THE REPRODUCTIVE SEQUELAE FOLLOWING ONE-DAY-OLD

EXPOSURE TO INFECTIOUS BRONCHITIS VIRUS

by

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INTRODUCTION

Infectious bronchitis is an acute, highly contagious, pandemic, respiratory disease of chickens. The disease occurs in all age groups. It was first recognized in North Dakota in 1930 and in the subsequent 30 years has been reported from many divergent areas of the world.

Since 1940 it has been recognized that the greatest economic losses associated with this disease concerned a reduction in egg production and quality. Quality, both external and internal, is reduced, as is hatchability. The age of the bird at the time of infection has an important bearing on the clinical signs, lesions and mortality rates. Severe lesions of the reproductive organs of the domestic chicken have been reported following early exposure to infectious bronchitis virus.

The purpose of this experiment was to study the pathogenesis of this disease as it is related to the reproductive tract in chickens.

LITERATURE REVIEW

Infectious bronchitis was first reported by Schalk and Hawn (1931) who had observed the disease in North Dakota in the spring of 1930. During the following ten years several reports of its presence in the U.S.A. appeared in the literature (Beaudette and Hudson 1933; Bushnell and Brandly 1933; Beach 1934; Beach and Schalm 1936). The disease was reported in Britain by Asplin (1948), in Japan (Sato et al. 1955) and in the Netherlands (Bijlenga 1956).

Van Roekel et al. (1939) reported that the greatest loss suffered from the disease when present in mature birds was reduced egg production.

Gordeuk and Bressler (1950) also referred to poor egg production but in addition mentioned poor egg quality, both external and internal, following outbreaks of the disease. They inoculated laying hens intranasally and intratracheally with virulent infectious bronchitis virus. In a 10 week period post inoculation the rate of lay in the treated group was 31 per cent while it was 55 per cent in the control group. Only 52 per cent of eggs laid by the exposed group during the experiment were suitable for commercial handling, due to their poor quality. Specific gravity was measured and for the first month post inoculation there was little difference between the exposed and control groups. Following this, however, the exposed group had a lower specific gravity.

Hill and Lorenz (1956) presented results from field and experimental outbreaks of infectious bronchitis. They found in the majority of cases that egg damage did not appear until some time after cessation of clinical signs. Thereafter, reproductive abnormalities were prolonged or became permanent.

McDougall (1968) also referred to this delayed effect. He demonstrated that the loss of internal quality did not become apparent until well into the recovery period some two weeks after the end of clinical signs. McMartin (1968) showed the same delay. He found egg production did not decline until respiratory symptoms were waning and egg abnormalities did not become evident until four weeks or more after exposure to infectious bronchitis virus.

Van Roekel et al. (1950) reported that in Massachusetts the primary loss from infectious bronchitis occurred in mature flocks that were in egg production. A rapid decline in production may occur together with a marked alteration in the external and internal quality of the egg. Eggs from affected flocks were bleached, misshapen, rough and thin shelled, with watery albumen. Recovery to preinfection levels of egg production rarely occurred. Likewise the abnormal external quality often continued as long as the flock was maintained.

Broadfoot and Smith (1954) investigated field outbreaks in southern Minnesota and northern and central Iowa. Their

findings concurred with earlier work but also showed that
infectious bronchitis will cause a statistically significant
decrease in hatchability and an increase in percentage of
unsettable eggs.

Broadfoot and Smith (1954) reported on field cases of
infectious bronchitis in young chickens. A total of 14,000
pullets were involved during the first 5 to 7 days of age.
They suffered severe respiratory symptoms with a mortality
varying from 5 to 35 per cent within flocks. Later at seven
months when peak production was reached the birds were pro-
ducing at less than 50 per cent, when 70 to 80 per cent egg
production would be expected from this particular variety
of bird.

Many eggs exhibited poor internal quality. Flock owners
observed that a certain percentage of birds laying no eggs
went to the nests with the same regularity as birds producing
normally. The outward appearance and behavior of the non-
layer was no different than the layers except for failure to
lay. Necropsies performed on nonlayers, revealed yolks of a
cheesy consistency, with roughened and pitted surfaces in
the abdominal cavity and the abdominal wall was lined by a
heavy layer of mottled oily fat. The oviducts were poorly
developed and some were nonpatent. The membranes of the
abdominal cavity were thickened and opaque. The ovaries in
all nonlayers were active.

Broadfoot et al. (1956) carried out an experiment to verify what he had earlier reported from field trials. He divided 750 White Leghorn pullets into six groups and exposed them to virulent infectious bronchitis virus at 3 to 4 day intervals from 1 to 18 days of age. Typical respiratory clinical signs were seen. The chickens were reared under standard conditions and came into lay at 20 weeks. The flock peaked at 70 per cent egg production at seven months. This was lower than the expected peak for this type of chicken. Nonlayers were necropsied and 64 per cent were found to have nonpatent oviducts. The earlier the birds were exposed the greater the incidence of nonlayers. Furthermore, the incidence of nonpatency within each group of nonlayers decreased as the age at time of exposure to the virus increased. Measurements of the infundilulum, magnum, isthmus, uterus and vagina of nonpatent oviducts showed them to be considerably shorter than normal. There were varying amounts of yolk material in the abdominal cavity. Patent oviducts found in nonlayers were also shorter than normal and did not show the normal nonglandular, translucent ring which demarcates the border between the magnum and isthmus. In the nonpatent oviduct, the caudal portion of the isthmus and cephalic portion of the uterus were absent. In most of these the sac-like remnant of the magnum was glandular but the uterine wall was nonglandular, translucent, cystic and con-

tained a clear fluid with concretions suspended in it.

Cole and Hutt (1953) in a study on "Normal ovulation in nonlaying hens" described findings similar to those of Broadfoot et al. (1956). They were dealing with large populations of White Leghorns from which nonlayers were identified by trapnesting. However, since this work contained very little history on health, the etiology of the condition could not be associated with any particular disease. They found 8 percent of nonlayers to have incomplete oviducts. In most of these the infundibular portion of the oviduct persisted while a section from the magnum was degenerated. In some cases this also involved the vagina. The normal mesosalpinx usually showed faint outlines of the missing parts of the oviduct. Some birds lacked only a short segment of the oviduct, but in others, several portions were underdeveloped. Since those parts of the oviduct which were developed were now closed at both ends, they accumulated fluid and became cystic.

Finne and Vike (1951) reported a hereditary atresia of the oviduct in White Leghorns. The ovary was normally developed and maturation and ovulation of ova occurred. The yolks passed into the abdominal cavity where they caused peritonitis, resulting in death at 5 to 6 months of age. The isthmus was involved in the atresia. In one male bird, presumed to bear factors for atresia isthmii which were transmitted to its progeny, atresia of the seminal duct

caudal to one of the testes was observed.

Hutt et al. (1956), in an investigation of nonlaying hens, found 28 per cent of nonlayers to have incomplete oviducts. The most common area involved was the magnum, either completely or partially. On occasions there were several underdeveloped areas in the oviduct. Generally, portions of the oviduct became cystic due to the constrictions. They suggested the discontinuous oviducts probably resulted from accidental degeneration of part of the müllerian duct during the development of the embryo. Evidence of a genetic basis was not conclusive in their study.

Goldhaft (1956) found massive cysts in the abdominal cavities of seven month old pullets. He found the majority of them involving the dorsal ligament of the oviduct but some were in the oviduct. The anterior two-thirds of the oviduct appeared normal. Then the oviduct terminated in a blind sac. The terminal portion of the oviduct was present but there was a clear and distinct separation between the two segments. Cultures of the cystic fluid were negative. He suggested the lesions were associated with some hereditary or congenital fault.

The age of exposure to infectious bronchitis virus has a bearing on subsequent egg production. Urban and Goodwin (1953) compared three groups of chickens that were exposed to the virus at different ages. The first group contained

chickens 19 to 22 weeks of age at 20 per cent production. The second group was 18 weeks old and only a few eggs had been laid. The third group was 11 to 12 weeks of age and did not lay any eggs for one month following infection. Egg production dropped sharply in the oldest group at a time when it would normally be increasing rapidly. It gradually recovered but never to a satisfactory level. The second group was only slightly interrupted in egg production but future production was noticeably reduced. Egg production in the third group did not appear to be greatly affected.

Box (1964) reported a drop of 35 per cent in overall egg production following exposure to infectious bronchitis virus at onset of laying. He found the extent of production loss within a flock seemed to be related to the time interval between infection and point of lay. Birds having been infected during the first 6 weeks of life showed suboptimal production and had a higher proportion of inferior quality eggs than expected. The greater the interval between infection and point of lay the less significant the drop in production.

Sevoian and Levine (1957) showed that infectious bronchitis in the laying bird produced many changes in the oviduct and subsequent egg production. Egg production was decreased and never regained expected levels. Egg quality was reduced and remained so until the end of the experiment.

During the acute phase of the disease, the oviduct decreased in size to 20 per cent of normal in some birds and 50 per cent in length. Decreases in weight and length were seen until the 21st day after inoculation. Soft, white, granular material was found attached to the magnum. This material was also seen in eggs laid by the hens. No difference in appearance of the epithelial surface was observed in fresh oviducts longitudinally cut. Upon fixation in formalin however, about one-sixth of the group showed a line of demarcation between the isthmus and uterus more clearly than could be seen in the oviducts of control birds. In these birds epithelial surface color of the uterus appeared darker than that of the isthmus. The longitudinal folds, especially of the isthmus, appeared more plump and closer together than those normally found at the junction of the isthmus and uterus.

Microscopically the cells of the epithelial lining of the oviduct decreased in height and acquired a cuboidal shape during the most active phase of the disease. This was especially true of the goblet cells. The decrease in height of the epithelial cells was not uniform in the various parts of the same oviduct. The cilia, which normally cover the surface of the epithelium, decreased in number and in many places were entirely absent. The decreases in epithelial height occurred mainly between the 7th and 21st days after infection. Re-

covery was slow and no bird had normal height of epithelium at all levels of the oviduct prior to 21 days post exposure. Eighty per cent had epithelial cells of normal height after seven weeks. By this time also the cilia had been restored to their normal appearance although they were sparse on occasional surface areas. Sixty per cent of the tubular glands from the infected group exhibited glandular dilation. The epithelium and contents of the glands appeared normal; however, the size, incidence and distribution of these glands were greater. These were located from the middle portion of the infundibulum to the posterior region of the uterus. The incidence was greatest in the magnum.

Lymphocytic foci and cellular infiltration in the lamina-propria and the intertubular stroma of the oviducts were more extensive in treated chickens compared to controls. The nodules were of considerable size but the glands around them did not appear to be significantly compressed. The diffuse area of cellular infiltration was made up primarily of plasma cells, mononuclear cells and lymphocytes. Varying degrees of fibroplasia and edema were observed in the lamina propria and the intertubular connective tissue of the entire oviduct in about two-thirds of the infected birds. Where fibroplasia was extensive, glandular elements were few in number or entirely absent. The portion of the oviduct that had been most affected by this change was the uterus. The muscular

layer of the oviduct showed little significant change except there was a heavier infiltration of the inflammatory cells in the infected birds as compared to the controls.

The oviduct is also liable to damage from another common virus, namely, Newcastle disease virus. Newcastle disease causes a sudden and drastic drop in egg production (Beach 1942). The greatest loss among laying birds suffering from Newcastle disease frequently results from reduced production and impaired egg shell and albumen quality. (Lorenz and Newlon 1944; Berg et al. 1947; Knox 1950; Parnell 1950; Quinn et al. 1956). Platt (1948) stated that egg production returns to normal within 4 to 5 weeks after infection.

Biswall and Morrill (1954) investigated the pathology of the reproductive tract of laying pullets affected with Newcastle disease. He confirmed previous work with regard to egg production and quality. Macroscopically during the acute stage of the disease some of the oviducts were found shrunken. When opened, the oviduct lacked the glistening appearance of normal oviduct mucosa. A mild to moderate edema in the oviduct and ovarian ligaments was observed. The histopathological changes observed in the oviducts were variable, not only in the birds of the same period post inoculation but also in birds of the same group and from segment to segment of the same oviduct. In the infundibulum there was a mild degree of hyperplasia in the lymphocytic aggregates. The

magnum showed cellular infiltration, mainly heterophilic granulocytes, edema and focal necrosis during the early stages of infection. In the later stages a mild to moderate hyperplasia of the lymphocytic aggregates was seen.

The isthmus had a heavy infiltration of heterophilic granulocytes. The glands showed cystic dilatation and capillaries were somewhat hyperemic. Mild hyperplasia of the lymphocytic aggregates was also noted. The heterophilic granulocytes appeared in the cells of the epithelial lining.

In the uterus the lining epithelial cells were markedly infiltrated with heterophilic granulocytes. The usual closely packed tubular glands were widely separated by edema. The mucosa was characterized by pericapillary hemorrhage. In the later stages there was lymphocytic hyperplasia and a mild intertubular connective tissue proliferation. The reaction in the vagina was milder than in the other segments and con-sisted mainly of a diffuse infiltration with inflammatory cells.

Domermuth and Gross (1962) demonstrated that Mycoplasma gallisepticum was capable of producing salpingitis in chickens. They inoculated day-old chickens via the umbilical orifice with the organism and found 64 per cent of the them developed a salpingitis grossly characterized by plugs of caseous matter in the oviduct. M. gallisepticum could be consistently isolated from these oviducts through the 25th

week post inoculation. Histopathological examination re-
vealed that the caseous plugs were composed of necrotic
heterophiles and fibrin and that the walls of the oviduct
appeared normal. They also investigated the route of
infection to the oviduct. They concluded that the oviducts
were infected by mechanical transfer of mycoplasmas from
the yolk or air sac to the oviduct and that it was unlikely
that mycoplasmas were carried via the blood stream.

Domermuth et al. (1967) repeated their previous work
and on this occasion isolated pathogenic Mycoplasma
gallisepticum from eggs, from grossly normal oviducts and
from caseous material from the oviduct of mature chickens
which had been inoculated with the organism via the umbilicus
at one day of age. Histopathologically infected oviducts
exhibited diffuse lymphoid areas and small lymphoid follicles
throughout the oviduct wall.

Gross (1958) recognized that Escherichia coli may produce
a salpingitis in the chicken. He inoculated the organism
into the air sacs and subsequently on necropsy found
salpingitis and isolated the organism from the oviduct. He
proposed that the bacterial infection was produced by direct
spread of the bacteria from the air sacs through the short
mesosalphinx into the oviduct.

Berg et al. (1951) found that after recovery from
coccidiosis laying birds produced eggs whose albumen quality

was better than it was prior to the onset of the disease. These same chickens, however, produced eggs with an excessive amount of thin albumen during the time interval between the start of clinical signs of coccidiosis and temporary cessation of lay. They considered it probable that the improvement in the amount of thick albumen that was observed after the birds returned to production was the result of the "rest" that they received while out of production. This indicated however, that there was no permanent damage to the oviduct.

Sherwood (1958) in a review discussed the factors affecting egg quality. They were divided into four main classes: nutritional, chemotherapeutic, toxic and infectious diseases. Each of these was discussed in detail. The disease conditions affecting egg quality have already been mentioned in the above literature review.

Surface (1912) described the histology of the oviduct of the domestic hen. The oviduct consisted of five segments in the adult bird, namely, infundubulum, magnum, isthmus, uterus and vagina. Two muscular layers, an outer longitudinal and an inner circular can be distinguished in all parts of the oviduct. The inner surface of the oviduct was thrown into a number of primary longitudinal ridges. The epithelium over these ridges formed secondary folds. Three types of glands were described: (1) Unicellular epithelial glands occurred between the ciliated cells in all parts of the ovi-

duct except the anterior portion of the infundilulum. (2)
Glandular groove cells were situated at the bottom of the
grooves between the secondary folds of the epithelium.
These were only found in the infundibular portion. (3) In
all parts of the oviduct between the infundilulum and vagina,
there was a thick layer of glands beneath the epithelium
which was called tubular glands. These consisted of long
convoluted and branched tubules which opened to the lumen of
the oviduct by short epithelial ducts. The tubular gland
cells consisted of large epithelial cells which in the magnum
and isthmus had small, irregularly shaped, dark staining
nuclei which were located toward the basal ends of the cells.
Coarse granules of varying sizes were present in the cytoplasm.
The line of demarcation between the magnum and isthmus was
characterized by the absence of these tubular glands. The
cells of the tubular glands were similar histologically in
the magnum and isthmus. The tubular gland cells of the
uterus had nuclei which were large, with regular outlines
and which were situated near the center of the cells. The
cytoplasm was very finely granular. The vagina contained
no tubular glands and only unicellular epithelial glands
were present.

Biswal (1954) demonstrated additional histological
findings in the reproductive tract of the chicken. He found
lymphocytic aggregates in the oviducts. They were found in

all five segments of the oviduct and in each of the healthy

birds sampled. They varied from clusters of only a few

cells to much larger aggregates. There did not appear to be

any definite relationship between the number and size of the

lymphocytic aggregates and the segments of the oviduct in

which they were found. The nodules did not appear to have a

supporting stroma. Scattered lymphocytes and plasma cells

were present in the lamina propria, muscularis and subserosa.

Ganglion cells were found occurring singly or in groups of

two or three in the vaginal wall. There are additional

references to oviduct anatomy in the literature (Kaupp

1918; Sturkie 1965; Bradley and Grahame 1960).

Ball et al. (1969) compared the morphological response

of the turkey oviduct to different pathogenic agents. They

demonstrated that as the level of exposure and pathogenicity

increased there was an increase in the numbers of lymphoid

foci and plasma cells in the oviduct. These changes were

quantitative rather than qualitative.

The literature contains many references to avian

lymphoid tissue (Lucas 1949; Biswal 1954; Sevoian and Levine

1957; Biggs 1957; Denington and Lucas 1960; Ball et al.

1969). In many instances the same structures have been

called different names such as lymphocytic foci or aggregates

and lymphoid follicles, foci or nodules.

Fine structure studies of oviduct have been carried out

by several authors. Aitken and Johnston (1963) reported on the ultrastructure of the infundibulum. Hendler et al. (1957) carried out a cytological study of the magnum. Johnston et al. (1963) described the fine structure of the uterus. Van Krey et al. (1966) investigated the structure of the vagina with special emphasis on the sperm glands.

Amiya Bhuson Kar (1947) described an occluding plate in the oviduct and its development. This plate occludes the junction between the oviduct and the cloacal lumen. It is perforated and finally destroyed during the process of laying the first egg.

METHOD OF PROCEDURE

Experimental Chickens

The experiment was carried out at the Veterinary Medical Research Institute, Iowa State University. Chickens were hatched at the Institute from eggs obtained from a mixture of three flocks, two inbred and one crossbred, of White Leghorn chickens. These flocks have been free of infectious bronchitis for many years and have been tested at regular intervals for evidence of infectious bronchitis antibodies. On all occasions these tests have been negative. Infectious bronchitis vaccines have never been administered to them and they were maintained under isolation conditions. The flocks have also been free of Newcastle disease and no vaccines have been used against this disease. Over the last 11 years no reactions to Salmonella pullorum, Salmonella typhimurium, Mycoplasma gallicepticum or Mycoplasma synoviae have been found on testing.

After hatching, the chickens were sexed by visual examination of the cloaca. The females were divided into two groups and brought to their respective isolation areas. There were 220 chickens in the experimental group and 83 in the control group. They were housed in batteries with approximately 40 in each battery until five weeks of age. Both groups of chickens were managed in the same way but they were attended by different caretakers.

Experimental Inoculation

The experimental group was exposed to infectious bronchitis virus at 24 hours of age using the Massachusetts type, strain 33, in its 7th embryo passage. Prior to inoculation the virus was passed twice through growing chickens. This virus was originally isolated at the New York State Veterinary College by Dr. M. S. Hofstad. It was administered to the chickens as an aerosol by a hand-pumped Peralta vaporizer.[1] The chickens were collected in the enclosed portion of the batteries and the viral aerosol was pumped in over their heads through small openings in the sides of the batteries. The chickens were maintained in this area for ten minutes. Each received approximately 10,000 chick-infective doses of infectious bronchitis virus.

Experimental Procedure

During the acute clinical stage of the disease no treatment was administered to the chickens. At 5 weeks of age the chickens were put in conventional housing on concrete floors covered with wood shavings. They remained under these conditions until 18 weeks of age. They were then put into individual battery cages.

At 11 weeks of age evidence of coccidiosis within the exposed group became apparent. The birds were immediately

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[1]Distributed by Peralta Hospital, Oakland, California.

moved into an adjoining area on fresh litter. They were moved alternately between the two areas twice weekly for three weeks. Each area was cleaned and covered with fresh shavings before the chickens were returned. No chemo-therapeutic agents were administered and fecal examinations were made during the weekly sacrifices and subsequent necropsies. The control group showed clinical signs of coccidiosis two weeks after it was noted in the exposed group. The same procedure was used to eliminate the disease as with the exposed group. A small number of the control group showed clinical signs of fowl pox at 17 weeks of age. The remainder of the group was immediately immunized by a homologous vaccine produced at the Veterinary Medical Research Institute from the same outbreak. The exposed group was immunized at 18 weeks of age using the wing web method. The control group was transferred to individual cages in the same house as the exposed group at 21 weeks of age. At the conclusion of the experiment when the birds were one year old all remaining birds were sacrificed and necropsied.

Tissue Collection

All birds which died were necropsied. A weekly sacrifice schedule was followed until 20 weeks of age. Birds were sacrificed by dislocation of the atlanto-occipital joint. During the first week 14 birds were sacrificed, 10

birds during the second, and 9 during the third week. The number was gradually lowered to two birds during the 10th week. It remained at this level until the 17th week when the number was increased to 5 birds per week. All sacrificed birds were necropsied and their reproductive organs placed in Carnoy's fluid (Lillie 1965, p. 42). The oviduct was very small in the young chickens. It lay on the surface of the left kidney and for ease in handling the entire left kidney was dissected out with the oviduct remaining on its surface. In this way the oviduct was removed from the carcass with the minimum of handling. This was continued until the 10th week of age, by which time the oviduct could be dissected by itself with the minimum of damage to it.

Control chickens were sacrificed at the same time as those exposed but the numbers were reduced by approximately one-half. Necropsies were performed and the reproductive organs were placed in Carnoy's fluid.

Electron Microscopy Procedures

Tissues were collected for electron microscopic studies during the acute stage of the disease. At 40 hours post exposure, two exposed and three control birds were sacrificed, while three exposed and three control birds were sacrificed at 108 hours post exposure. The oviducts were removed immediately after sacrifice and immersed in 2.5 per cent glutaraldehyde, then minced into 1 mm segments. These were

fixed in the glutaraldehyde for 45 minutes and then stored in phosphate buffer at pH 7.4. Post fixation in 1 per cent osmium tetroxide was carried out for 45 minutes. The material was dehydrated in the following grades of alcohol for ten minutes each: 50, 70, 85, and 95 per cent, and two final ten minute dehydrations, each in absolute alcohol. The tissue was then embedded in Epon 812. Sections of between 500 and 700 Angstroms were cut with a glass knife on an LKB ultratome[1] and stained with uranyl acetate or lead citrate. They were examined on a Hitachi HS6[2] or a Hitachi HS8[3] electron microscope.

Histological Procedure

Tissues were collected and immersed in Carnoy's fluid within five minutes after sacrifice. The tissue remained 18 hours in the fixative and was then washed in alcohol and stored in 70 per cent ethyl alcohol. Tissue was embedded in Paraplast[4] tissue embedding medium, sectioned at 6 u and mounted on glass slides with Permount[5] mounting medium. Sections were stained with Harris's hematoxylin and counter-

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[1]L.K.B. Produkter A.B. Stockholm, Sweden.

[2]Hitachi, Ltd., Tokyo, Japan.

[3]Available through the courtesy of Dr. D. Croghan, U.S.D.A., Ames, Iowa.

[4]Scientific Products, Chicago, Illinois.

[5]Fisher Scientific Company, Fairlawn, New Jersey.

stained with eosin Y as described in the U.S. Armed Forces Institute of Pathology Manual of Histological and Special Staining Technics (1969). The entire oviduct from the young chickens was included in the embedding medium. From eight weeks onwards samples of each segment were taken and the remainder stored.

Viral Titration Procedures

At 65 hours post exposure tissues were collected for viral titration. There were seven tissues sampled, namely, oviduct, ovary, lung, trachea, air sac, kidney and bursa of Fabricius. Each sample represented pooled tissue from four chickens. The tissues were ground in a mortar with pestle and alundum and suspended in tryptose phosphate broth. They were frozen at -30° C until chicken embryos were available for inoculation. All eggs used were produced by the Veterinary Medical Research Institute supply flock. Ten day embryonated chicken eggs were used for inoculation The respiratory tissues were diluted to 10^{-6} while the remainder were diluted to 10^{-4} in typtose phosphate broth[1] and 0.1 ml of dilution was inoculated into the allantoic sac. Six eggs were inoculated per dilution. Titration end points were based primarily on mortality and typical stunting of the embryos. At 42 days post exposure samples were

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[1]Difco Laboratories, Detroit, Michigan.

harvested from sacrificed birds and the same procedure was performed as previously for the isolation of virus.

Serological Examinations

Blood samples were collected from chickens at 50 and 65 days post exposure from both groups for serological tests. Plate agglutination tests were performed for Mycoplasma gallicepticum and Mycoplasma synoviae, using stained inactivated antigen.[1]

A hemagglutination inhibition test was run on samples collected 65 days post exposure for evidence of antibodies against Newcastle disease virus. The Beta procedure (National Academy of Sciences 1963) was performed. A serum neutralization test to show the presence of antibodies against infectious bronchitis virus was run on the same sera. Another serum neutralization test was performed at the conclusion of the experiment for evidence of infectious bronchitis viral antibodies.

To determine the susceptibility of the chickens to infectious laryngotracheitis, five chickens were placed in separate isolation at 18 weeks of age and exposed to infectious laryngotracheitis by swabbing the trachea with infective material.

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[1] Connaught Medical Research Laboratories, Toronto, Canada.

Egg Quality Evaluation

Egg production records were kept for each bird. At six months of age an experiment was performed to evaluate egg quality. Twenty previously exposed and 20 control chickens were selected at random from the two groups and the following data were collected: Haugh units, specific gravity, shell thickness, visual observations of the shell and internal quality. The experiment was conducted over a seven week period during which time three consecutively laid eggs were examined from each bird per week. The Haugh units were calculated as recommended by Haugh (1937), which consisted of measuring the albumen heights and relating them to the eggs weights. The specific gravity was observed by the egg flotation method in salt solutions (Miller and Marion 1969) and the shell thickness was measured by a micrometer.

RESULTS

The chickens developed clinical signs of the disease as early as 36 hours post inoculation and within 72 hours all birds had signs of varying degrees of severity. The characteristic features of the disease were seen. These consisted of gasping, rales, coughing and nasal discharge. As the disease progressed a large proportion of the birds became weak and depressed and tended to congregate in the vicinity of the heat source. Lacrimation was observed in some birds. The first mortality occurred three days post inoculation. The rate quickly rose and then leveled off for the following ten days. It then gradually dropped until the 20th day post inoculation. There was a 25 per cent mortality due to infectious bronchitis during this period.

Lesions Between Inoculation and 20 Days of Age

Macroscopic

Chickens which died showed a serous exudate in the trachea, nasal passages and sinuses during the first five days post inoculation. Following this a catarrhal exudate was more commonly noted in the respiratory tract. The majority of birds which died beyond eight days following inoculation had a yellow caseous plug in either the lower trachea or bronchi. Small areas of pneumonia were observed. The kidneys of many birds appeared congested and the ureters

were filled with urates in 20 per cent of the birds which died. The air sacs were characterized by a moderate degree of fibrinous inflammation which gave them a cloudy appearance. In the sacrificed exposed birds many of the same findings were observed but the lesions were less severe than in the birds which had died of the disease. The oviduct of young birds was very small. In all birds of this age group it appeared normal macroscopically.

Microscopic

The trachea was thickened due to edema and inflammatory cells in the submucosa. The tracheal and bronchial lumens contained necrotic exudate and on occasion this was found to completely block the lumen. The kidneys appeared congested with erythrocytes in 30 per cent of the birds which died. Many of the ureters were completely occluded by urates.

Lymphoid cell infiltration was seen on the 2nd day post inoculation in the oviducts of treated chickens. They were scattered in the submucosa but on occasion they tended to occur in greater concentrations near the blood vessels. The submucosa also exhibited a moderate degree of edema. On the 11th day post inoculation a lymphoid nodule was observed in the submucosa. As time proceeded these nodules were seen in increasing numbers in the exposed group. In the control group they were not observed until seven weeks of age and then only in small numbers. They appeared to be encapsulated

and some seemed to have lost a proportion of their cells as
empty spaces occurred within the nodule. This could possibly
happen during the tissue processing. During this stage it
was impossible to identify characteristic segments of the
oviduct by histological means.

Lesions Between 20 and 40 Days Post Inoculation

Macroscopic

As the birds became older the respiratory tract lesions
became less severe. Small caseous plugs were found in the
bronchi, which were partially occluded in 30 per cent of the
exposed birds. There was little evidence of any pneumonic
changes and the kidneys appeared normal. The oviducts were
larger than in the previous age range but appeared normal
macroscopically.

Microscopic

The tracheal and bronchial reactions were changed to a
chronic nature. There were large lymphoid and plasma cell
infiltrations in the submucosa of the trachea and in the
lungs beneath the primary and secondary bronchi. Lymphoid
nodules of the same type as were found in the oviduct were
observed in the same regions as the cellular infiltrations.
There were numerous lymphoid nodules in the kidney.
On the 22nd day post inoculation a hypoplastic area was

observed in the oviduct. This was seen on microscopic examination only. Serial sections of the oviduct in the region showed the oviduct lumen gradually becoming smaller. Eventually, the oviduct epithelium disappeared and this was followed by complete absence of the lumen. Two mm caudally, the lumen reappeared as a small space. This gradually increased to a normal size with the attainment of normal epithelium.

A lymphoid nodule occurred in the hypoplastic region. During this period lymphoid and plasma cells together with lymphoid nodules were commonly seen in the submucosa of all segments of the oviduct.

Lesions Between 40 and 60 Days Post Inoculation

Macroscopic

All tissues except the oviduct appeared normal during this period. On the 44th day post inoculation a small cyst was found in the oviduct. The oviduct appeared normal in its cranial portion and ran caudally on the surface of the left kidney. Approximately in its mid-portion the oviduct came to an abrupt termination. Three mm caudally, the cyst commenced and continued caudally to the cloacal region. The cyst was within the oviduct lumen and had a constant width of 5 mm throughout most of its length. The anterior portion of the cyst wall was transparent while it became translucent as it progressed towards the cloaca. It was filled with a

clear serous fluid. The area between the apparently normal
oviduct and the cyst was connected by portions of the dorsal
and ventral ligaments of the oviduct. They contained a
faint remnant outline of the oviduct which joined the two
sections. On subsequent necropsies many birds were found
to have cysts of a similar nature. They were all accompanied
by hypoplastic areas anterior to them.

In some cases the cysts were divided by further
hypoplastic regions into two segments. The length of the
hypoplastic area varied between 2 and 5 mm during this
period. Twenty-two per cent of treated birds showed
hyperplasia of the oviduct.

Microscopic

Apart from the oviduct, the only evidence of past infec-
tion during this period was the large number of lymphoid
nodules which occurred in the submucosa of the respiratory
system and smaller numbers which occurred in the kidneys.
The oviduct of exposed chickens still contained numerous
lymphoid nodules as well as free lymphoid and plasma cells.
In the cystic oviducts, the anterior intact portion of the
oviduct appeared similar to the oviducts in other treated
birds. The cyst walls were thin but had an intact internal
layer of surface epithelium. Cilia were still present on
many epithelial cells. The walls contained lymphoid and
plasma cells plus a small number of lymphoid nodules.

On average the wall was 8 to 10 cells thick. The
hypoplastic areas were varied in nature. In the majority
of cases some remnant of the oviduct could be recognized
in the ligaments. There was often an increase in the
connective tissue elements of the ligaments associated with
the hypoplastic regions. In 20 per cent of cases no evidence
of oviduct tissue could be seen.

Lesions Between 60 Days and Start of Lay

Macroscopic

During this period approximately the same percentage of
chickens were recognized to have cystic oviducts. The cysts
became larger with advancing age as did also the hypoplastic
region. This region varied from bird to bird but in 80 per
cent of cases it involved the middle one-third of the oviduct.
The remainder involved portions of the caudal one-third, with
a few exceptions where over 60 per cent of the oviduct was
hypoplastic. In 15 per cent of cases two hypoplastic
regions were observed with two cysts resulting, one caudal
to each area.

Between 12 and 15 weeks of age, the time when glandular
development normally starts, partial glandular development
took place in the cyst walls. This was most pronounced in
the posterior portion of the cysts. After 14 weeks of age
the posterior portions of the oviduct showed a decrease in
cystic size. At 19 weeks of age there was seldom cystic

involvement of the vagina and in 75 per cent of the cystic
oviducts the uterus showed a moderate degree of glandular
development. The cysts contained a clear fluid but large
quantities of white flaky material were suspended in it.

Microscopic

During the early stages of this period the cyst wall
appearances were as described in the previous age group. At
12 weeks of age glandular development started to take place
and contained until 18 weeks of age but at a slower than
normal rate. In the hypoplastic regions little evidence of
oviduct tissue could be recognized. There was an increase
in the connective tissue between the cyst and anterior
oviduct in the dorsal and ventral ligaments. There was
normal glandular development of the oviduct in areas
anterior to the hypoplastic region.

Lesions in Adult Chickens

Macroscopic

During this period egg records were kept on each hen.
Twenty-six per cent of the previously exposed chickens never
laid and 20 per cent laid consistently poor quality eggs.
All the control chickens consistently laid normal quality
eggs. The exposed chickens were thus classified into three
groups: (1) nonlayers, (2) poor quality layers, (3) normal
layers.

Figure 1. Reproductive organs of a chicken 52 days old,
           previously exposed to infectious bronchitis
           virus at one day of age. A cyst with a
           hypoplastic region (arrow) anterior to it is
           shown. The dorsal ligament of the oviduct is
           held up by needles to show the cranial portion
           of the oviduct to advantage

Figure 2. Two oviducts of sexually mature previously
           exposed chickens, with varying lengths of
           hypoplastic areas. The lower oviduct has an
           ovary cranial to it. The upper oviduct has a
           short area of hypoplasia in its magnum while
           caudal to this the oviduct shows mild cystic
           distension. The lower oviduct shows the most
           extreme form of hypoplasia in which two-thirds
           of the oviduct is involved. The ovary of this
           bird is quiescent
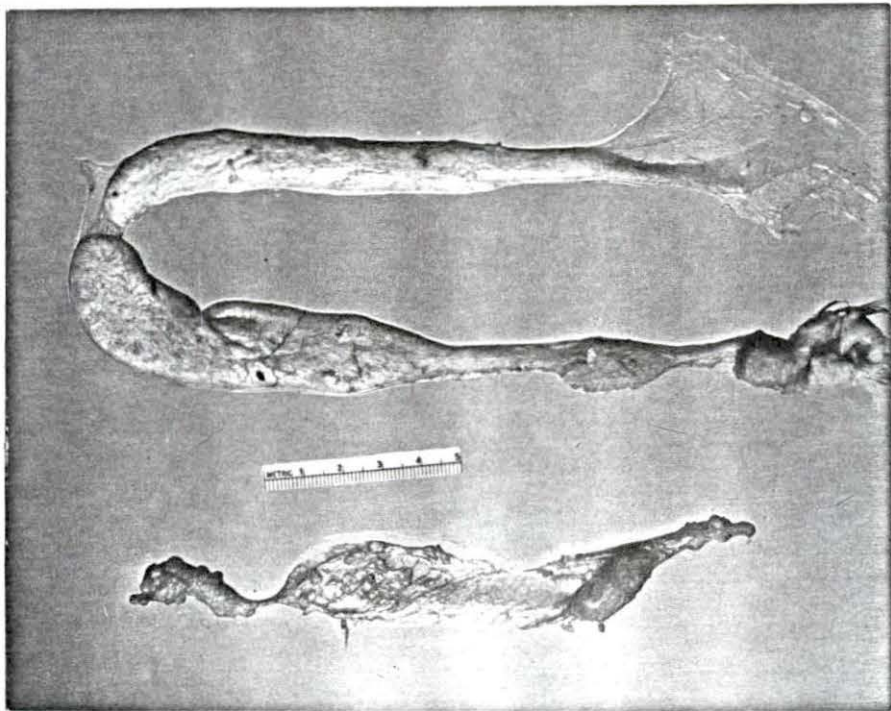
Figure 3. Reproductive organs of a sexually mature
previously exposed chicken, with two areas of
hypoplasia. A cyst occurs between the two
hypoplastic regions. The ovary is active
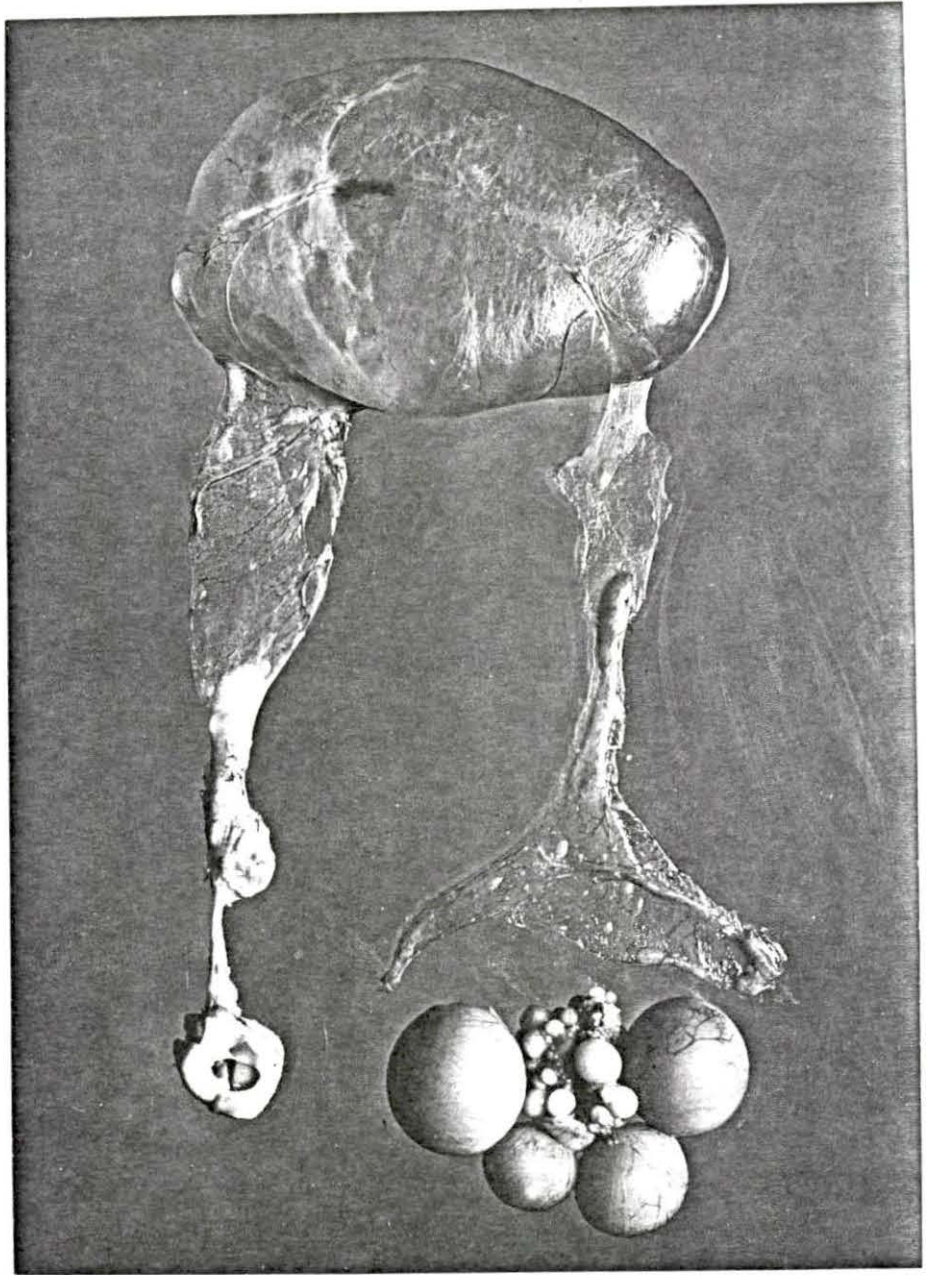
Figure 4. Reproductive organs of a sexually mature previously
           exposed chicken with one area of hypoplasia in the
           magnum. A cyst occurs caudally to the hypoplastic
           region. The cyst wall is transparent in its
           anterior portion, through which a sedimentary
           material may be seen. The uterus is only partially
           involved in the cyst and the vagina is not in-
           volved

Figure 5. Reproductive organs of a sexually mature control
           chicken. An egg is present in the uterus
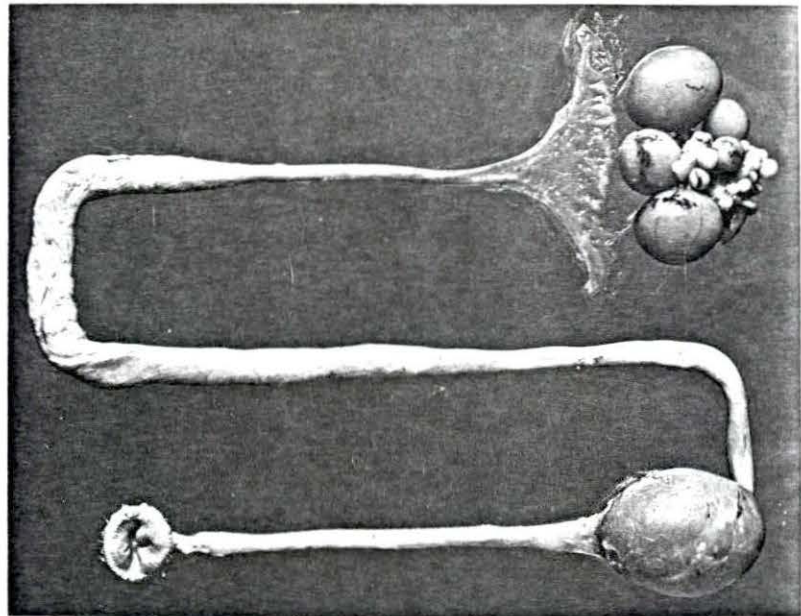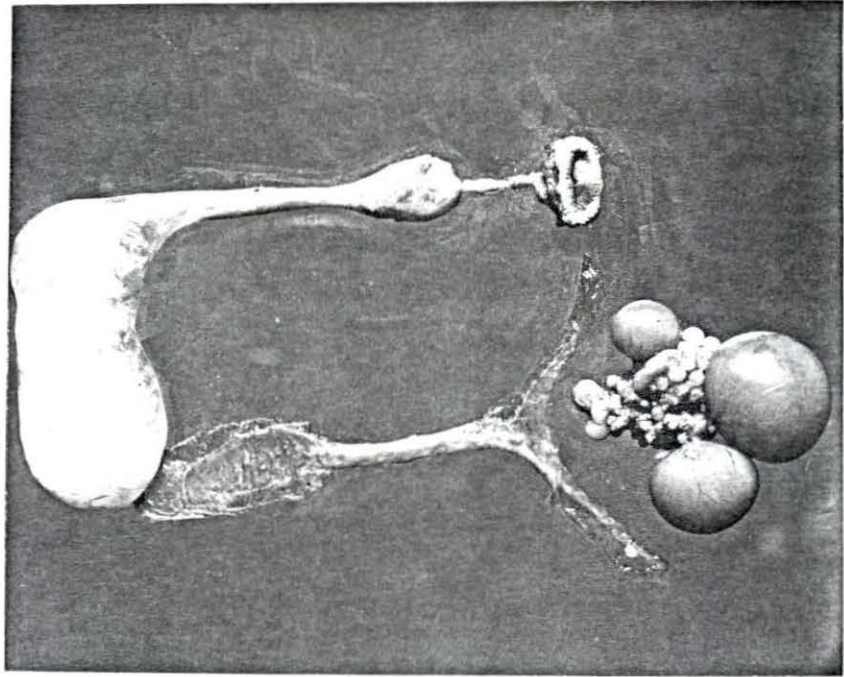
Figure 6. Reproductive organs of a sexually mature control chicken with an egg in the lower magnum. The junctional line (arrow) between the magnum and isthmus occurs a short distance caudally to the egg

Figure 7. Reproductive organs of a sexually mature previously exposed chicken. The junctional line between the magnum and isthmus is absent
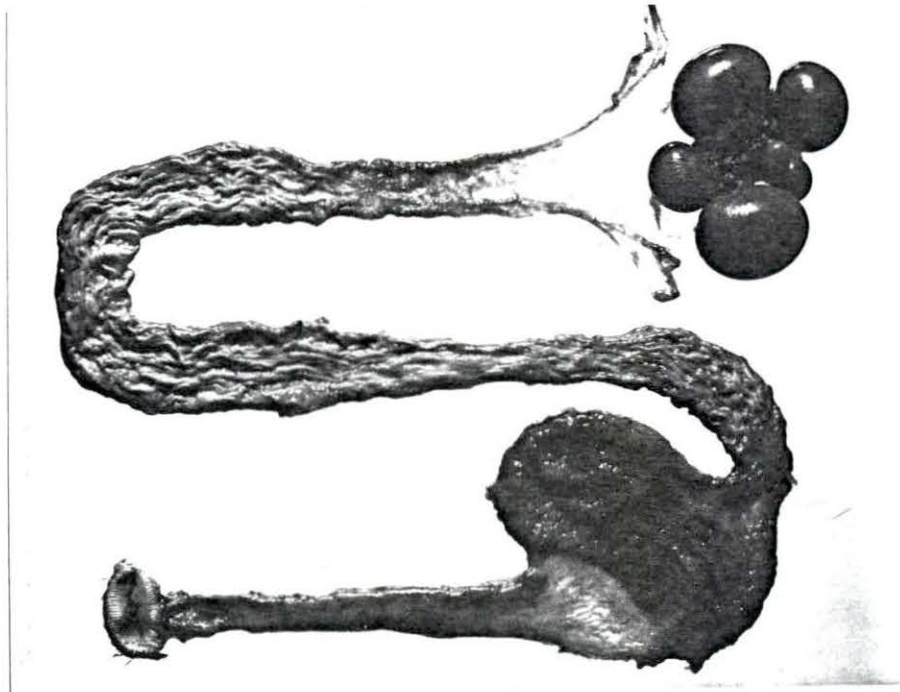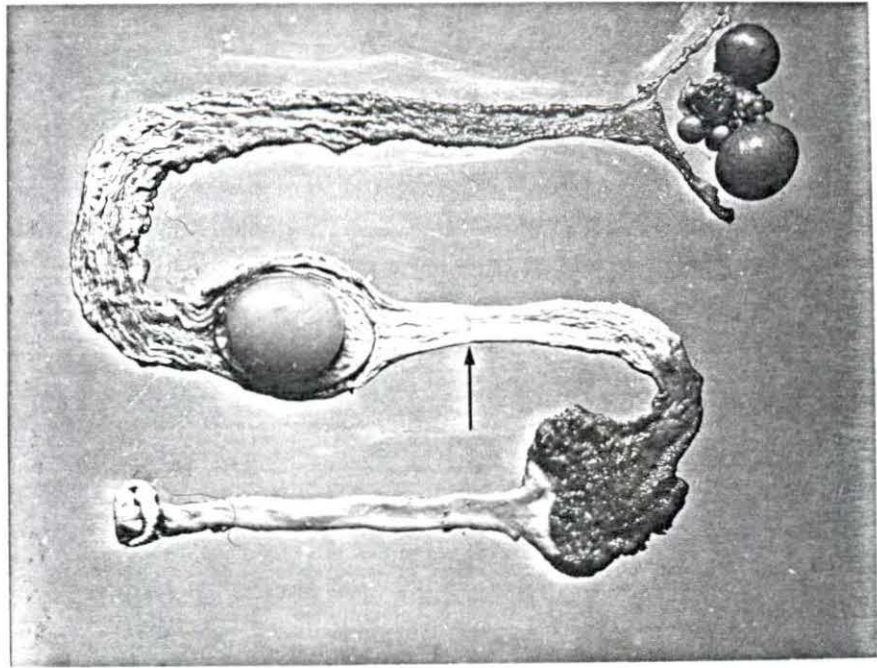
Figure 8.   Reproductive organs of a sexually mature
             previously exposed chicken, which had laid poor
             quality eggs. A partially developed egg is
             located in the lower magnum. The outline of
             the yolk may be seen through the localized
             hypoglandular wall of the oviduct
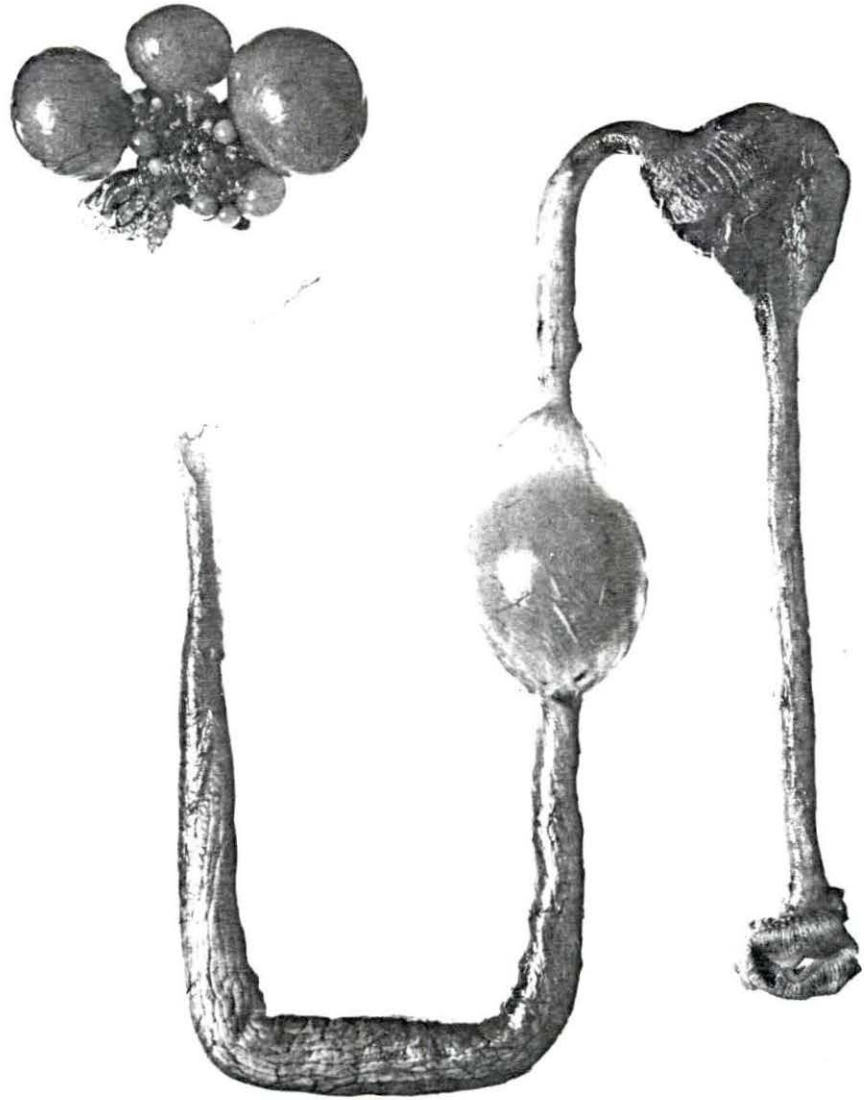
Figure 9.   Reproductive organs of a sexually mature previously
            exposed chicken which had laid poor quality eggs.
            This oviduct is the oviduct shown in Figure 8.
            It has been dissected and the egg removed. The
            glandular elements on either side of the junctional
            line are hypoplastic

Figure 10. Reproductive organs of a sexually mature control
            chicken. A partially developed egg is located
            in the lower magnum. The yolk outline cannot
            be seen through the oviduct wall
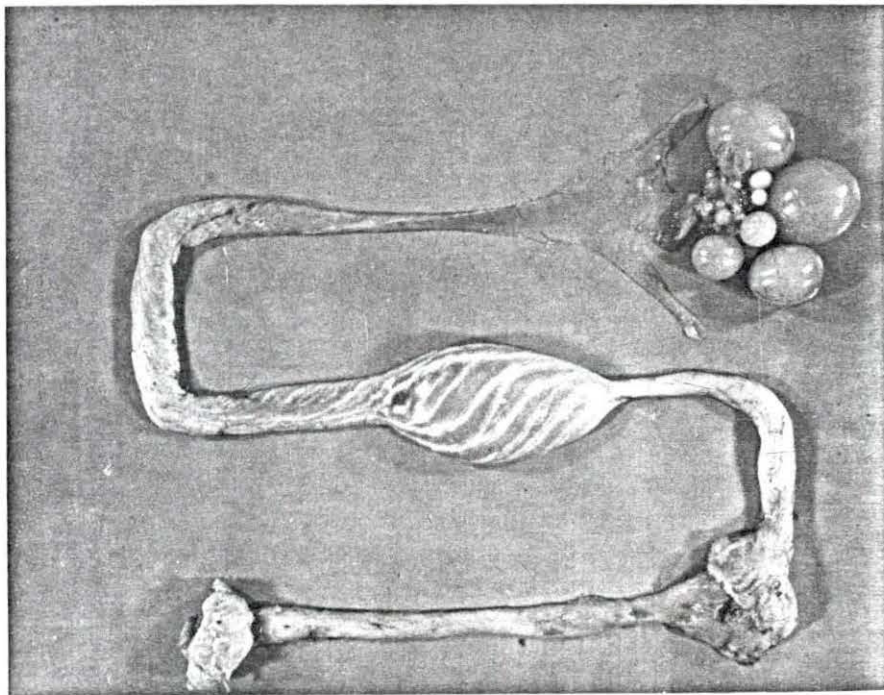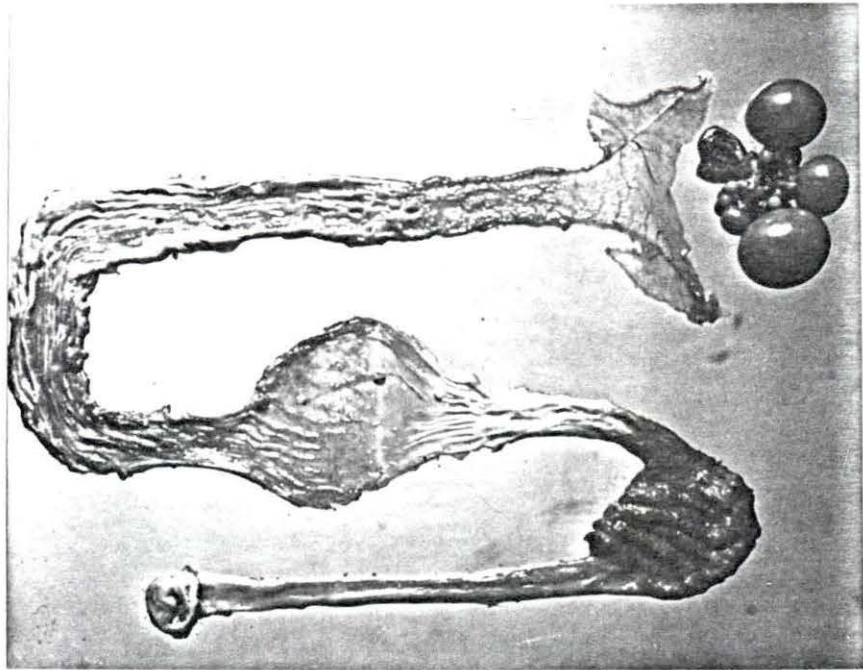
Figure 11.   Lymphoid cell infiltration of the oviduct wall
             at two days post inoculation. Hematoxylin and
             eosin stain. X 265

Figure 12.   Cross section of the oviduct from a three-day-
             old noninfected control chicken. Hematoxylin
             and eosin staining. X 280
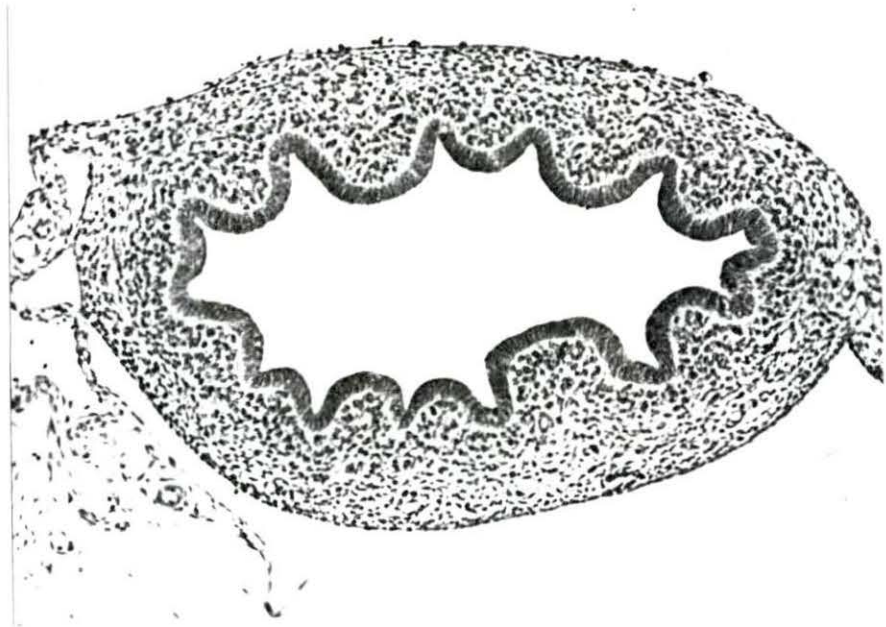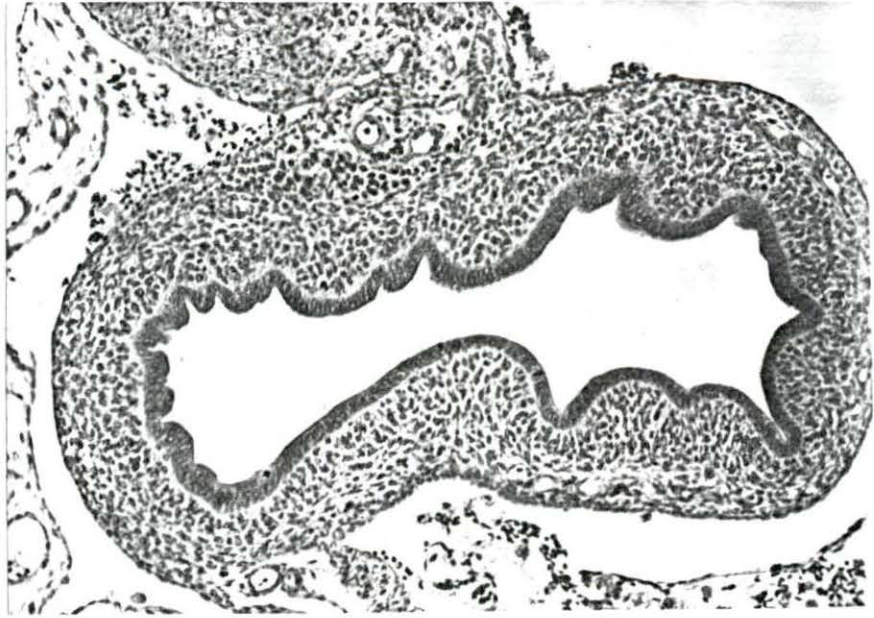
Figure 13.   Lymphoid cell infiltration of the oviduct wall
             at two days post inoculation. Hematoxylin and
             eosin stain.   X 460

Figure 14.   Cross section of the oviduct from a 23 day old
             previously exposed chicken. The section is
             located 2 mm anteriorly to the hypoplastic
             region. Hematoxylin and eosin stain.   X 126
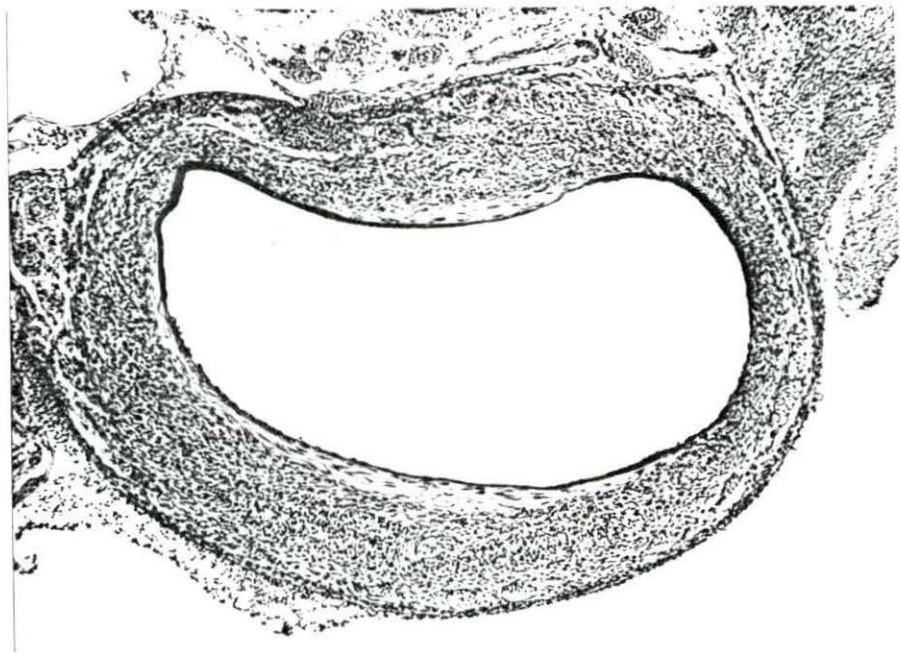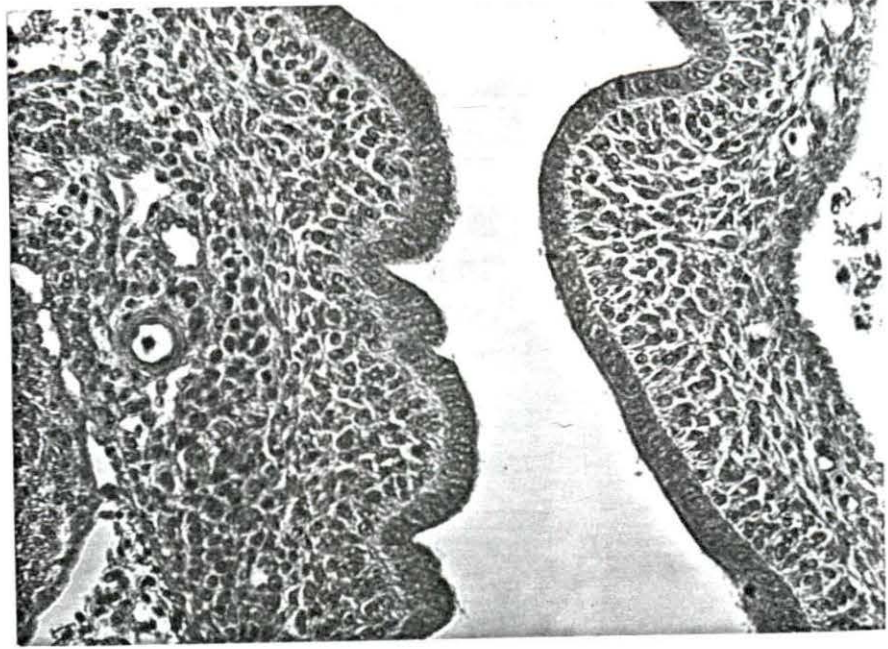
Figure 15.   Cross section of an oviduct with hypoplasia
             in a 23 day old previously exposed chicken.
             There is a total loss of luminal epithelium.
             A lymphoid nodule (arrow) is present in the
             oviduct wall. Hematoxylin and eosin staining.
             X 315

Figure 16.   Cross section of the oviduct from a 23 day old
             previously exposed chicken. The section is
             located posteriorly to a hypoplastic region.
             The oviduct lumen is greatly reduced in width.
             A lymphoid nodule is present in the oviduct
             wall. Hematoxylin and eosin. X 215
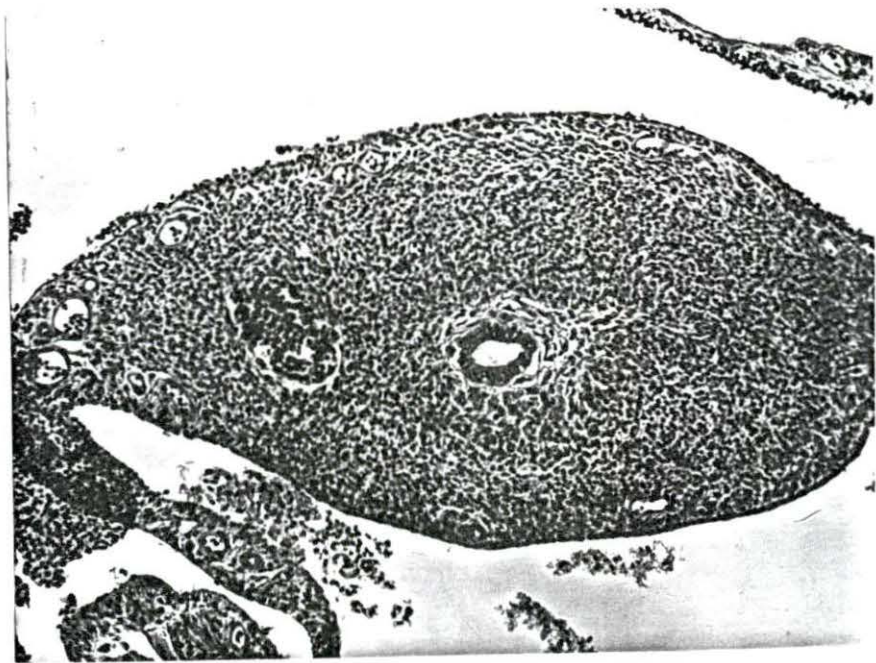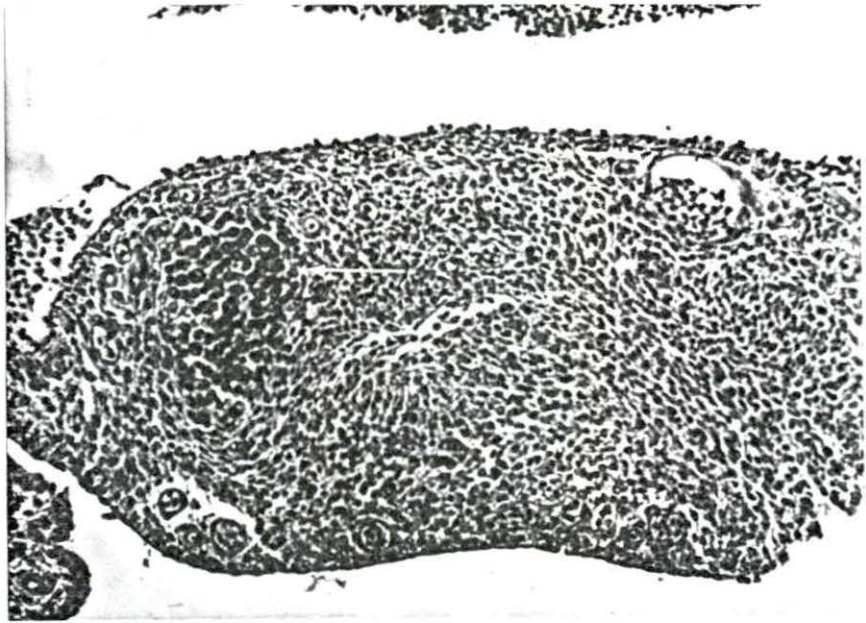
Figure 17.   Cross section of oviduct from a nine week old
             previously exposed chicken. The section is
             located in a region of incomplete hypoplasia.
             There is epithelium present where the lumen
             is patent. Hematoxylin and eosin. X 185

Figure 18.   Section of cystic wall of an oviduct from an
             eight week old previously exposed chicken.
             The epithelium is intact and the oviduct wall
             width is reduced. Hematoxylin and eosin.
             X 430

Figure 19.   Section of cystic wall of an oviduct from a
             one year old exposed chicken. There is
             partial glandular development in the cyst
             wall. Hematoxylin and eosin. X 185
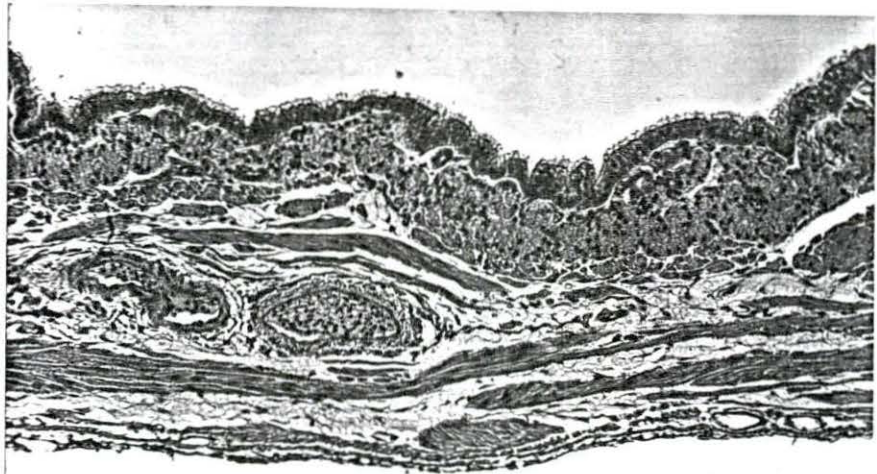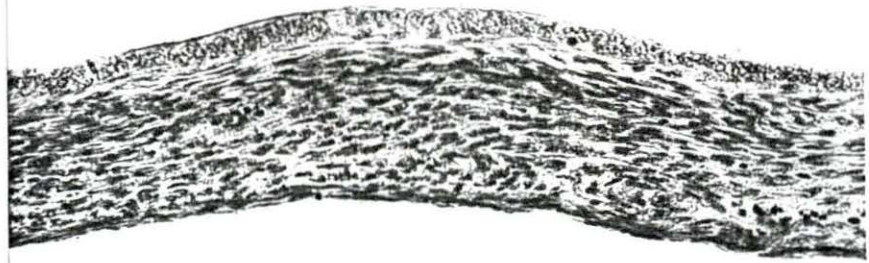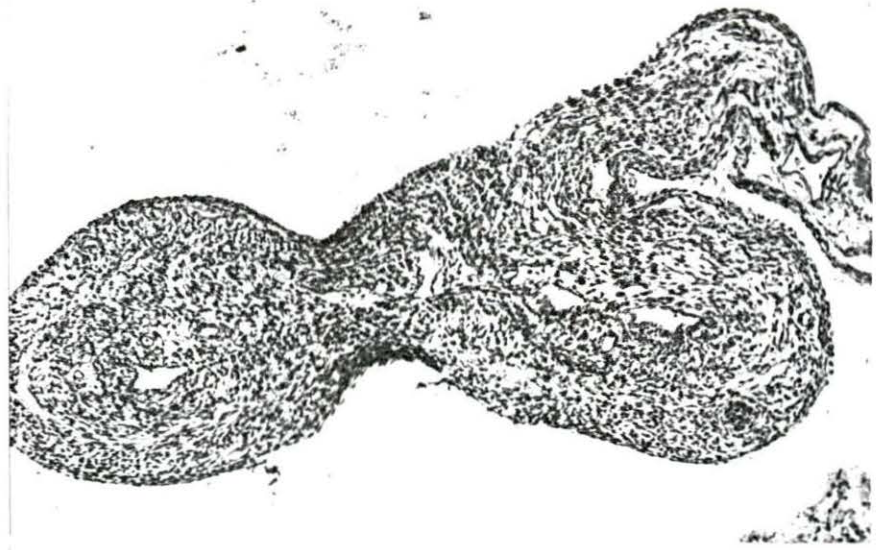
Figure 20.   Section of isthmus from a control, laying
             chicken with glands and arrangement of folds
             in the tissue. Hematoxylin and eosin.   X 55

Figure 21.   Section of magnum from a one-year old exposed
             chicken. with hypoglandular areas in the
             oviduct. The left-hand portion of the figure
             contains glandular elements of a normal
             appearance but in the remainder of the figure
             the glandular elements are greatly reduced.
             Hematoxylin and eosin staining.   X 75
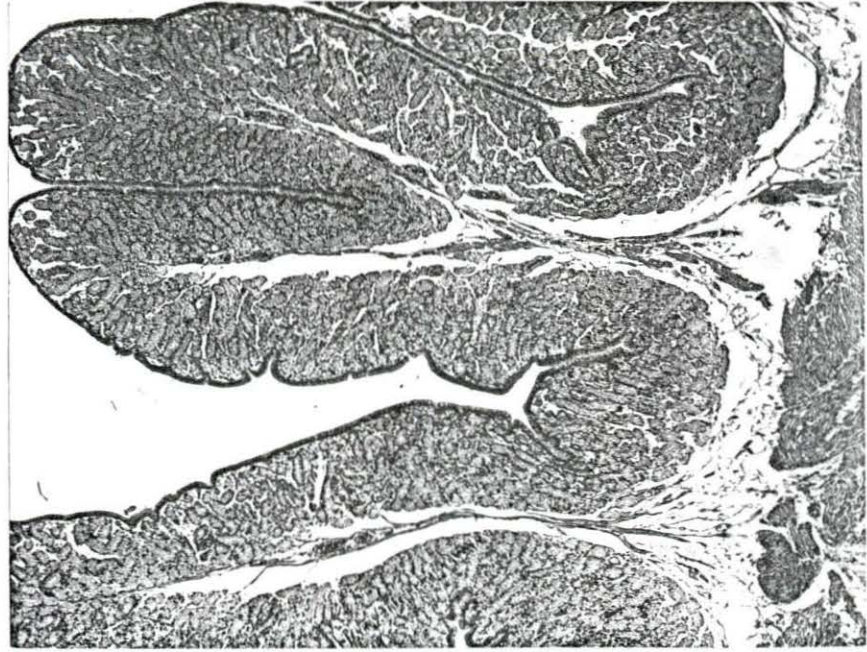
On external examination all three groups of birds had
the appearances of good layers; however, the nonlayers
often had large pendulous abdomens which were not found in
the other two groups.

On necropsy 83 per cent of nonlayers were found to have
nonpatent oviducts. These oviducts showed hypoplastic areas
of varying lengths. Posterior to the hypoplastic region
the oviduct was cystic. The cyst sizes varied greatly.
The large cysts often contained 200 ml of fluid while small
cysts on occasions contained less than 20 ml of fluid. The
latter oviducts often had well advanced glandular development
and had the appearance of an oviduct of a hen out of pro-
duction. However, they were still cystic as there was no
exit for secretions. In the large cysts the greater propor-
tion of the cyst lining had a transparent, nonglandular
character. The majority of the cysts were intermediate
between the two extremes.

The hypoplastic regions varied in length from a few mm
to 5 mm in most cases. Sometimes a faint outline of the
oviduct could be seen in the ligaments while on other
occasions nothing could be seen. Ninety per cent of
the hypoplastic regions involved the caudal half of the
magnum and cephalic half of the isthmus. The remainder
involved the other segments of the oviduct except the
infundibulum, where no hypoplastic lesions were ever ob-

served. In 3 birds, cysts approximately 5 mm in diameter were found on the infundibular surface. These did not block the lumen. The oviduct anterior to the hypoplastic regions appeared normal for a nonlaying hen. No macroscopic lesions were observed in 17 per cent of nonlayers.

The ovaries of all nonlayers were active with ova of varying developmental stages. On ovulation the ova were discharged into the abdominal cavity. Evidence of this was seen as intact ova in the abdominal cavity. On no occasion was an ovum found in the anterior portion of a hypoplastic oviduct. The ova were quickly absorbed in the abdominal cavity. The abdominal fat of these birds had a deeper yellow color compared to controls. In chickens which had a thick layer of fat, a color gradient was obvious in it. The layer next to the abdominal cavity had a deep yellow color but became progressively lighter towards the exterior. Thirty per cent of the birds had a layer of yellowish-green viscous material lining the abdominal air sacs and other structures of the abdominal cavity. In the remaining two-thirds, apart from the color change, no other lesions were found.

The oviducts of hens which laid poor quality eggs had definite macroscopic lesions. Ninety per cent of these lesions were located in the caudal half of the magnum and cranial half of the isthmus. These areas showed either a

complete absence of glandular tissue or only partially developed glands. The oviduct was thin and transparent in these areas. When a developing egg was present within the oviduct beneath such an area a distinct outline of the yolk could be recognized. There was no evidence of any constriction in the oviduct. The glandular hypoplastic areas varied in size and shape. In some they formed a circumscribed ring around the oviduct while in others it consisted of a horizontal band running parallel with the direction of the oviduct up to 8 cm in length. The normal constriction between the magnum and isthmus was not seen in 40 per cent of this group. In one bird a transparent oviduct wall was observed in a portion of the vagina. There was no evidence of internal laying in any of these hens.

The exposed birds which laid normal eggs had normal appearing oviducts macroscopically. The overall percentage of exposed chickens showing nonpatency of the oviduct was 22. This figure refers to the period between first observation of nonpatency on the 22nd day post inoculation and the termination of the experiment. The right oviduct persisted in chickens both exposed and control at 24 per cent and 16 per cent respectively. In 80 per cent of the cases cyst formation was present. Generally the cysts did not exceed 5 cm but in 20 per cent of cases the cysts were large containing up to 100 ml of a clear serous fluid. No cysts were

found on the oviducts (left) of control chickens.

Microscopic

The histopathologic changes in the oviducts of chickens
which were hypoplastic were similar to those already described
for the earlier age group. However, there was greater glan-
dular development both in the anterior segment of the oviduct
and in the cystic area. The hens which laid poor quality
eggs had areas of glandular hypoplasia. The surface epithe-
lium was intact with the presence of a normal ductal system.
There was a lack of glandular elements in association with
the ducts. In some areas the ductal system did not extend
very deeply and in these areas the oviduct wall was narrow
and would correspond to the transparent area seen macro-
scopically. These oviducts had the same degree of cellular
infiltration and numbers of lymphoid nodules as the treated
chickens which were laying normal eggs. There was no evidence
of any increase in connective tissue elements in the hypo-
glandular areas.

Oviducts in both the exposed and control adult chickens
contained infiltrations of lymphoid and plasma cells together
with lymphoid nodules. This was much less pronounced in
the control birds.

Figure 22.   Luminal epithelial cells from the oviduct of
             a control chicken at five days of age. Portion
             of the lumen is shown. X 21,775
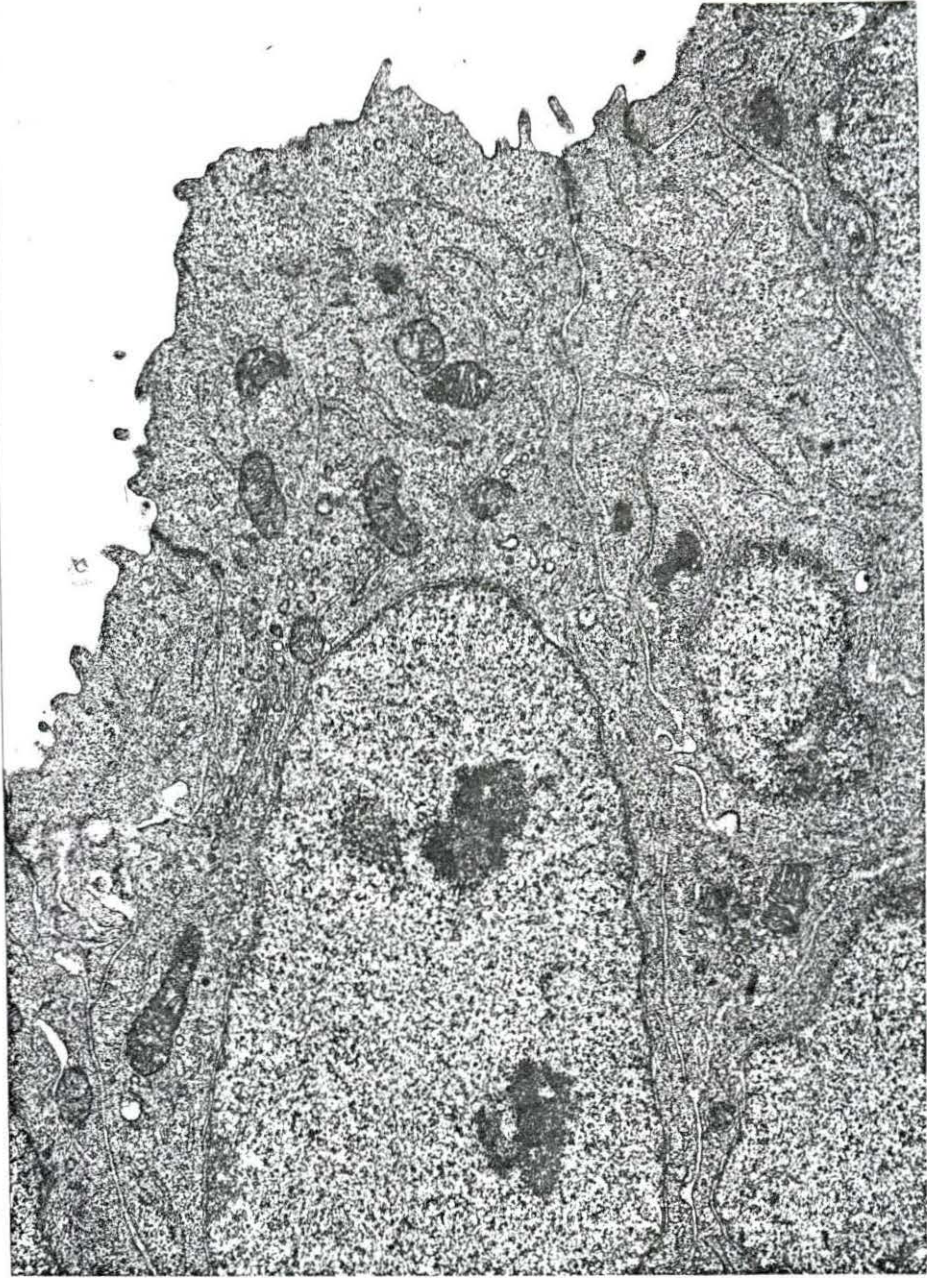
Figure 23. Luminal epithelial cells from the oviduct of
a previously exposed chicken at four days post
inoculation. There is dilatation of the
rough-surfaced endoplasmic reticulum (arrows).
The contents of many of these structures con-
tain a granular material of varying electron
density. A small area of lumen is shown at
the lower center edge. Compare this micro-
graph with Figure 22 showing the normal
structural arrangement. X 21,775
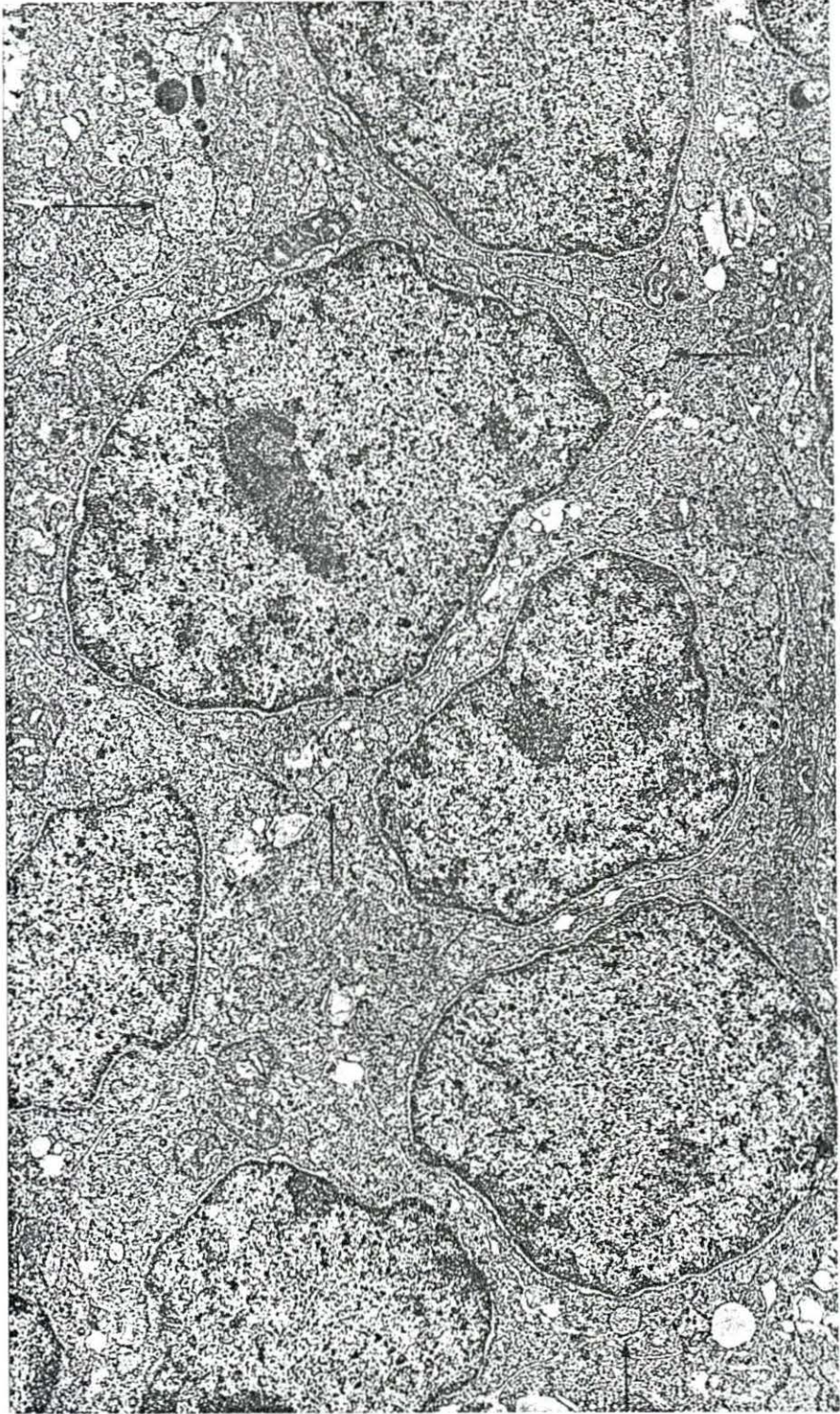
Ultrastructural Findings

No significant changes were seen in the infected tissue examined at 40 hours post exposure. At 108 hours post exposure dilation of the rough surface endoplastic reticulum was observed in the epithelial lining cells of the oviduct. In the same cells a distension of the perinuclear space was noted.

Virus titration

Table 1 shows the virus distribution in tissues collected 65 hours post inoculation. At this time the virus concentration was highest in the respiratory system. The virus was present in all tissues examined. The titration results of tissues collected 42 days post inoculation were all negative as shown in Table 2.

Test results

The plate agglutination tests against both Mycoplasma gallicepticum and Mycoplasma synoviae were all negative. The hemagglutination inhibition test for the presence of Newcastle disease antibodies was negative. The five chickens exposed to infectious laryngotracheitis virus all showed classical signs of the disease within three days. The serum neutralization test for the presence of infectious bronchitis viral antibodies was positive for the exposed group and negative for the controls. Subsequent to the

Table 1. Titrations for infectious bronchitis virus in
          tissues collected from chicks 65 hours after
          inoculation

Tissue	Log_{10} titer[a] of virus per 0.1 ml
Air Sac	4.83
Lung	4.14
Trachea	4.12
Oviduct	3.34
Ovary	3.20
Testes	3.60[b]
Kidney	2.36
Bursa	2.00

[a]Average in tissue suspensions from three chickens.

[b]This figure is of interest, because pooled semen from ten recovered adult roasters resulted in a fertility rate of approximately 65 per cent when inseminated into control hens as compared to a figure of approximately 75 per cent from six control roasters. Histological evaluations of testes and vas deferentia from recovered and control roasters are pending.

Table 2. Titrations for infectious bronchitis virus in
          tissues collected from exposed chicks at 42 days
          post inoculation

Tissue	Log_{10} titer of virus per 0.1 ml
Air sac	0.0
Lung	0.0
Trachea	0.0
Oviduct	0.0
Ovary	0.0
Kidney	0.0
Bursa	0.0

12th week only one chicken died. The etiology of this death

was not determined.

Table 3. Egg production of 70 chickens previously exposed
to infectious bronchitis by aerosol at one day
of age and 26 control chickens[a]

Age	Exposed %	Control %
8 months	55.7	76.3
9-1/2 months	50.0	76.6

[a]Averages over test period of seven days.

Table 4. Clinical and pathological data on chickens
exposed to infectious bronchitis virus at one
day of age

	Numbers	Percentage
Mortality rate due to I.B.V.	55/220	25[a]
Nonpatent oviducts	31/141	22[b]
Hypoglandular oviducts	15/70	21
Nonlayers	18/70	26
Nonlayers; patent oviducts	3/18	17
Nonlayers; nonpatent oviducts	15/18	83
Normal layers	37/70	53
Mortality cause unknown	2/220	-
Total treated group	220	-

[a]Calculated to nearest whole number.

[b]This figure refers to all birds examined between the
first observation of nonpatency and termination of the
experiment. Thus it is only a projection for the entire
treated group as all birds which died or were sacrificed
prior to 22 days are not included.

Table 5.   Analysis of visual egg examination from 20
           sexually mature previously exposed chickens and
           20 controls[a]

	Exposed %	Control %
Calcareous deposits on shell	14.4	0.5
Thin shell	3.7	0.0
Ridges on shell	14.3	0.0
Faulty shape	2.2	0.2
Watery albumen	10.0	3.0
Yolk separation from thick albumen on breakage	15.5	0.7
Albumen adhering to shell membrane on breakage	19.3	0.0

[a]In all the foregoing parameters except one, $P <$
0.005.   In the case of faulty shape, $P < 0.05$.

Table 6. Arithmetic means of egg measurements from 20
         sexually mature previously exposed chickens and
         20 controls

	Exposed	Control
Haugh units[a]	90.42	93.99
Specific gravity[b]	1.0888	1.0879
Weight (grams)	52.78	53.33
Shell thickness (mm)	.341	.334

[a]The Haugh units were lower in the exposed group
compared to controls, but the difference was not significant.
However, the variability among the recovered groups was over
ten times as great as in the controls.

[b]There was a highly significant positive correlation
between specific gravity and shell thickness within
individuals; in effect eggs from individuals which con-
sistently were of lower specific gravity also had reduced
shell thickness. Furthermore such parameters tended to be
consistent over the seven week observation period, as was
also the case in the controls.

DISCUSSION

Localized hypoplasia of the oviduct was found to be the main lesion in this study. The experiment indicates that the oviduct is liable to severe damage by exposure to infectious bronchitis virus at one day of age. The results agree in general with the findings of Broadfoot et al. (1956). However, the severity of lesions was greater in this work. They reported 18.9 per cent incidence of incomplete oviducts compared to 22 per cent in this study. Differences could possibly be due to the different types of virus involved in the two studies. It is not known if different types of the virus have different pathogenicities for the oviduct. Another factor was a 5° F drop in the brooder temperature for seven hours immediately post inoculation due to an electrical problem. This may have stressed the birds.

The viral titration results indicated the presence of the virus in the oviduct. The titers reached suggest the virus is actually multiplying in the oviduct rather than being passively present following a viremia or direct contact from the abdominal cavity. The strain of virus used did not have as great an affinity for reproductive tissue as for respiratory tissue, as evidenced by tissue viral titers during the acute stage of the disease. The reproductive lesions are not due, therefore, to enhanced tropism of this strain of virus for reproductive tissue, if one uses

the criterion of viral concentration in the tissue as indicative of pathogenicity. The viral levels in the respiratory system and other tissues examined are in agreement with the finding of Hofstad and Yoder (1966). They found great difficulty in isolating the virus from the blood.

Domermuth and Gross (1962) showed that mycoplasmal salpingitis resulted from mechanical transfer of mycoplasma from the yolk and air sacs. They suggested it was unlikely that mycoplasma was carried by the blood stream to the oviduct. Gross (1958) showed that Escherichia coli infection of the oviduct occurred in conjunction with air sac infection by this organism. The same route of infection may occur in infectious bronchitis.

Twenty-two per cent of the treated group had non-patent oviducts, while not a single case of nonpatency was observed in the control group. The hypoplasia varied considerably in its extent throughout the treated group. In a few instances two-thirds of the entire oviduct was hypoplastic while in others only a few mm were involved. The virus did have an affinity for the production of lesions in one area of the oviduct, namely, the caudal half of the magnum and cranial half of the isthmus. Ninety per cent of the hypoplasias involved these areas. This is in contrast with Broadfoot et al. (1956) who reported the caudal portion of

the isthmus and cranial half of the uterus to be involved in the hypoplasia in the majority of cases. However, his analysis included chickens exposed at varying intervals of time up to 18 days of age. It is of interest that the majority of the hypoplasias of the oviduct were centered around the junction of the magnum and isthmus, an area where there is no glandular development. The reason for this localization is unknown.

While the virus was present in the oviduct and simultaneously in the respiratory system, no evidence of hypoplasia was seen until 22 days post inoculation. The lesions seen up to this time were of a nonspecific nature. From later evidence it seems the epithelial lining cells are the primary areas of viral attack. The cellular pathogenesis is not known. There are several reports in the literature regarding the effects of infectious bronchitis virus on tissue culture (Wright and Sagik 1958; Cunningham and Spring 1965; Akers and Cunningham 1968). Beaudette's chicken-embryo adapted cultures of infectious bronchitis virus have been used in most studies. However, Buthala (1956) reported that cells from chicken embryo trachea and lung were not able to support the multiplication of Massachusetts strain, type 33 of infectious bronchitis virus. In general the cytopathogenic effect produced by infectious bronchitis virus has been described as degenera-

tive and necrotic in nature. The production of syncytia has also been noted as a special criterion of cellular response to infectious bronchitis not seen in the other "coronaviruses" (Akers and Cunningham 1968).

There is evidence in this experiment which suggests that a similar type of effect may occur in the oviduct. In the earliest nonpatent oviducts observed, the epithelial cells in areas adjacent to the hypoplastic region were pale and had lost much of their basophilic staining character. They were also larger compared to epithelial cells at some distance from the hypoplastic region. Secondly the limited ultrastructural findings suggest a nonspecific degenerative type of reaction. The connective tissue elements beneath the epithelium remained unaltered even in the hypoplastic areas until 40 days post inoculation except for the infiltration of cells. Once the epithelial cells become necrotic the connective tissue elements beneath it will produce closure of the oviduct lumen by organization. Thus the oviduct will become permanently nonpatent.

When the oviduct becomes occluded any secretion of the oviduct caudal to it will have no means of escape. The first nonpatency was observed on the 22nd day. However, the first cyst was not seen until the 44th day post exposure; at this particular time secretion is not very active. The cysts were small at first and gradually increased in size.

The suspended material in the cysts was most likely a secretion of the partially developed oviduct glands. All secretions of the cranial portion of the oviduct had a route of escape into the abdominal cavity.

The only other documented report of any etiological factor leading to cystic oviducts in chickens is by Finne and Vike (1951). They observed a hereditary factor to be involved in the cause of the cysts they found. They did not associate them with any infectious disease. Hereditary factors can be ruled out as the primary cause of the cysts in this experiment since no control was observed to have a cyst in its oviduct. Segmental aplasia of the uterus occurs in all species of domestic animals but is seen most frequently in cattle (Spriggs 1946; Arthur 1958), and swine (Warnick et al. 1949; Wiggins et al. 1950). Sheppard (1951) stated that uterus unicornus occurs in approximately one of 1,000 cats. Bloom (1954) reported that a similar ratio exists in the bitch. There appears to be a hereditary basis for the development of segmental aplasia of the uterus in cattle (Fincher and Williams 1926; Gilmore 1949). Little is known about the other species and the etiology is regarded as either hereditary or due to genetic defects in the Müllerian ducts. There may be either inhibited development of various segments of the duct or defects in the pattern of fusion.

Roberts (1950) observed concretions of a "putty like

consistency" in cystic portions of a bovine uterus which exhibited aplasia. These concretions are not unlike the sediment observed in the avian cysts following infectious bronchitis.

Hydrosalpinx generally is a sequel of salpingitis rather than a congenital malformation of the tube in mammals. Failure of the hymenal membrane to perforate has been reported by Fielder (1954), in the mare.

Characteristic lesions were observed in all hens which laid poor quality eggs. While these lesions had many aspects in common with those observed in the nonpatent oviduct, morphologically and functionally they were different. In both cases there was a failure of a localized area of the oviduct to develop properly. Secondly, the areas of hypoplasia were located in the same regions of the oviduct. In the hens which laid poor quality eggs the hypoplasia was confined to the glandular elements of the oviduct. There were no constrictions of the lumen and the surface epithelium was intact throughout the entire length of the oviduct. The ductal system was moderately well developed.

While the virus was present only when the chickens were young, there was no evidence of hypoglandular areas until the chickens came into lay. The virus could not be isolated from the tissue samples harvested 42 days post inoculation. The virus thus prohibits the normal development of glandular

elements in localized areas. The mechanism whereby this is accomplished is unknown. The virus may inhibit future differentiation of the epithelium into glandular elements or destroy cells already differentiated into primordial gland cells. A possible example of the former could occur if localized areas of the oviduct became insensitive to estrogen, whose stimulation is essential for complete development. The estrogen itself must be unchanged since the remainder of the oviduct appears normal. The avian oviduct may show a 40-fold increase in size following continued estrogen administration while the birds are fed normal rations (Sturkie 1965). This response to estrogen is diminished if the ration is deficient in folic acid (Hertz 1945). This diminished response is not due mainly to inanition, although this is a factor, but possibly to the fact that folic acid is involved in the enzyme system through which estrogen exerts its effects (Kline and Dorfman 1951).

The results indicate that each portion of the oviduct is specialized to accomplish a definite function in the normal development of the egg. In the absence of any localized glandular area definite changes were observed in the eggs. The egg characteristics of each hen were approximately of a similar nature throughout the entire egg laying period. Thus it may be possible to correlate egg characteristics

with localized lesions of the oviduct. With the presence of significant egg shell changes and a minimum of uterine lesions, this would suggest that the isthmus has an influence on shell deposition since lesions of the isthmus were found at significant levels in the treated group. The isthmus is responsible for the secretion of the shell membrane and this membrane in turn may influence the pattern of the shell deposition. The results also indicate that exposure of one-day-old chickens will cause a significant drop in egg production ($P < 0.01$) together with a drop in both internal and external quality.

Structurally and functionally the virus did not appear to have any significant effect on the ovary. Even in chickens which had nonpatent oviducts the ovaries were of normal size and were apparently functioning normally. The serological results indicated the two flocks were free of all complicating diseases. There is no evidence in the literature to relate coccidiosis or fowl pox with any permanent damage to the oviduct. Thus it is unlikely that the outbreaks of either of these diseases changed the results of the experiment.

The histological reaction during the acute stage of the disease was nonspecific. Domermuth et al. (1967) described similar microscopic lesions in the oviduct wall of young chickens with mycoplasmosis. In the mature

chicken infiltration by heterophiles of the oviduct wall
was reported by Sevoian and Levine (1957). However, this
cell type was not seen in this study. Sevoian and Levine
(1957) also observed lesions in the epithelial cells and
areas of the oviduct where glandular elements were reduced
or almost absent. Thus, the virus has the same primary
target cells (epithelial cells) in both day old and sexually
mature chickens.

McIntosh et al. (1967) recovered six viral isolates
from patients with upper respiratory tract diseases, which
had a close morphological resemblance to both infectious
bronchitis virus and mouse hepatitis virus. However,
serologically they were found to be unrelated. Neutralizing
antibodies to infectious bronchitis virus have been found in
man (Miller and Yates 1968), but their significance is not
known at the present time.

Viruses may attack developing tissues in mammalian
embryos; e.g., hog cholera virus (Sautter et al. 1954),
blue tongue virus (Shultz and DeLay 1955) and rubella in
pregnant women (Gregg 1941). In these conditions the virus
produces a residual effect which results in hypoplasia and
developmental anomalies. While these occur in the mamma-
lian embryo, the one-day-old avian oviduct is very close
to an embryonic tissue and may possibly have some character-
istics in common with those of intra-uterine embryos.

The immunological status of the chickens is of importance. The chickens used in this experiment were free of parental antibodies. It is unlikely that chickens with parental antibodies would have the severe lesions found in this experiment. Under field conditions there are many flocks in isolated areas where parental antibodies are absent. In such flocks early exposure to infectious bronchitis virus would make the flock relatively unprofitable.

This work indicates that there are many questions still to be answered with regard to infectious bronchitis virus and its relationship with the oviduct. It is generally accepted that different types of infectious bronchitis virus have characteristic pathogenicities for the respiratory tissue. At present, there are no reports in the literature regarding the pathogenicities of different types for reproductive tissue. The age of the bird at exposure determines the severity of the lesions to a great extent. However, most of the work to date in this area has been reported from field outbreaks where it is often difficult to determine the exact age at exposure.

Further electronmicroscopy may indicate organelle changes in the epithelial cells which may help in the characterization of the pathogenesis. The total length of oviduct which will become hypoplastic in a three-day-old

chicken is very small and as a result it may be difficult to locate the area of hypoplasia. Further work on egg quality and the morphological lesions of the oviduct could yield fruitful results as to the mechanism of poor internal and external egg quality.

SUMMARY

The purpose of this work was to demonstrate the reproductive defects reported to follow exposure to infectious bronchitis virus and to attempt to gain an understanding of the pathogenesis of the defects produced. Exposure of 220 one-day-old pullets to Massachusetts type, strain 33 of the virus resulted in heavy mortality (25 per cent) and subsequently a high percentage of reproductive defects. The virus did not show any increased affinity for the oviduct compared to other tissues.

At the cellular level, during the acute stages of bronchitis in the young chick, histological and ultrastructural changes were those of a relatively mild acute inflammatory process in which heterophils were not conspicuous. The ultrastructural changes, in particular were of a nonspecific type.

Localized hypoplasia of the oviduct was found to be the main lesion in this study, manifested in two types. In one, the hypoplastic areas resulted in nonpatent oviducts. This was found in 22 per cent of previously exposed chickens. In the other type, the hypoplasia resulted in macroscopic and microscopic hypoglandular areas in oviducts which remained patent.

The vast majority of these hypoplastic areas involved the caudal portion of the magnum and cranial portion of the

isthmus. Poor quality eggs were laid in significant numbers by the treated group and there was a positive correlation between inferior egg quality and presence of hypoglandular areas in the oviducts. The reduction in egg quality was due to albumen and shell abnormalities. The economic implications of these effects are much greater than has been previously realized.

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