PATHOLOGY OF THE CHICK EMBRYO INFECTED WITH INFECTIOUS BRONCHITIS VIRUS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Ladd Nixon Loomis

1949

PATHOLOGY OF THE CHICK EMBRYO INFECTED WITH INFECTIOUS BRONCHITIS VIRUS

By

Ladd Nixon Loomis

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 Bacteriology and Public Health

1949

TABLE OF CONTENTS

	Page
Acknowledgment	1
Introduction	1
Review of Literature	3
Materials and Methods	11
Tissue Technic for Preparation of Specimens	14
Results	16
Candling Observations	16
Gross Pathological Appearance of Embryos	16
Gross Lesions of Embryo Organs	20
Microscopic Lesions of Embryo Organs	23
Discussion	28
Summary and Conclusions	32
References	33
Photographic Plates	36

ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation for the advice and guidance of Dr. C. H. Cunningham, Dr. Frank Thorp Jr., Dr. H. J. Stafseth and Dr. R. A. Runnells; also to Mr. M. L. Grey for taking the photographs.

INTRODUCTION

Infectious bronchitis is an important virus respiratory disease of chickens characterized by a rapid spread and short duration with typical symptoms of sneezing, coughing and respiratory rales. Gross lesions consist of congestion and edema of the lungs, mucus in the bronchi and lower trachea and, in some cases, cloudiness of the air sac membranes with accumulations of cheesy material. Facial swelling may be observed in young chicks. In some chickens a nasal exudate may be present.

The morbidity rate is usually high but the mortality rate varies with the age of the affected chicken. In chicks the mortality rate may be as high as 60 to 70 per cent while in semi-mature and adult chickens it is negligible. In laying flocks a lowered egg production may persist for as long as two months before returning to the previous level of production.

Chickens recovered from the disease are refractory to subsequent infection with the virus. Naturally acquired passive immunity may be present for as long as four weeks in chicks hatched from eggs layed by immune hens. Inoculation of embryonating chicken eggs with suspensions of infected materials from chickens suspected of having the disease is one method for isolation and identification of

the virus. This study was undertaken as fundamental research on the pathology of the chick-embryo as a possible aid in the differential diagnosis of avian respiratory diseases.

REVIEW OF LITERATURE

Schalk and Hawn (1931) in North Dakota were the first to describe this disease from observations made of approximately 25,000 chicks. Morbidity rates were from 25 to 75 per cent and mortality rates were from 40 to 90 per cent.

Gross lesions of the lungs were described as acute congestion with a sero-mucoid liquid exudate. A similar exudate was present in the sinuses.

Berkefeld V, N and W filtrates of blood tissues and exudates of the respiratory tract of infected chickens were capable of producing the disease. Transmission experiments were complicated by infection of control chickens. This was overcome by housing the control birds in separate buildings and using separate attendants.

Beach and Schalm (1936) reported that Berkefeld V, N and W filtrates of nasal, tracheal and bronchial exudates were capable of causing infection.

Chickens recovered from the disease were refractory to further infection but no cross-immunity existed against coryza and laryngotracheitis.

Beaudette and Hudson (1937) were successful in

propagating infectious bronchitis virus on the chorioallantoic membrane of embryonating chicken eggs. The virulence of the virus increased by adaptation and embryo deaths increased with each successive passage especially from the seventh passage on. Infected embryos weighed one half as much as normal embryos of the same age. The virus did not produce pock or placque lesions on the chorioallantoic membrane. The authors reported that infected chorioallantoic membranes were thinner and more adherent to the inner shell membrane than normal chorioallantoic membranes. Yolk material was semi-solid.

Delaplane and Stuart (1939) reported certain similarities in the symptoms of infectious bronchitis and laryngotracheitis. The coughing, sneezing and respiratory distress were similar but in semi-mature and adult chickens infectious bronchitis did not cause the hemorrhage and false membranes in the larynx such as were noted in laryngotracheitis.

Chickens recovered from infectious bronchitis were immune to further infection. No cross-immunity existed between infectious bronchitis, laryngotracheitis or infectious coryza. Bronchitis immune serum was capable of neutralizing infectious bronchitis virus as demonstrated by infectivity tests with chickens.

The authors reported that one adult chicken was a carrier for as long as eight weeks, thus suggesting one

phase of the epidemiology of the disease.

The virus was propagated successfully on the chorioallantoic membrane of embryonating chicken eggs. No specific lesions of the membrane were noted.

Delaplane and Stuart (1941) presented detailed protocols for the modification of infectious bronchitis virus when propagated on the chorioallantoic membrane of embryonating chicken eggs.

During the first eight passages the virus failed to produce characteristic lesions of either the chorioallantoic membrane or of the embryo. None of the embryos were killed by the virus. With each succeeding passage the virus became more pathogenic for the embryo and at the 70th passage the virus was so adapted that all embryos were killed by the end of the second day. Gross lesions reported were whitish foci on the liver and congestion and swelling of the kidneys. Whitish opaque lesions of the chorioallantoic membrane were observed.

The virus survived storage in the fresh frozen state in the freezing compartment of an electric refrigerator for four and one-half months and at room temperature for five to seven days.

Delaplane (1943) discussed the differential diagnoses of avian respiratory diseases and stated that the

principal mode of dissemination of infectious bronchitis was through contact exposure with infected or "carrier" chickens and with contaminated equipment.

Typical lesions consisted of mucus, catarrhal and purulent accumulations in the trachea and bronchi with marked congestion and edema of the lungs. The air sacs might be clouded or show accumulations of a cheesy material, but in most cases no changes were noted. Facial swelling might be observed in young chicks.

Thirty to fifty per cent of infected chickens showed a serous or catarrhal nasal exudate after the sixth day of infection. Chickens recovered from the disease were immune but individual "carriers" might exist.

Hofstad (1945) reported that he was unable to detect carriers of infectious bronchitis by housing susceptible and recovered chickens in the same pen for three weeks or longer.

Further experiments by Hofstad (1947) showed that chickens recovered from infectious bronchitis could be carriers.

Cunningham and Stuart (1946) reported the effects of certain chemical agents on an egg-adapted strain of infectious bronchitis virus. Virus-infected allantoic fluid was mixed with the chemical agent and after a reaction period of three minutes the mixture was injected into

eggs by the allantoic sac route. Survival of the embryo was used as the criterion for the inactivation of the virus.

The following agents were effective against the virus; phenol, 3 per cent; liquor cresolis saponatus, 3 per cent and one per cent; tincture of metaphen, undiluted and one per cent; potassium permanganate, 1:1000 and 1:10,000; ethyl alcohol, 95, 70, 40 and 25 per cent; mecuric chloride, 1:1000; tincture of zepherin, 1:1000; Lugol's solution, 1 per cent; sodium hydroxide, 1:20; formalin, 1 per cent.

Boric acid (4.0%) and sodium hydroxide (.01%) were without effect.

Cunningham and Stuart (1947) reported that freezing and thawing of infectious bronchitis virus-infected allantoic fluid produced two types of precipitates, one soluble at room temperature and the other insoluble at room temperature. The insoluble precipitate could be sedimented by centrifugation.

The virus was more stable when stored at -25 C or -70 C than at -10 C.

No significant differences were observed when Difco nutrient broth, Difco tryptose phosphate broth, 0.85 per cent sodium chloride, or M/10 phosphate buffer (pH 7.0) were used as diluents for titration of the virus.

Delaplane (1947) found the allantoic sac route superior to the chorioallantoic membrane route for initial isolation of the virus, as evidenced by dwarfing of embryos on the first passage.

Streptomycin (0.25 gram per one ml.) decreased bacterial contamination in tracheal exudates used to inoculate embryonating chicken eggs for isolation of the virus.

Levine and Hofstad (1947) showed that infectious bronchitis virus could be air-born for as far as five feet. Ultra-violet irradiation for sterilization of the air proved to be of little or no value for control of the disease.

Reagan, Hauser, Lillie and Craige (1948) demonstrated by electron microscopy that the infectious bronchitis virus was round in shape with a mean diameter of 90 mu. A filamentous projection similar to that of Newcastle disease virus was present.

Jungherr and Terrell (1948) demonstrated by serum neutralization tests that naturally acquired passive immunity to infectious bronchitis could be present in chicks hatched from eggs laid by hens recovered from either the naturally or artificially induced disease.

Neutralizing antibodies in the yolk of embryonating eggs were found to decline after the eleventh day of in-

cubation, whereas after sixteen days a rise in neutralizing antibodies was observed in the egg content minus the yolk and albumen.

The neutralizing antibody level in chicks was high for the first two weeks after hatching but then declined rapidly and at the fifth week the chicks were susceptible to infection. Subsequent exposure of the chicks to the virus resulted in a marked increase of antibody.

Fabricant (1949) examined 6,000 embryos infected with the virus and emphasized the "curled" appearance of the embryo and the characteristic "dwarfing".

Dwarfing and curling appeared in 46 per cent of the infected embryos on the first passage, 33 per cent on the second, 20 per cent on the third and 1 per cent on the fourth passage.

Groupe (1949) found a thermostable material present in the allantoic fluid from infected embryos which had been stored at 36° C for 24 hours after death. This material interfered with the multiplication of infectious bronchitis virus in subsequent egg passages. Dilutions of the material caused a corresponding increase in chick embryo death rates.

Inoculation of heat-inactivated allantoic fluid containing interfering material delayed embryo deaths when inoculated into the allantoic sac 30 minutes before injection of active infectious bronchitis virus.

The author was unsuccessful in reproducing the interference phenomenon by the following methods: first, storage of infected allantoic fluid *in vitro* for 24 hours at 36 C; second, by storage of infected allantoic fluid *in vitro* in the presence of normal chorioallantoic membranes for 24 hours at 36 C; and third, by dilution of infectious bronchitis virus in allantoic fluid harvested from normal thirteen day old embryos killed by chilling and subsequently stored at 36 C for 24 hours before harvest.

MATERIALS AND METHODS

The virus used was a strain of infectious bronchitis virus designated as Lot 258 and supplied by Dr. Henry Van Roekel, Department of Veterinary Science, University of Massachusetts. This strain has been used for vaccination against infectious bronchitis in the Massachusetts control program. (Van Roekel, 1949)

Lot 258 had been isolated in May, 1941 from laboratory birds that had contracted a natural infection. Since that time the virus had been periodically propagated by passage in susceptible chickens, but had not been propagated in embryonating avian eggs.

The virus sample was received as a saline suspension of washings from the trachea and bronchi of an adult chicken showing typical symptoms of the disease on the third day after inoculation. Two passages in susceptible chicks were made using intranasal and intratracheal routes of inoculation. The supernatant fluid of pooled lung and tracheal tissue suspensions from three chicks of the second passage were used for the initial egg inoculations. Prior to inoculation the inoculum was treated with penicillin (10,000 units per ml.) and streptomycin (0.01 gm. per ml.). The inoculum was 0.2 ml per 10-day embryonating egg via the allantoic sac. Infected allantoic fluid was collected on the third post-inoculation day and used as inoculum for

the next passage. This procedure was followed for subsequent passages of the virus.

Ten-day embryos were used throughout the study. Inoculation was via the allantoic sac. Eggs were transilluminated for selection of an area of the chorioallantoic membrane free from large blood vessels about 3 mm. below the air cell. A small hole was drilled through the shell, without piercing the shell membrane, by means of a small drill attached to the chuck of an electric motor. Another hole was drilled through the shell over the top of the air cell. Tincture of metaphen was painted over the holes and allowed to dry. The shell membrane over the top of the air cell was punctured with a sterile teasing needle to allow equalization of pressure within the egg when the inoculum was injected into the allantoic sac and to prevent leakage of the inoculum from the site of injection. After injecting the inoculum, using a B-D Yale 1-cc. capacity tuberculin syringe, fitted with a 27 guage, ½-inch needle, the holes in the shell were sealed with melted paraffin and the eggs returned to the incubator. All incubation was at 99° F. in an electric forced-draft incubator.

At the time of collection of materials the shell over the air cell was painted with tincture of metaphen and then cracked and removed with forceps. The allantoic fluid was collected with a 5-ml. syringe and needle. The chorioallantoic membrane was then ruptured and the egg inverted to deposit the embryo in a Petri dish. The allantoic

fluid was collected on the third day after inoculation and 0.2 cc. of this fluid was used per egg for the next series of egg inoculations. Bacterial sterility tests of inoculums were negative.

TISSUE TECHNIC FOR PREPARATION OF SPECIMENS

Normal and pathological specimens were processed in an identical manner to insure uniformity for histological examination. Specimens were collected from living embryos only and fixed immediately to avoid post-mortem changes.

The method used was to candle the eggs and select living control embryos and living infected embryos. The embryos were removed from the shells and placed in Petri dishes for comparison and for photography. The abdominal cavity was then opened to insure penetration of the fixative and the embryos were dropped into labeled fixation jars containing Zenker's fixative. Formal-saline, Zenker's-formal (Helly's) and Bouin's solution were also used.

In an attempt to obtain optimum conditions, tissues were fixed for 4 to 24 hours and washed for 12 to 36 hours. The tissues were dehydrated by successive transfers in ethyl alcohol dilutions, ranging from 30 to 80 per cent; followed by 95 per cent and absolute ethyl alcohol. Cedar oil was used for 24 to 48 hours with one change. Tissues were embedded and sectioned in the usual manner.

The egg albumen method for tissue section fixation on the microscope slide was used.

The staining procedure described by Mallory (1937)

was the standard Hematoxylin-eosin method, using Harris' aqueous alum hematoxylin 1 per cent and acidified aqueous eosin 1 per cent. The regressive staining procedure using acid alcohol (ethyl alcohol 95 per cent, HCl 1 per cent) was used. Also, Wolbach's variation of the Giemsa stain (Lillie, 1948) was used especially to detect myelogenous tissue. However, the sharper hematoxylin-eosin stain was preferred for routine tissue examination.

A rapid acetone method of tissue preparation was used as follows: formalin one hour, three changes of acetone in one hour and one change of paraffin in one hour (all in the paraffin oven). No difference in staining quality could be detected by the writer between the standard hematoxylin-eosin method and the rapid acetone hematoxylin-eosin method.

RESULTS

Transillumination of Infected Embryonating Eggs.

Gross alterations of the infected embryos could be detected easily by candling the egg. The infected embryo had a typical curled position and did not have normal free motion when the egg was sharply rotated. Large vessels of the extra-embryonic membranes were more prominent than those of normal embryos. The infected chorioallantoic membranes had an anemic appearance, which was possibly due to impaired capillary circulation. This change was most noticeable on the third or fourth day after inoculation.

Gross Alterations of Infected Embryos.

In general the gross alterations agree with the description of Delaplane and Stuart (1941) and Beaudette and Hudson (1937). The most marked characteristic was the curled position of the embryo and its small size as compared with the normal embryo of the same age. Movements of the infected embryo were noticeably slower and weaker.

The embryo was drawn up with its feet over its head into a firm round shape and was dwarfed in size, to 50 per cent the size of the normal embryo.

The thickened amnionic membrane adhered to the

embryo and resisted removal. When the amnionic membrane was removed a dry fibrotic surface was left on the inside of the amnionic membrane and the feathers of the embryo were drier than normal.

Some embryos showed jaundice and all embryos showed decreased feather development. Infected embryos exhibited deformed feet that were compressed over the head and also a wry neck which had a characteristic lateral curvature.

The organs remained outside the abdominal cavity with greater frequency when the embryo was infected. About 33 per cent of the embryos showed excess bile deposits. Ten per cent of the embryos had a distended white cloaca filled with fat droplets.

Approximately one to two per cent of the embryos were resistant to infection with this strain of infectious bronchitis virus. These embryos resembled the normal embryos in every respect.

No abnormal odor was noticed from the infected embryos. Occasionally small petechial hemorrhages were noted in the skin of a few infected embryos, as mentioned by Delaplane and Stuart (1941).

SUMMARY OF GROSS PATHOLOGICAL APPEARANCE OF INFECTED EMBRYOS.

1. The chorioallantoic membrane was adherent to the inner shell membrane and appeared thinner than normal.

2. Living infected embryos were sluggish and weak.

3. The embryos were dwarfed as much as one half normal size.

4. The embryo assumed a ball-like shape characterized by a curled position with the feet deformed and compressed over the head.

5. The dry fibrotic amnionic membrane resisted removal from the embryo. Feathers of the embryo were not as moist as normal.

6. The feather development was immature.

7. The embryo skin ~~and feathers were~~ were icteric.

8. Residual yolk material was of greater volume and of firmer consistency than normal.

9. The cloaca was distended with fat droplets, which caused a white appearance. This occurred in about 25 per cent of infected embryos.

10. A characteristic wry neck was observed in all infected embryos.

11. Incomplete involution of abdominal organs occurred in about 4 per cent of the infected embryos. The organs remained external to the abdominal cavity.

12. No abnormal odor was detected.

13. Gross lesions of internal organs: Livers were either hemorrhagic, icteric, or contained necrotic foci; kidneys were usually swollen and some showed foci of necrosis, or icterus; lungs were small, pale, and viscid; heart developed to half normal size.

14. Approximately 33 per cent of the infected embryos showed bile discoloration of liver and kidneys.

15. Approximately one to two per cent of the embryos did not exhibit pathological alterations.

GROSS LESIONS OF EMBRYO ORGANS

Heart

The heart in all infected embryos was noticeably decreased in size. No other lesions were observed either grossly or microscopically.

Liver

Extensive pathological lesions of the liver were noticed in every case. Grossly the liver showed an extensive hepatitis with hemorrhages in the tissue or under the serosa. Excess bile production and or bile obstruction were seen. About 33 per cent of the livers exhibited marked biliary discoloration. Instead of a normal yellowish fatty appearance the livers were usually dark red to purple. One-third of the livers examined showed varying shades of green, either at the edges or throughout the liver, especially in the ventral area adjacent to the gall bladder.

Whitish or yellowish necrotic areas alternating with hemorrhagic areas were noticed on the surfaces of some livers.

Lungs

Lung development was markedly retarded. Infected lungs were of soft consistency and pale pink in color. About 70 per cent of the lungs were found adherent to the thoracic walls by fibrotic strands that increased the difficulty of removing them from the embryo both when fresh or fixed.

The infected lungs were of a sticky texture which was easily detected when they were placed on a glass plate. A thin tenacious serous exudate was invariably present. A 50 per cent decrease in the size of the lung was of common occurrence in infected embryos.

Kidney

The kidneys of infected embryos were swollen and exhibited yellowish foci; they were somewhat firmer than normal kidneys. The swellings were due to edema and cellular infiltration of myelogenous cells. Part of the yellowish deposits were considered to be urate crystals. Some kidneys were dark green in color, due to excessive bile. The livers of these embryos were also bile stained and were dark green in color.

Chorioallantoic Membrane

The chorioallantoic membrane was thickened by proliferation of many layers of mesoderm. Edema of areas of junction of the allantoic membrane to the amniotic membrane were noticed. The marked proliferation of islands of tissue that could be produced by strains of virus isolated by Delaplane and Stuart (1941) were not seen in tissues infected with this strain of virus.

Despite the appearance of thinness and its tenaciousness to the shell, the infected chorioallantoic membrane was thicker and more opaque when fixed than the normal.

Yolk Sac Membrane

The outstanding gross feature was extreme friability of the yolk sac which invariably ruptured when the embryo was removed. This was true also in the normal embryo but to a lesser degree.

Amniotic Membrane

This appeared to be thickened, opaque and to ~~con-~~ strict embryo movement.

Bones

The bones of the infected embryo were softer and uniformly appeared to be several days behind the normal in length, rigidity and diameter.

Brain

No lesions were observed.

MICROSCOPIC LESIONS OF EMBRYO ORGANS

Lung

Pneumonia characterized by congestion, granulocytic and monocytic infiltration, together with a serous exudate in the bronchial sacs was found in all infected lungs. These reactions were distributed evenly throughout the entire lung tissue and the condition was apparent from as early as the fifth day of infection.

While moderate desquamation of epithelial cells of the bronchial sacs occurred, no areas of extensive necrosis or abscessation were found. The infiltration of ~~vesicular~~ elements, serous exudate and desquamation, progressed in severity as hatching age was reached but without the occurrence of the severe tissue reaction observed in the liver and kidneys. A pulmonary tissue-virus equilibrium seemed to be established in the lungs.

The bronchioles and bronchi contained a serous exudate which was composed of fine granular eosinophilic particles, granulocytes, monocytes and epithelial cells. No diphtheritic membrane, extensive necrosis or abscess formation was seen in any bronchioles or bronchi examined.

The smooth muscle cells at the edges of the bronchial sacs were hyalinized to a uniform and extensive degree especially after the sixth or seventh day. Later, these

hyalinized areas were infiltrated with granulocytes and monocytes. Interstitial edema was not detected.

Liver

Early manifestations of virus activity were produced on the hepatic vascular system. Severe congestion with perivascular "cuffing" was seen throughout the liver by the sixth day. At this time pyknosis and karyorrhexis of the nuclei of hepatic cells began to appear. Coagulation necrosis with nucleolysis was present on about the eighth day. This condition was observed to involve whole lobes of the liver. By the nineteenth day extensive abscessation occurred in many embryo livers. The periphery of those abscesses was ringed by granulocytic and monocytic cells, nearby areas exhibited hemorrhage and also congested hepatic arterioles and capillaries. Some livers became extensively stained with bile pigments, but the microscopic lesions of this type of liver advanced with the same degenerative stages as the hemorrhagic reaction with the exception that much more bile pigment was present in the tissue section.

Normal fatty changes of the liver did not take place in the majority of infected livers. Fat vacuoles present in the normal hepatic cells were noticeably absent in the pathological hepatic cells.

Extensive hemorrhage beneath Glisson's capsule was frequently noted.

The interlobular bile capillaries underwent the same degenerative changes, and at the same time, as the hepatic cells.

The reaction of the reticular-endothelial system (Kupffer cells) was masked by the severe blood vascular reaction.

Kidney

Interstitial nephritis with edema and distention of the proximal convoluted tubules with large hemoglobin casts were the early lesions observed.

Most changes occurred in the metanephros. Due to the immaturity of the tissue, the granular appearance of some tubular epithelium was difficult to evaluate; while in other areas marked extrusion of cytoplasm from the ruptured epithelial cells into the lumen of the tubules was seen.

The majority of the glomeruli did not seem to be altered. The intraglomerular space was clear and Bowman's capsule was of normal thickness and was not adherent to the glomerular tuft. However, a few sections of the kidneys showed dilated glomerular spaces, some containing desquamated epithelial cells surrounded by granulocytic cells. Other glomeruli were noticed to be enlarged with swollen capillary tufts plugged with granulocytes. Vacuolization of endothelial cells in these capillaries was noted.

Also a few sections contained areas of dissolution of the glomerular tuft that left an intact basement membrane surrounding tissue debris. This occurred near areas of focal necrosis of tubular epithelium.

The renal vascular system exhibited extreme congestion, of both arteries and capillaries. Some large areas of both subcapsular and interstitial hemorrhage were observed surrounded by layers of granulocytic and monocytic cells. Erosion of arterioles in the vicinity was considered to be the cause.

Spleen

The spleen was enlarged to twice normal size. Excess hemoglobin was present. Also many hyaline-like clumps of material were present. No areas of necrosis were seen, but some capillary congestion was observed. (The clumps of eosinophlic material indicated excessive debris from erthrocyte destruction)

Brain

Slight capillary congestion was the only abnormality observed.

Chorioallantoic Membrane

Marked proliferation of the cells was observed in both mesothelium and ectoderm. Edema was marked. No areas of hemorrhage or necrosis were observed.

Amnionic Membrane

Edematous swelling and proliferation of the endo and mesothelium were seen.

Yolk Sac Membrane

The yolk sac membrane showed capillary congestion.

Bone

No cellular changes were observed.

DISCUSSION

Fabricant (1949) in a recent report emphasized the "curled" position of embryos infected with infectious bronchitis as well as the dwarfing mentioned by Beaudette and Hudson (1937), Delaplane and Stuart (1939), and Delaplane (1947).

This report confirms both alterations and it is the author's opinion that these changes together are pathognomonic of the disease.

Inoculation via the allantoic sac using allantoic fluid (treated with antibiotics) proved a very satisfactory method for virus propagation. The pathological alteration of embryos was noted on the first egg passage. The lesions were considered to be due to the virus since the preparations were bacteria-free.

Brandley, Thorp and Prickett (1949) reported that intravenous inoculation of normal whole blood or leukotic material caused extensive lesions to develop in embryos. However, those pathological manifestations were considered different from those reported in this investigation.

While perivascular infiltration did occur in the material studied by Brandley et al the much more severe hepatic lesions caused by infectious bronchitis virus was

evident. Brandley et al reported no kidney lesions except a decrease in size; whereas the infectious bronchitis virus infected kidneys were enlarged and showed extensive lesions. Brandley et al found marked bone changes such as enlargement of the shafts of long bones to three times normal diameter. The bones of infectious bronchitis infected embryos exhibited a decrease in length and diameter as well as rigidity.

The kidney lesions resembled those reported by Jungherr (1943) for avian monocytosis. Enlargement of kidneys, uric nephritis, desquamation of the epithelium of the proximal convoluted tubules, dilation of tubules with hyaline-like casts, or plugs of hemoglobin and crystalloid deposits are all similar findings. However, no thickening of the basement membrane or protein precipitate in Bowman's space was seen in the kidneys in this investigation.

Extensive necrosis and marked vascular reaction indicated a more severe tissue reaction, in infectious bronchitis.

Although "cuffing" of hepatic vessels is comparatively rare, Murphy (1916) reported the same reaction from the use of sterile tissues as the inoculum. He concluded that "cuffing" was the result of splenic stimulation. His explanation of this reaction was "That grafts of adult spleen, bone marrow, liver, and kidney placed in the outer membrane of a chick embryo cause stimulation of the embryo

spleen and lead to proliferation of certain leukocytic elements in the mesoderm, subcutaneous tissues and around vessels in the liver and kidney".

The spleens in this report were increased to twice normal size but not to the size described by Murphy and without the microscopic changes described by Dantschakoff (1920).

In this study "cuffing" occurred in approximately thirty per cent of the livers examined. Combined with the gross embryo changes and the other microscopic lesions discussed, "cuffing" should be considered as an aid in diagnosis but not as a specific lesion determining a diagnosis.

No inclusion bodies were found in the tissues examined. (Lucas, 1949)

Delaplane (1941) mentioned that infected embryos did not live after hatching. This was readily understood when the extensive lung, liver and kidney changes were evaluated. These tissues would be unable to meet the increased demands made of them to support the active life of a newly hatched chick.

In view of the liver lesions that occur in infected embryos, perhaps if virus derived from only liver tissue were used in successive egg passages, a viscerotrophic adaptation might take place and a liver vaccine for adult

chickens might be developed. The physiological reserve of the adult chicken liver might be able to withstand the infection without a high mortality and an active immunity might be produced.

SUMMARY AND CONCLUSION

Infectious bronchitis virus caused a characteristic pathological effect on chick embryos. A distinctive curled position and dwarfing was consistently found. Fifty per cent of the embryos died within five days after inoculation via the allantoic sac. Approximately one to two per cent reached hatching age. Approximately one per cent of the embryos were completely refractive to the virus (in eight passages). The first and subsequent passages caused dwarfing and curling of embryos. No increase in mortality rates was detected during the eight passages. A failure to grow to one-half normal size was regularly produced in the heart, liver, and lungs. The kidney and spleen were enlarged to twice normal size or more.

Pneumonia with marked serous exudation was a constant lung lesion. Hemorrhagic hepatitis merging into necrosis and abscessation was uniformly observed. Interstitial nephritis degenerating into necrosis and abscessation occurred. The spleen exhibited congestion and increased activity. The brain tissue was congested.

The gross and microscopic tissue changes combined with the distinctive alteration of position and size of chick embryos are of diagnostic significance in the differentiation of virus activity in avian respiratory diseases.

REFERENCES

Beach, J. R. and Schalm, O. W. (1936).
A filterable virus, distinct from that of Laryngotracheitis, the cause of a respiratory disease of chicks. Poul. Sci. 15: 199-206.

Beaudette, F. R. and Hudson, C. B. (1937).
Cultivation of the virus of infectious Bronchitis. Jour. A.V.M.A., 90: 51-60.

Beveridge, W. I. B., and Burnet, F. M. (1946).
The cultivation of viruses and ricketsiae in the chick embryo. Med. Research Council (Brit.), Special Rept. Series, No. 256.

Brandly, C. A., Thorp, Frank, Jr. and Prickett, C.O. (1949).
Response of Chicken Embryos to Tissues of Chickens Affected with the Avian Leukosis Complex and to Tissues of Normal Birds. Poul. Sci., 28: 486-497.

Cunningham, Charles H. and Stuart, H. O. (1946).
The Effect of Certain Chemical Agents on the Virus of Infectious Bronchitis of Chickens. Am. J. Vet. Res., 7: 466-469.

Cunningham, C. H., and Stuart, H. O. (1947).
Cultivation of the virus of infectious bronchitis of chickens in embryonated eggs. Am. J. Vet. Research, 8: 209-212.

Cunningham, C. H., and El Dardiry, A. H. (1948).
Distribution of the virus of infectious bronchitis of chickens in embryonated chicken eggs. The Cornell Vet., 38: 381-388.

Dantschakoff, Vera (1930).
Myeloid metaplasia of the embryonic mesenchyme in relation to cell potentialities and differential factors. Carnegie Institution of Washington, Contributions to Embryology, No. 49: 3-32.

Delaplane, J. P. and Stuart, H. O. (1939).
Studies of Infectious Bronchitis. Rhode Island Agric. Exper. Sta. Bull. 273.

Delaplane, J. P. and Stuart, H. O. (1941).
The Modification of Infectious Bronchitis Virus of Chickens as the Result of Propagation in Embryonated Chicken Eggs. Rhode Island Agric. Exper. Sta. Bull. 284.

Delaplane, J. P. (1947).
Technique for the isolation of infectious bronchitis or Newcastle virus including observations on the use of streptomycin in overcoming bacterial contaminants. Mimeo. report Nineteenth Annual Pullorum Disease Conference. Raleigh, N. C. June 11-12-13.

Fabricant, Julius (1949).
Studies on the Diagnosis of Newcastle Disease and Infectious Bronchitis of Fowls. II. The Diagnosis of Infectious Bronchitis by Virus Isolation in Chick Embryos. The Cornell Vet., 39: 414-431.

Groupe, Vincent (1949).
Demonstration of an Interference Phenomenon Associated with Infectious Bronchitis Virus (I.B.V.) of Chickens. J. of Bact., 58: 23-31.

Hofstad, M. S. (1945).
A Study of Infectious Bronchitis in Chickens. The Cornell Vet., 35: 32-35.

Hofstad, M. S. (1947).
A Study of Infectious Bronchitis in Chickens. The Cornell Vet., 37: 29-34.

Jungherr, E. L., and Terrell, N. L., (1943).
Naturally acquired immunity to infectious bronchitis in chicks. Am. J. Vet. Research, 9: 201-205.

Levine, P.P., and Hofstad, M. S. (1947).
Attempts to Control Air-borne Infectious Bronchitis and Newcastle Disease of Fowls with Sterilamps. The Cornell Vet., 37: 204-211.

Lillie, R. D. (1948).
Histopathological Technic. The Blakiston Co., Philadelphia.

Lucas, A. M. (1949).
Personal communication.

Mallory, F. B. (1938).
Pathological Technique. W. B. Saunders Co., Philadelphia.

Murphy, J. B. (1916).
The effect of adult and chicken organ grafts on the chick embryo. J. Exper. Med. 24: 1-5.

Schalk, A. F. and Hawn, M. C. (1931).
An apparently new respiratory disease of baby chicks. Jour. A.V.M.A., 21: 413-422.

Van Roekel, H. (1949).
Personal communication.

Fig. 1. The curled position of an embryo pathognomonic of infectious bronchitis virus. 17-day-old embryo, 7th day of infection. Kodachrome print.

Fig. 1

Fig. 2. The embryo on the left is a seventeen-day-old embryo, 7th day of infection. It illustrates the dwarfing effect of the virus. Notice the decreased feather development, anemia, the deformed feet. The cloaca is distended with fat droplets. Normal embryo of the same age is on the right.

Fig. 3. The two dwarfed embryos are infected. All three are nineteen-day-old embryos. This illustrates the individual variation in size of the two infected embryos on the left. The alteration of the necks and the compressed feet are typical. The normal embryo of the same age is on the right.

Fig. 2

Fig. 3

Fig. 4. Heart, liver, lungs and kidneys (pelvic girdle) from two normal embryos on the left and from two infected embryos on the right. The difference in organ size is apparent. The group of organs third from the left shows a hemorrhagic hepatitis with focal necrosis of one lobe. Undeveloped lungs with areas of pneumonia. The kidneys are spotted with necrotic areas and are swollen.

The group of organs on the extreme right illustrates the bile discolored type of liver reaction. Underdeveloped lungs and the kidneys contain bile pigments in correlation with the excessively bile stained liver. All embryos are nineteen days old.

Fig. 5. Organs (heart, liver and lungs) of the infected embryo are on the left. Notice the hemorrhagic hepatitis with a necrotic area on the tip of the left dorsal lobe. The lungs are pneumonic and the tenacious serous exudate is evident about the lungs.

The normal organs on the right illustrate the normal fatty appearing liver and the highly vascular lung tissue.

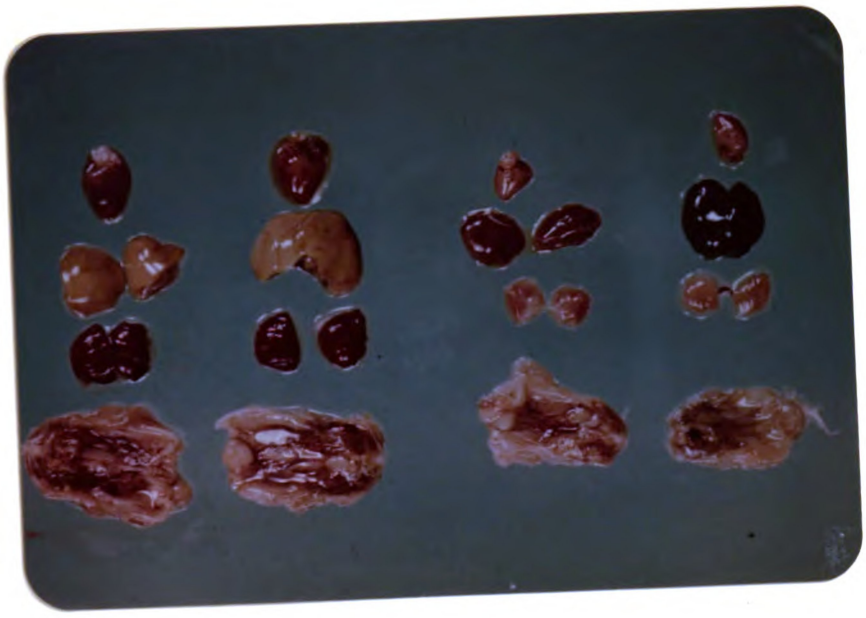

Fig. 4

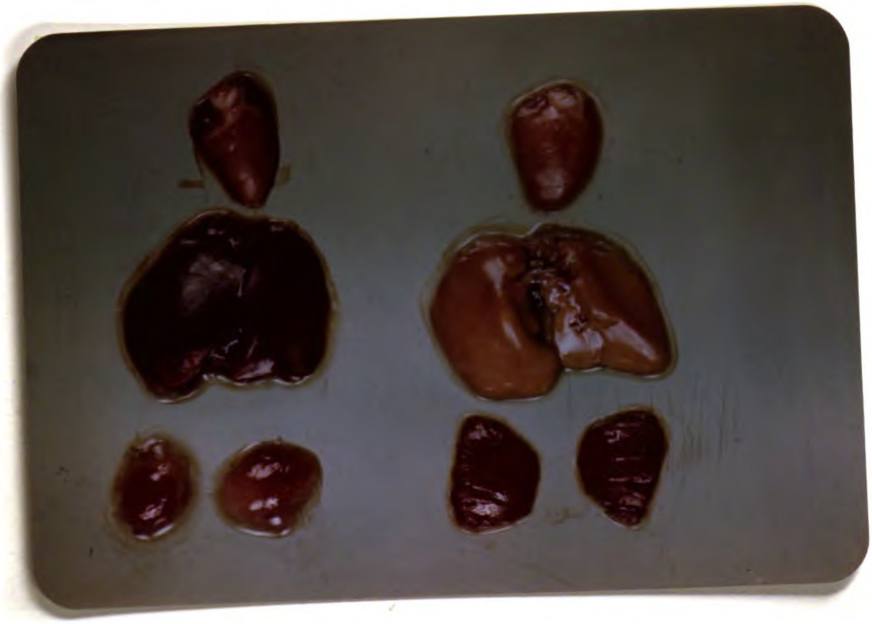

Fig. 5

Fig. 6. Normal kidneys above and the infected kidneys below. Notice the bile pigmentation of the infected lower left kidney. The lower right kidney illustrates the necrotic areas seen in some embryos. The kidney is enlarged. The difference in size of the pelvic girdle between normal and pathological embryo is shown.

Fig. 7. Photomicrograph of a kidney of an infected seventeen-day-old embryo showing plugging of a proximal convoluted tubule with a hemoglobin cast, in upper right. Edematous tissue with disintegrated tubules in center, and in lower right a dilated glomerular space containing desquamated cells with an enlarged and congested glomerular tuft. 175X Kodachrome print.

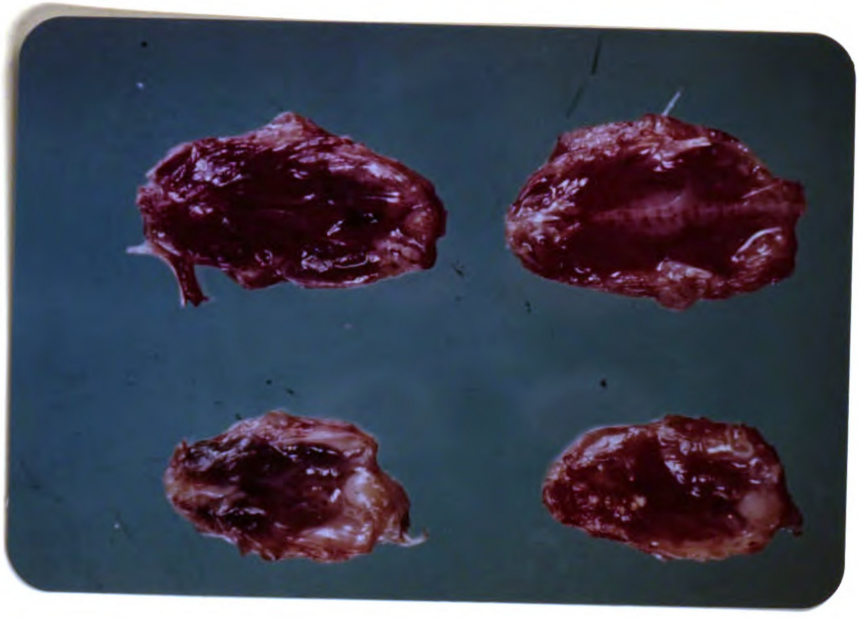

Fig. 6

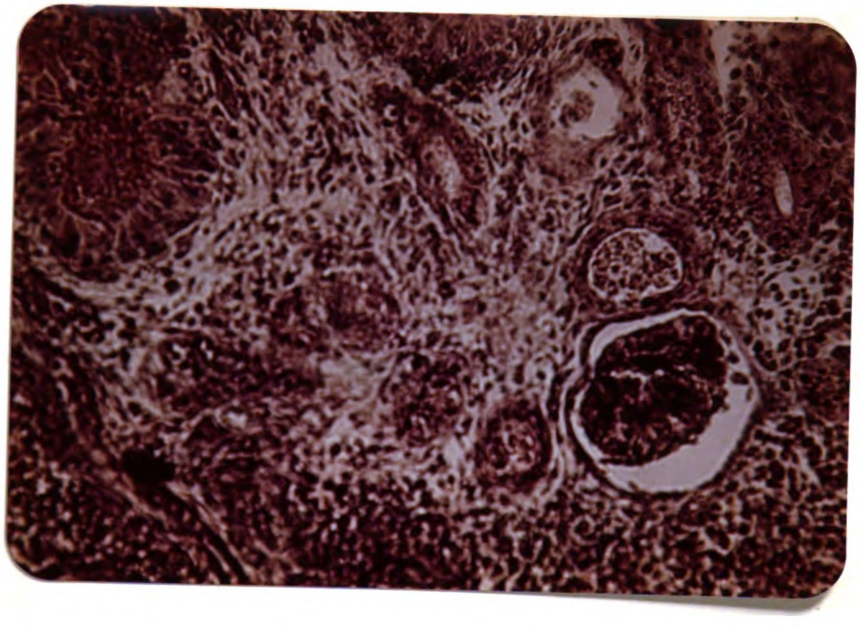

Fig. 7

Fig. 8. Photomicrograph of lung tissue of an infected eighteen-day-old embryo. Pneumonia with serous exudation and granulocytic and lymphocytic infiltration is shown. Congestion of pulmonary arteriole is seen in the lower right hand corner. The bronchial sacs are filled with serous exudate containing granulocytic and desquamated epithelial cells. 90X Kodachrome print.

Fig. 9. Photomicrograph of liver tissue of an infected embryo fifteen days old. Perivascular "cuffing" of cells of the granulocytic series is shown. 90X Kodachrome print.

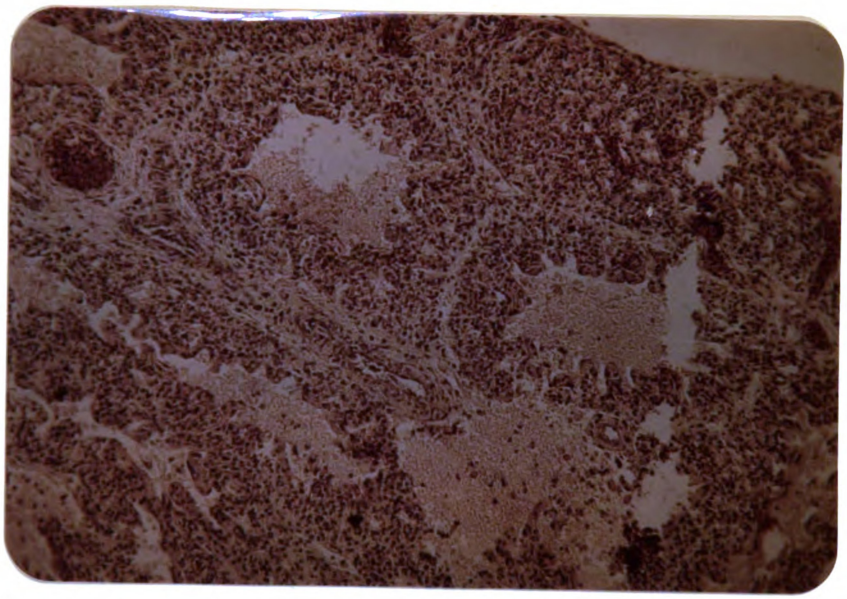

Fig. 8

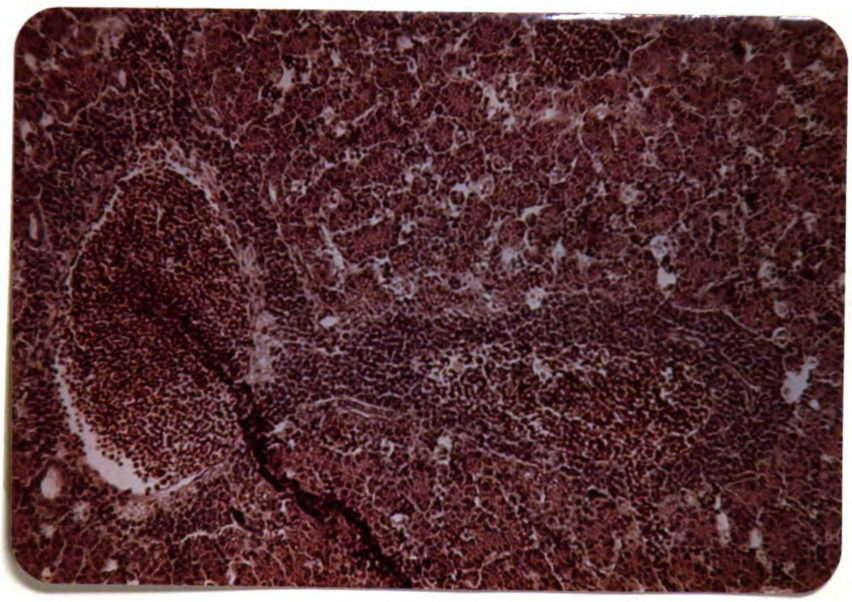

Fig. 9

Fig. 10. A normal chorioallantoic membrane from a 15-day-old embryo. 135X Kodachrome print.

Fig. 11. A pathological chorioallantoic membrane showing the cellular proliferation of the mesoderm and ectoderm, also edema. 135X Kodachrome print.

Fig. 10

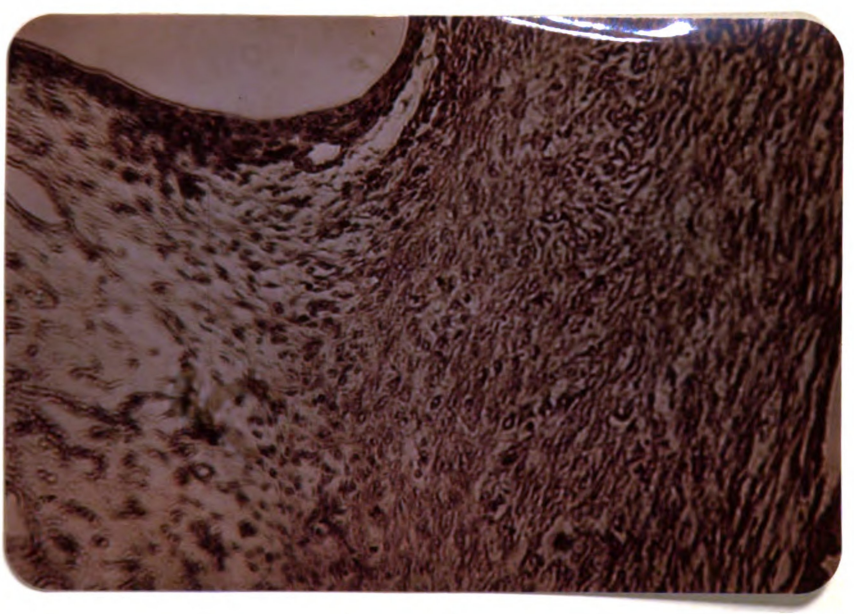

Fig. 11

Fig. 12. 13th day. First of a series of four photographs showing a comparison of size between an infectious bronchitis virus infected embryo and a control embryo the same age. Notice the cloaca distended with fat droplets. Also the deformed feet of the infected embryo.

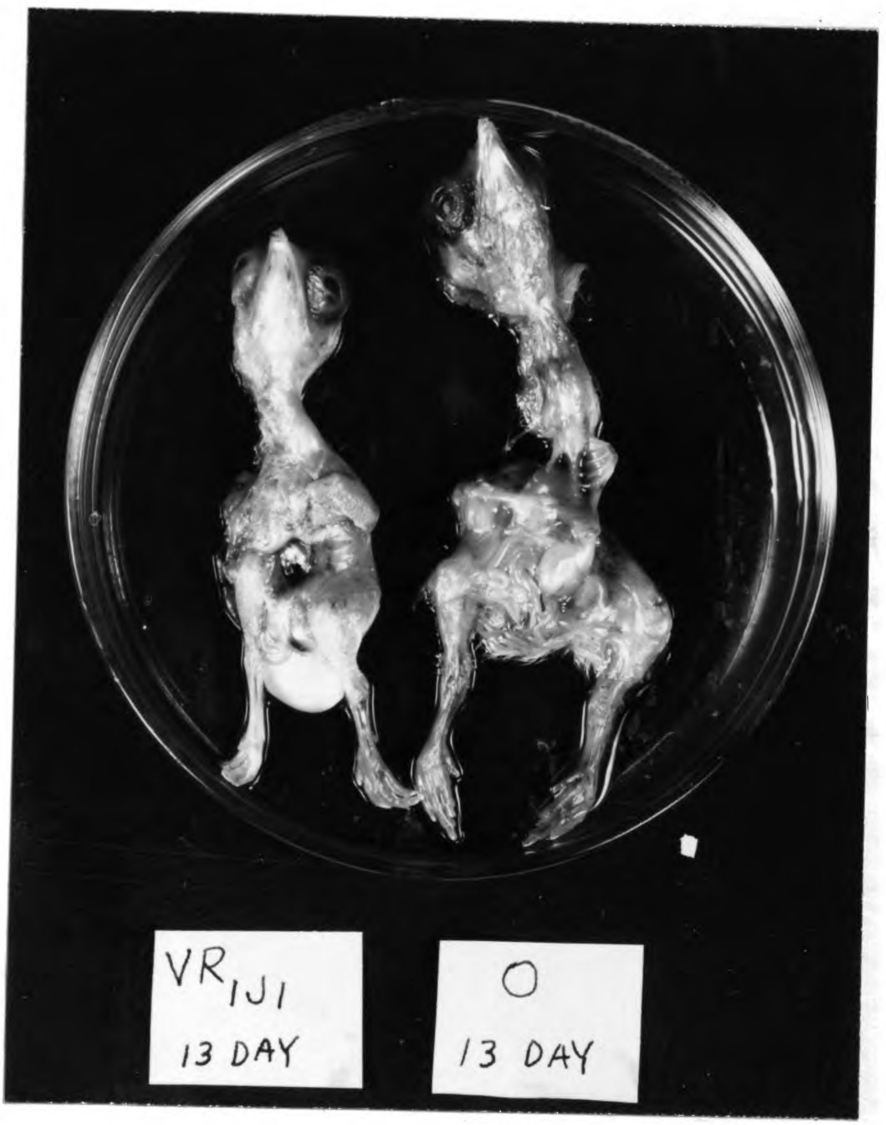

Fig. 12

Fig. 13. 14th day. The characteristic bending and shortening of the neck in relation to the body and head is seen, also the deformed feet. Notice the difference in feather development.

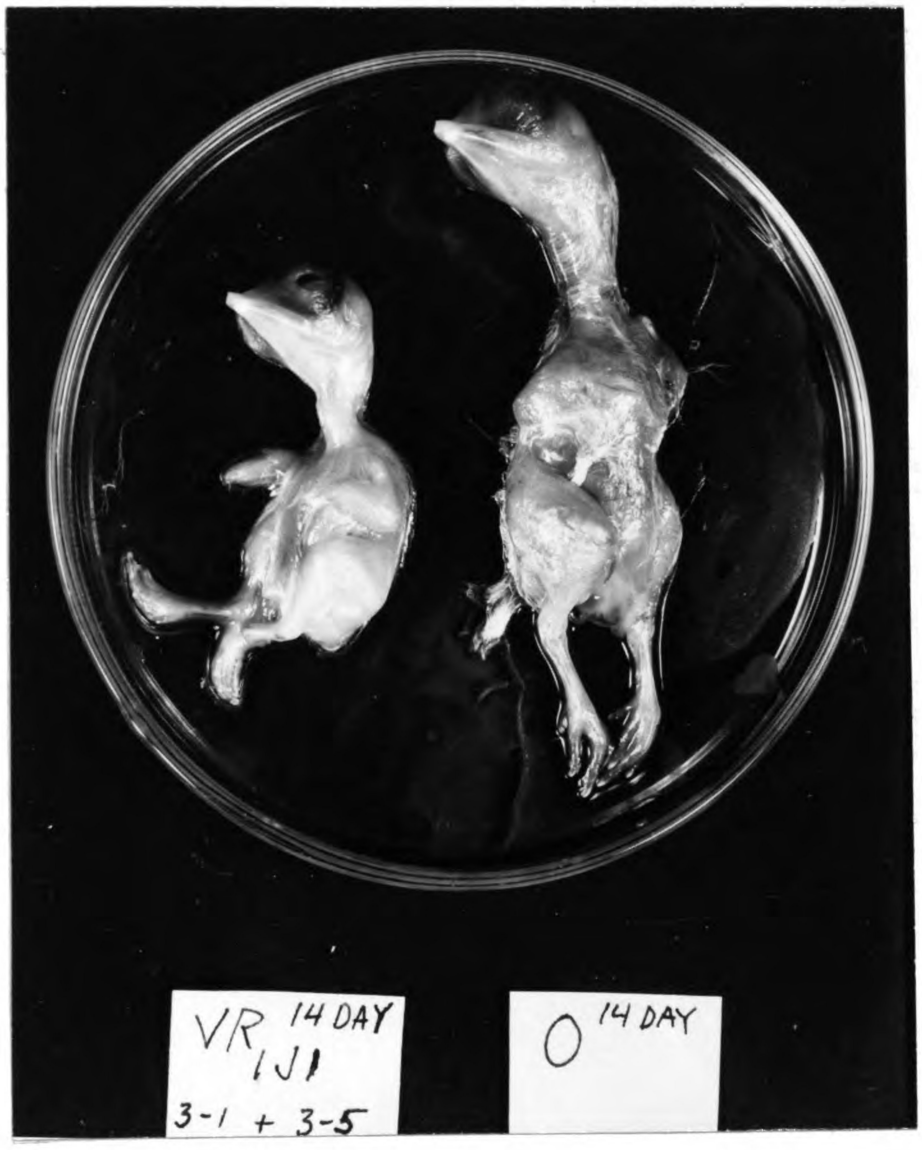

Fig. 13

Fig. 14. 15th day. A marked difference in size, feather development and foot posture is illustrated.

Fig. 14

Fig. 15. 16th day. Notice the alteration of body and head size to neck length in the infected embryo, also its size in comparison to the normal.

Fig. 15

Fig. 16. A comparison of embryo position and size upon removal from egg shell.
16th day.

Pathological Embryo
The curled position assumed by the infected embryo. The membranes are more opaque than the normal membranes. The yolk material is of a firmer consistency.

Fig. 17. Normal Embryo
16th day. Shows the transparency of the membranes and the fluid appearance of the yolk material.

Notice the freedom from constriction as compared to the pathological embryo, also the advanced feather development.

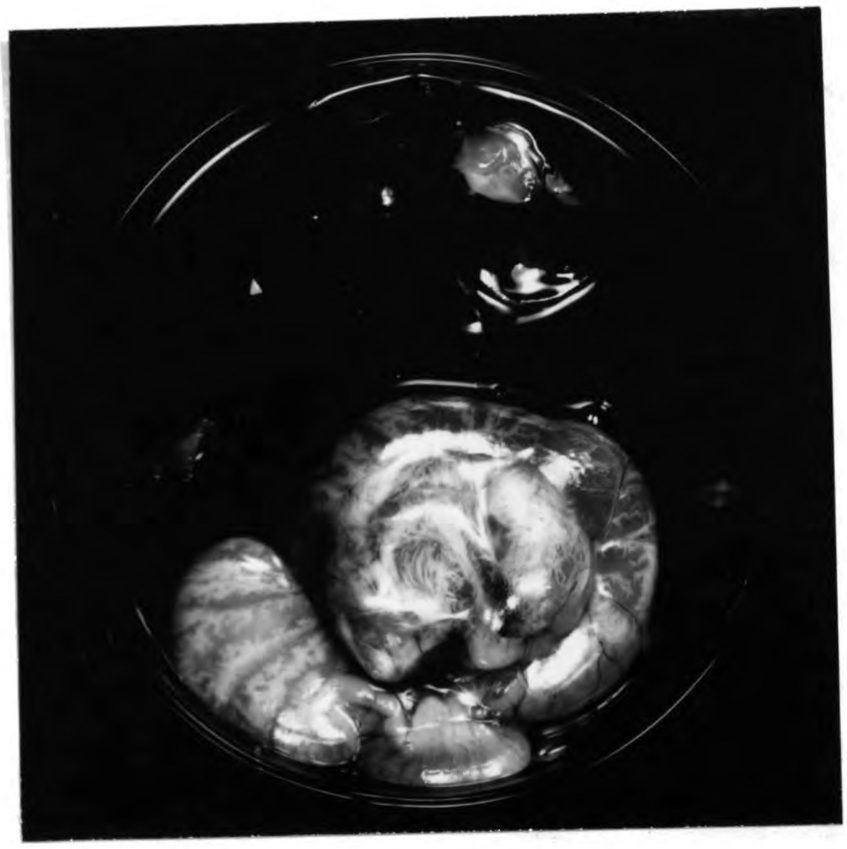

Fig. 16

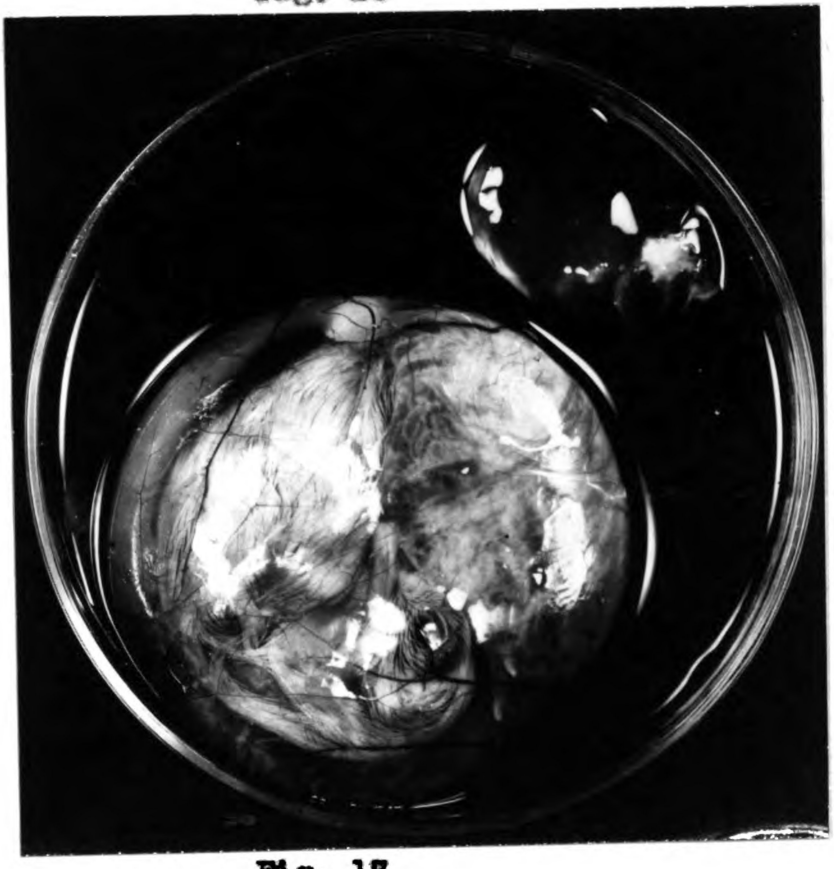

Fig. 17

Fig. 18. Comparison between embryos just broken out of shell. The top embryo is normal. The bottom embryo is a bronchitis infected embryo showing the thickened and opaque membranes.

Fig. 18

Fig. 19. Photograph in black and white the same embryo as Fig. 1.

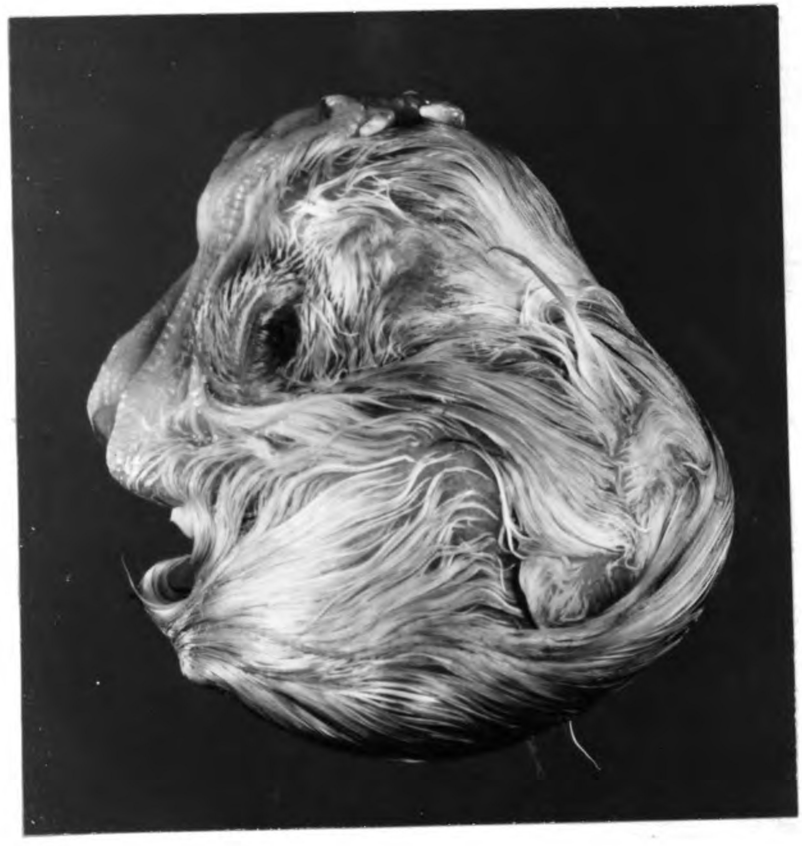

Fig. 19

Fig. 20. Photograph in black and white of same embryos as Fig. 2.

Fig. 21. Photograph in black and white of same embryos as Fig. 3.

Fig. 20

Fig. 21

Fig. 22. Photograph in black and white of same organs as Fig. 5.

Fig. 23. Photograph of normal and pathological kidneys of eighteen-day-old embryos. Normal kidney is on right. Notice the swollen appearance of the infected kidney, also the minute areas of necrosis. These kidneys had some areas of crystalline (urate) deposits.

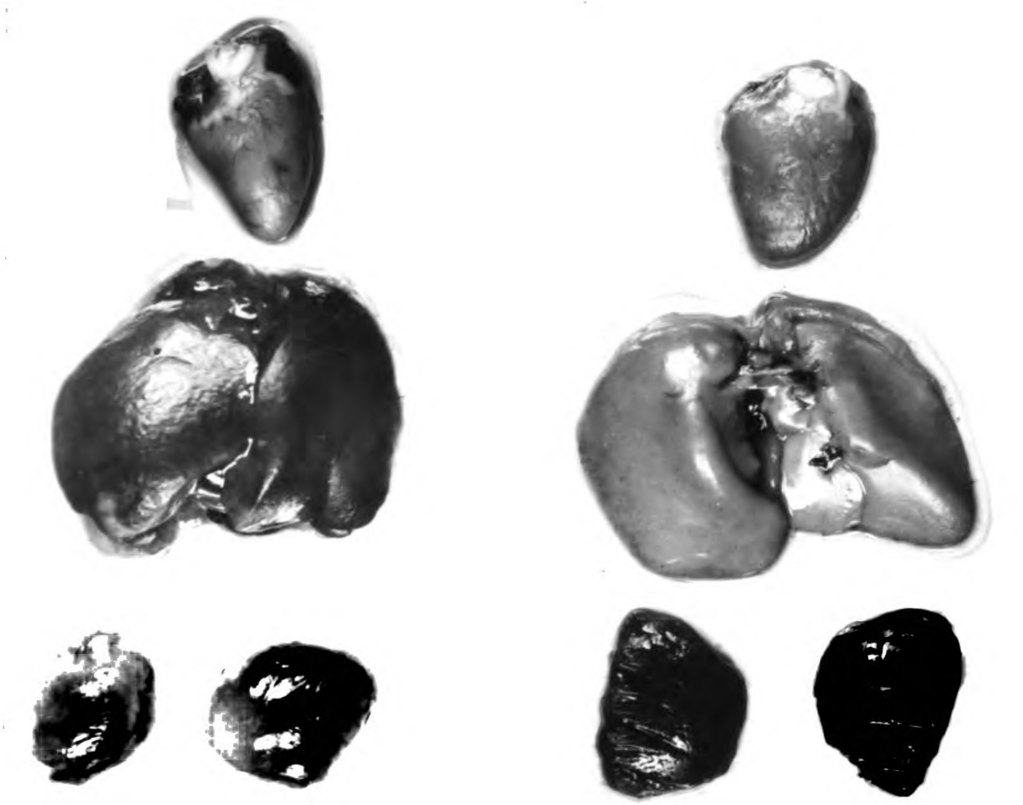

Fig. 22

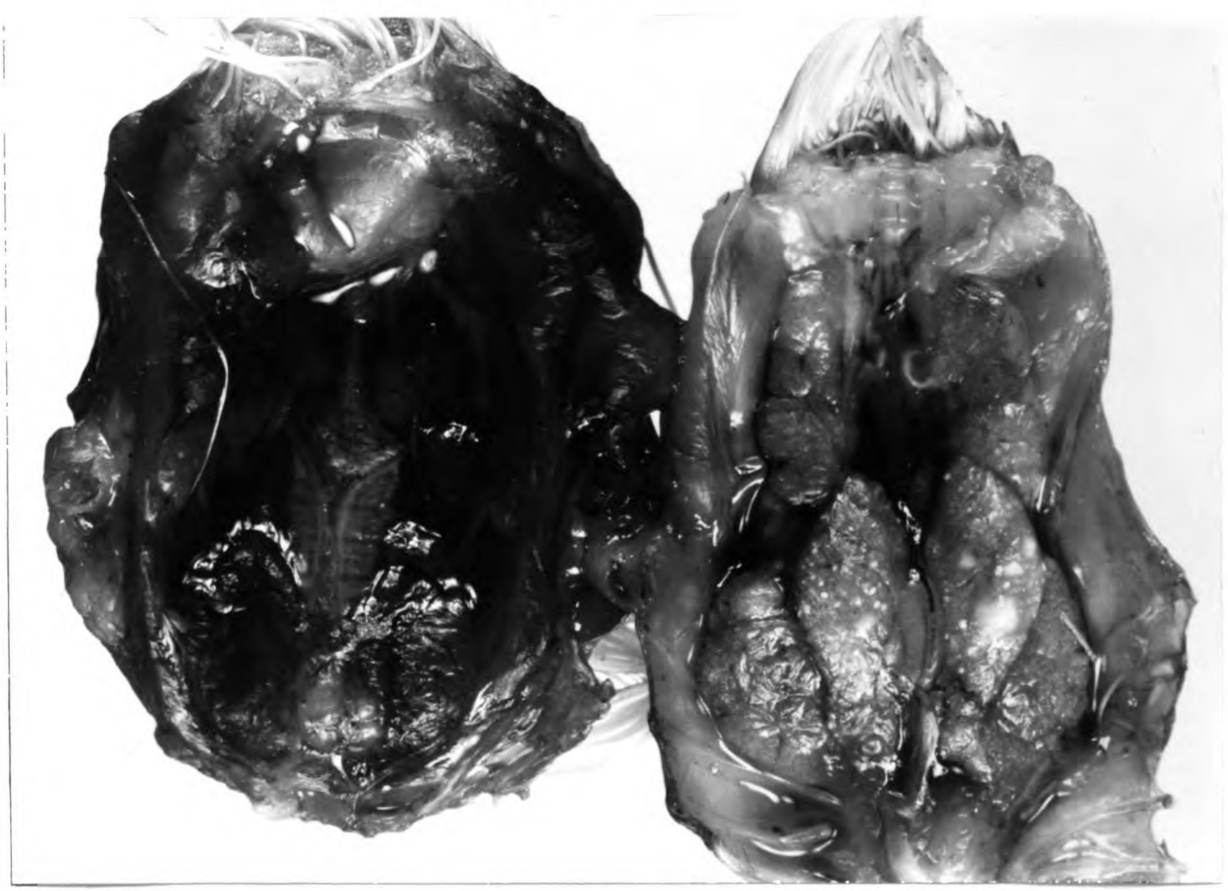

Fig. 23

May 9 '50