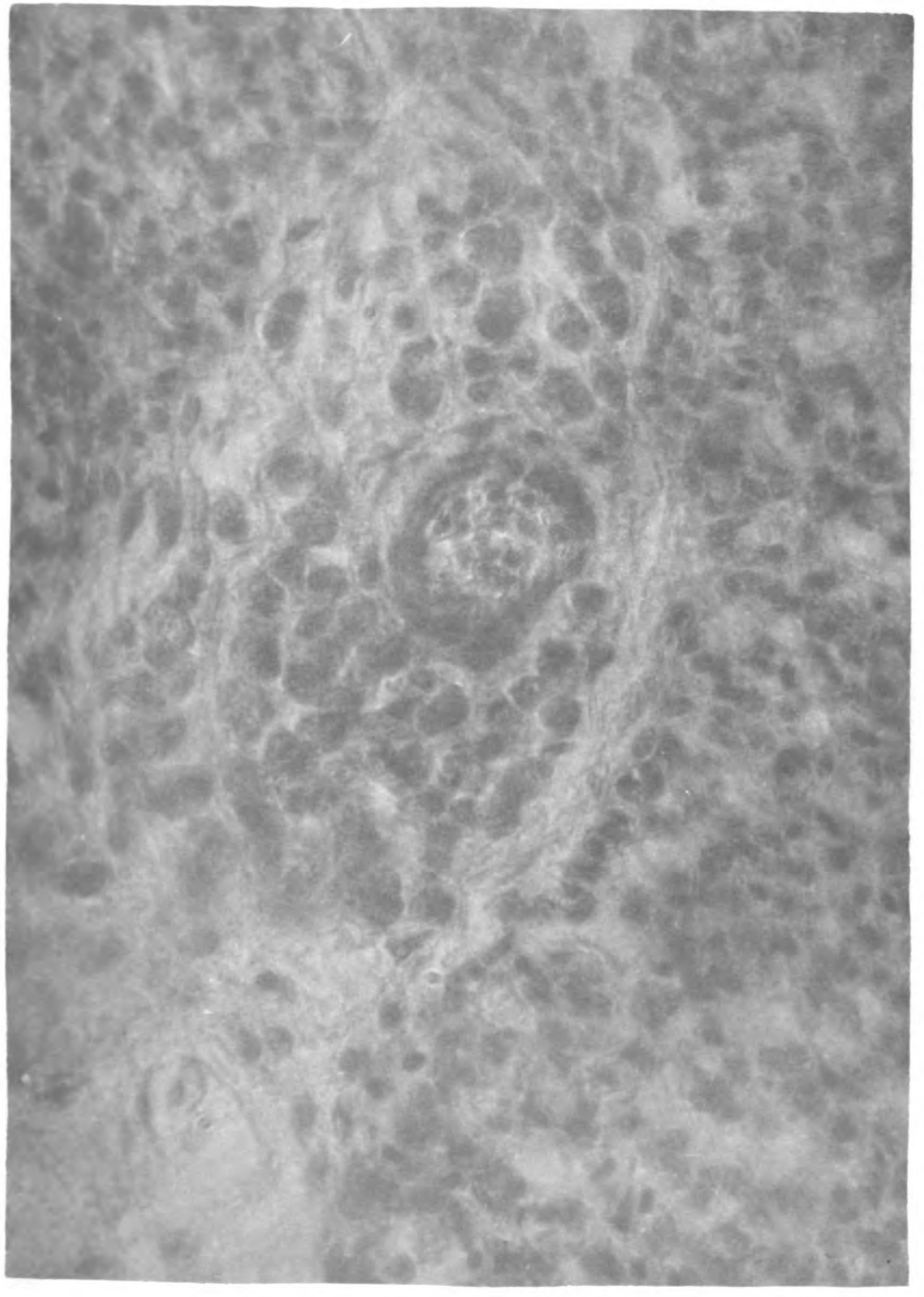

Fig. 13

Fig. 14. Showing hyalinization of muscle fibers at the site of the alveolar openings. Congestion of the capillaries is also evident. H&E. 480 X.

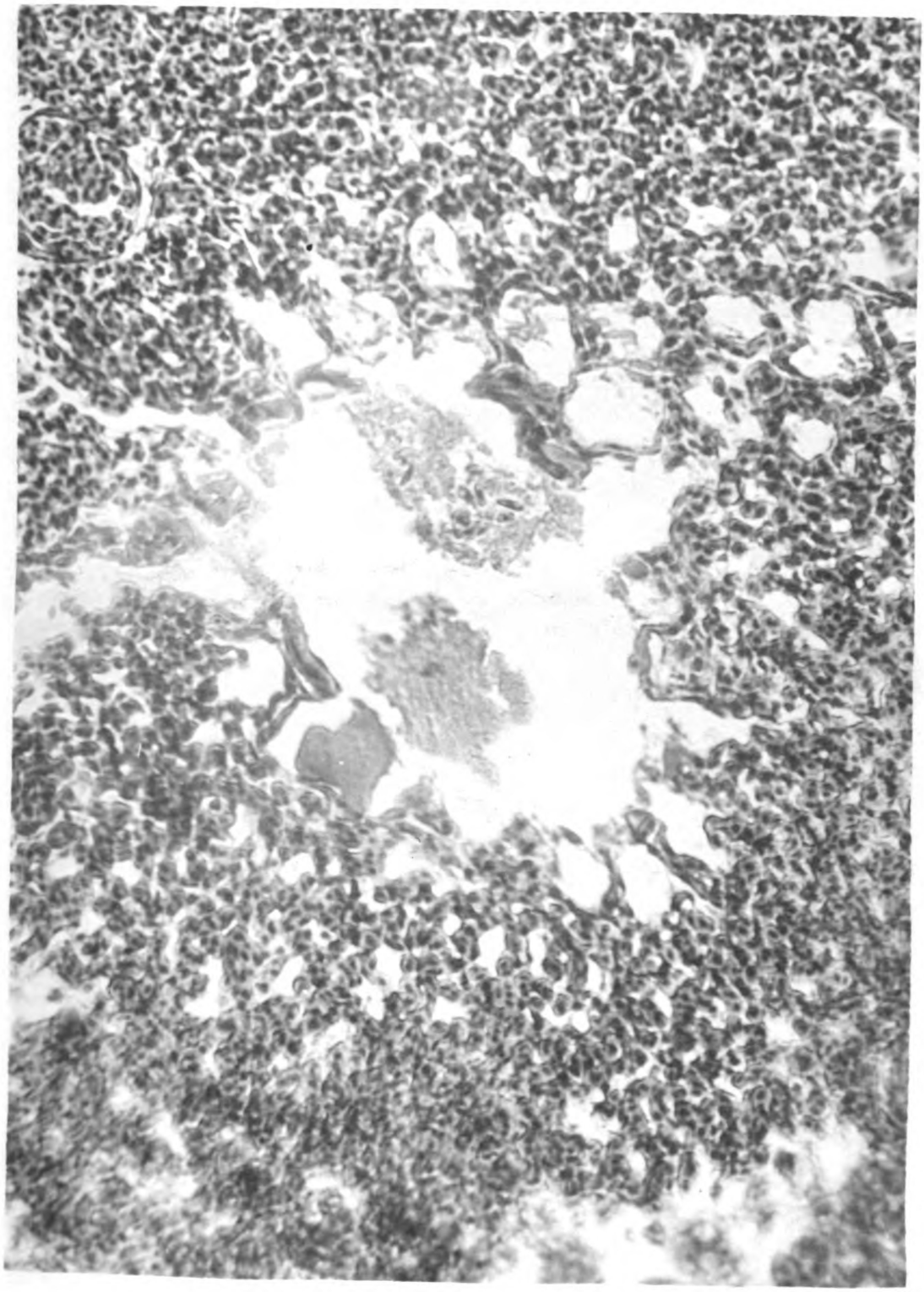

Fig. 14

Fig. 15. The pleura is covered with a thick layer of fibrinous exudate. Infiltration of lymphocytes is seen together with the fibrin. H&E. 100 X.

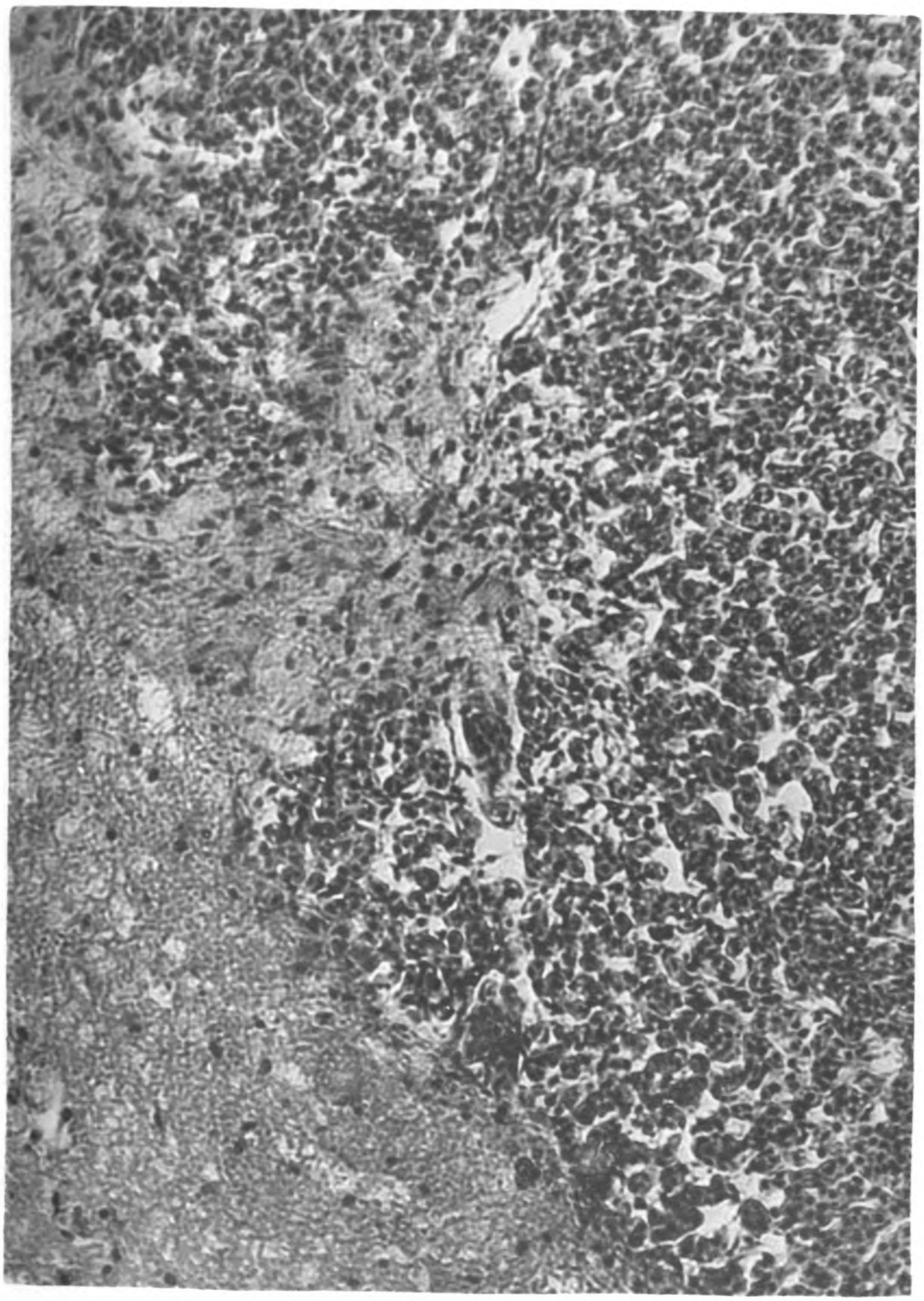

Fig. 15

Fig. 16. A bronchus showing increase in thickness of the lamina propia. Epithelial nests in this zone seem to be incarcerated by connective tissue. H&E. 170 X.

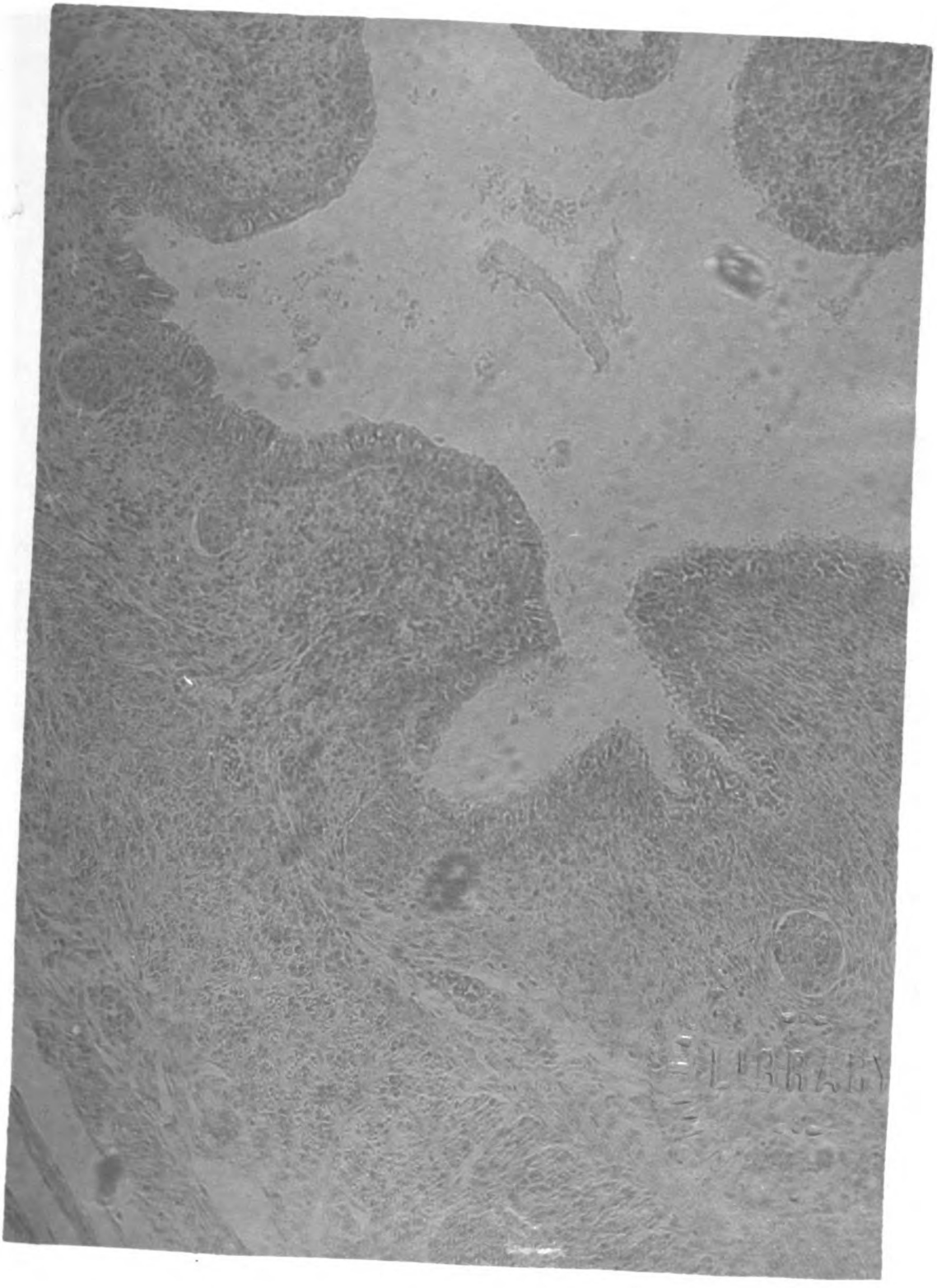

Fig. 16