Pathogenicity of four serotypes of avian infectious bronchitis virus for the oviduct of chickens

by

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INTRODUCTION

Avian infectious bronchitis is an acute, highly contagious respiratory disease of chickens characterized by tracheal rales, coughing and sneezing. In addition, young chickens may have a nasal discharge and in laying flocks there is a drop in egg production.

Since 1939 it has been recognized that the greatest economic losses associated with this disease concerned a reduction in egg production and quality, both external and internal, and reduced 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 evaluate the pathogenicity of different serotypes of infectious bronchitis virus for the oviduct and to ascertain the susceptibility of chickens exposed at different ages to the virus. Massachusetts type, strain 33, was evaluated at two chicken embryo passage levels, 7th and 55th. The methodology involved the evaluation of four parameters: gross lesions, microscopic pathology, virus titration in chicken embryos for the presence of virus in selected tissues and finally the localization of specific viral activity by immunofluorescence.

LITERATURE REVIEW

Infectious bronchitis (IB) 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 United States 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), in the Netherlands (Bijlenga, 1956) and in Australia (Cumming, 1963).

Van Roekel et al. (1939) reported that the greatest loss suffered from the disease when present in mature birds was reduced egg production. Early reports indicated that IB was primarily a disease of young chickens. However, it was later observed that the disease was common in semimature and laying flocks as well.

IB has a viral etiology, namely infectious bronchitis virus (IBV). The virus has recently been placed in a new group, "coronaviruses", (Almeida et al., 1968) of which IBV is the prototype. Other members of this group include mouse hepatitis virus and a number of viruses isolated from humans suffering from the "common cold." They share basic characteristics which include pleomorphic particles ranging in size from 76-250 nm with characteristic club-shaped surface projections, sensitivity to lipid solvents and heat and a ribonucleic acid core (Parker et al., 1970).

McIntosh et al. (1969) and Bradburne et al. (1970) studied the antigenic relationships between these viruses. Several relationships between various members of the coronaviruses have been revealed by neutralization,

complement fixation and gel diffusion tests. However, IBV was shown to be unrelated antigenically to any other member.

Miller and Yates (1968) showed that the sera of individuals associated with poultry had the ability to neutralize IBV compared to a lack of neutralization by sera of individuals not associated with poultry. This indicates either previous infection with IBV or the capacity of human serum to neutralize an avian virus which is antigenically related to a still undiscovered human agent.

Several different serological types of IBV have been distinguished. In 1956 Jungherr et al. described the immunological differences between two strains of IBV and proposed that they be designated the Massachusetts (Mass) and Connecticut (Conn) types. Hofstad (1958a) reported Iowa 97 and 609, and Winterfield and Hitchner (1962) described the Gray and Holte types. In 1964 Winterfield et al. described the JMK isolate as immunologically distinct from the above types. The Australian T (Aust) strain, isolated by Cumming (1963), and IBV-10 (Cuxhaven) have been compared by Von Bulow (1967) and he reported them to be separate serotypes, while he reported IBV-7 (Stuttgart) was a variant of the Mass type. Purchase et al. (1966) isolated RPL-IBV from a closed flock and reported it to be related to Iowa 97. Hopkins (1969) proposed a new serotype of IBV, SE-17. Von Bulow (1969) reported on another serotype, KA/Koblenz, isolated from the field. Berry and Stokes (1968) studied antigenic variations between eight English and three foreign IBV strains and concluded that the variation was minor and of subtype character. Their study did not include the American strains other than the Mass type.

While it is possible to demonstrate marked serological differences between types in virus neutralization tests using convalescent sera, the use of hyperimmune sera will usually indicate less antigenic variation[1]. Cross immunity studies in chickens indicate some cross protection to challenge between isolates even though virus neutralization tests indicate marked differences (Hofstad, 1961). The Mass type stimulates greater protection against challenge with heterologous strains than do the other types. Cross protection tests between the Aust T isolate and the Mass type indicate cross immunity when challenged soon after recovery[1].

Fritsche et al. (1969) compared serum neutralizating titers produced by the 52nd and 120th chicken embryo passage of a Mass serotype, H strain of IBV. Satisfactory immunity was produced serologically if "H-120" was given to 2-4 week-old chickens and if "H-52" was administered to the same chickens at 13 weeks of age. This vaccination protocol is now commonly used in Great Britain.

The various serotypes of IBV may be divided into two groups based on their ability to cause nephrogenic lesions. Gray, Holte, Aust T and JMK are nephrogenic. There is no evidence in the literature that the other types produce kidney lesions. In the United States the usual field strain encountered is designated as the Mass type. Several isolates commonly designated as the Mass type are the pathogenic chicken strain (41), the Beaudette embryo lethal strain and strain 33[1].

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[1]M. S. Hofstad, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa. Antigenic studies of infectious bronchitis virus. Personal communication. 1971.

A widely used technique in research and diagnosis for the detection of antigen in tissues is Coons et al. (1941) fluorescent antibody technique. Stultz (1962) first used this technique for the identification of IBV in tissue culture cells. It was used by Mohanty et al. (1964) to follow the growth cycle of the Beaudette strain of IBV in chicken embryo kidney cells. Corstvet and Sadler (1964) observed IBV antigens in tracheal smears, thoracic and abdominal air sac epithelial cells of chickens infected with egg adapted IBV. The specific fluorescence was seen as diffuse granules around the nucleus. These findings were confirmed by Braune and Gentry (1965).

Lukert (1966) demonstrated the relative sensitivities of primary chicken embryo cell cultures for IBV using immunofluorescence. He found the order of sensitivity from highest to lowest was kidney, lung, liver and fibroblasts. Lukert (1969) showed that it was possible to differentiate, though not necessarily identify, IBV serotypes by immunofluorescent staining on tissue culture infected cells. He suggested that the pattern of reciprocal immunofluorescent staining more closely resembled the results of cross protection tests than did cross neutralization test results.

Lucio and Hitchner (1970) confirmed the finding of Lukert (1969) but they were working with infected tissues rather than tissue culture cells. They found specific gravity in the larynx, trachea and lungs but failed to find it in any other tissue. Specific activity persisted at least eight days with Gray, JMK, Iowa 97 and Iowa 609 types, while Mass, Conn and Holte types persisted only five days. In their conjugation process they compared four different techniques and evaluated them.

Beaudette and Hudson (1937) were the first to cultivate IBV in chicken embryos on the chorioallantoic membranes. They observed that the first six passages were relatively nonlethal to embryos. An increase in virulence on further passage was noted, with death resulting in a majority of embryos. Gross lesions were dwarfing of the embryo, thinning of the chorioallantoic membrane and thickening of yolk material. Delaplane and Stuart (1941) confirmed these findings and in addition found white, opaque, circular lesions on chorioallantoic membranes at the point of inoculation. With successive passage in eggs, the virus increased in virulence for embryos, with a progressive decrease in time required to produce death of embryos. An increase in virulence for embryos was accompanied by a decrease in virulence for chicks.

Delaplane (1947) reported that the allantoic cavity route of inoculation was superior to chorioallantoic membrane route, for initial isolation of virus, as evidenced by dwarfing in first passage.

Fabricant (1949) confirmed findings of previous authors and, in addition, he described "curling" of embryos into a ball-like shape. This curling precedes dwarfing and occurs in infected embryos of normal size. Loomis et al. (1950) described the pathology of the chicken embryo infected with IBV. It included the presence of whitish foci of urates in the mesonephros. This observation was confirmed by Hitchner and White (1955) and Hofstad (1958b). These workers consider presence of urates of diagnostic importance in absence of other gross lesions.

Cunningham and El Dardiry (1948) reported on the distribution of IBV in chicken embryos inoculated via the allantoic sac. Highest concentrations of virus were found in chorioallantoic membrane, followed by

allantoic fluid, amniotic fluid and liver. Virus titer was greater in materials collected from living embryos compared to embryos dead at the same post inoculation interval. Fabricant (1951) stated that 24 hours after inoculation, titer of virus in allantoic fluid was at a maximum and that it decreases progressively after 36 and 48 hours.

IBV multiplies primarily in the respiratory tract (Hofstad and Yoder, 1966). Fabricant and Levine (1951) reported the presence of virus in eggs during active infection and for 36 days thereafter. Winterfield and Hitchner (1962) isolated IBV from kidneys. Hofstad and Yoder (1966) carried out an examination for the distribution of IBV in chicken tissues following experimental exposure to the virus. They recovered the virus from the trachea, lung, kidney, bursa of Fabricius, air sac, blood, spleen, pancreas and liver. The primary difference between IBV at different embryo passage levels was its gradual loss of invasiveness for nonrespiratory tissues at the higher passage levels. After 85 embryo passages there was apparently no multiplication of the virus in nonrespiratory tissue and even some loss of invasiveness for the tracheal and lung epithelium. No apparent differences were noted between the serotypes of IBV using the criterion of viral titration by embryo inoculation. The concentration of virus was greatest in the trachea, lung and air sacs, with lesser amounts in other tissues. Virus was present 24 hours post exposure and was found rather consistently through the 8th day. From the 9th to the 14th day virus was only occasionally detected. They noted that the virus tended to persist in the bursa of Fabricius.

Doherty (1967) also reported on the occurrence of IBV in tissues following experimental exposure. He recovered the virus from the trachea,

lung, heart, kidney, spleen and liver. However, he only once recovered it from the blood. This is in agreement with Hofstad and Yoder (1966) who also had difficulty in recovering the virus from the blood.

Cook (1971) reported IBV (Mass serotype) could be recovered from eggs laid by hens for up to six weeks after inoculation of IBV, and from a small number of day-old chickens hatched from such eggs. The virus was also recovered from semen collected from roosters up to two weeks after inoculation. The chickens which hatched showed no signs of infectious bronchitis and were susceptible to challenge with homologous virus at six weeks of age.

Winterfield and Fadly (1971) evaluated the criteria for examining the immune response to IBV. They found that immunofluorescent findings did not always correlate with clinical signs and virus titration results in embryos. Occasionally fluorescence, in varying degrees, was observed in tracheal mucosal cells, which they suggest may have been due to vaccine application at a prior date. No signs were seen and isolation attempts were negative.

Surface (1912) described the histology of the oviduct of the domestic hen. The oviduct consisted of five segments in the adult bird, namely, infundibulum, magnum, isthmus, uterus and vagina. Two muscular layers, an outer longitudinal and an inner circular could 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 oviduct except the anterior portion of the infundibulum, 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, and 3) in all parts of the oviduct between the infundibulum 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 line of demarcation between the magnum and isthmus was characterized by the absence of these tubular glands. 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. (1967) 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.

Gordeuk and Bressler (1950) referred to poor egg production and in addition mentioned poor egg quality, both external and internal, following outbreaks of IB. They inoculated laying hens intranasally and intratracheally with virulent IBV. In a ten week period post inoculation the rate of lay in the treated group was 31 percent while it was 55 percent in the control group. Only 52 percent 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 IB. 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 IB.

Broadfoot and Smith (1954) investigated field outbreaks of IB in southern Minnesota and northern and central Iowa. Their findings concurred with earlier work but also showed that IB will cause a statistically significant decrease in hatchability and an increase in percentage of unsettable eggs.

Broadfoot *et al.* (1954) reported on field cases of IB in young chickens. A total of 14,000 pullets were involved during the 5 to 7 days of age. They suffered severe respiratory symptoms with a mortality varying from 5 to 35 percent within flocks. Later at seven months when peak production was reached the birds were producing at less than 50 percent, when 70 to 80 percent 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 nonlayer 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 they had earlier reported from field trials. They divided 750 White Leghorn pullets into six groups and exposed them to virulent IBV (field strain) 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 percent egg production at seven months. This was lower than the expected peak for this type of chicken. Nonlayers were necropsied and 64 percent 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 infundibulum, 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 contained a clear fluid with concretions suspended in it.

Jones and Jordan (1970) using a Mass type IBV, strain 41, repeated the work of Broadfoot et al. (1956) and found nonpatent oviducts in adult chickens following day old exposure to the virus. They found a higher incidence of nonpatency compared to Broadfoot.

Crinion et al. (1971a) described the pathogenesis of oviduct lesions in immature chickens following exposure to Mass type IBV at one day of age. Virus titers 65 hours after inoculation were greatest in the respiratory tissues, with titers in the reproductive tissues being comparable to those in other organs, such as the kidney and bursa of Fabricius. Early changes consisted of mild lymphoid cell infiltrations in the oviduct wall and dilations of the rough-surfaced emdoplasmic reticulum of the epithelial cells. At the 22nd day post inoculation, microscopic evidence of oviduct hypoplasia became apparent. The lumen of the oviduct became progressively smaller and eventually became completely obliterated. Oviduct cysts were first observed on the 44th day post inoculation. Localized hypoplasia of the oviduct, accompanied by occlusion of the oviduct lumen and the development of cysts, occurred in 22 percent of the birds.

Crinion et al. (1971b) showed that exposure to IBV at one day of age resulted in permanent lesions and egg abnormalities in mature chickens. Twenty-two percent had focal nonpatent areas of hypoplasia involving the caudal magnum and cranial isthmus. These were of the same nature as those seen in the younger birds (Crinion et al., 1971a). Also, 21 percent of the chickens had hypoglandular areas in the same oviduct locations. Eggs from this latter group were significantly inferior in quality to those

from birds without such lesions. The reduction in egg quality was due to albumen and shell abnormalities.

Duff et al. (1971) placed day-old chickens in contact with other chickens which had just received H-52 IBV, a Dutch vaccine strain. Infection did not take place until the fifth day. When six weeks old, ten females were killed and the findings were similar to those of Jones and Jordan (1970) and Crinion et al. (1971a). They found three birds with cysts in the oviduct out of the ten killed. They also exposed chickens which were 12 weeks old and examined them six weeks later. The oviducts, in contrast to those from a noninfected group, were less uniform in their development, although individually they appeared normal.

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 eight 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 percent 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 IBV 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 percent 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 percent 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 six 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 percent of normal in some birds and 50 percent 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.

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. 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. Recovery was slow and no bird had normal height of epithelium at all levels of the oviduct prior to 21 days post exposure. Eighty percent 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.

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 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. Biswal and Morrill (1954) investigated the pathology of the reproductive tract of laying pullets affected with Newcastle disease.

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 percent of 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 revealed 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 disease. Each of these was discussed in detail. The disease conditions affecting egg quality have already been mentioned in the above literature review.

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 IB for many years and have been tested at regular intervals for evidence of IBV antibodies. On all occasions these tests have been negative. IBV 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 12 years no reactions to Salmonella pullorum, Salmonella typhimurium, or Mycoplasma gallicepticum have been found on testing.

The female chickens were held in isolation until ready for inoculation. Five groups of chickens were hatched at weekly intervals and the groups were exposed at the same time to the particular virus being used. This procedure was followed for each virus inoculation but only one virus inoculation experiment was carried out at any one time. All isolation facilities and cages were fumigated between each virus inoculation. Each group contained approximately 60 chickens.

IBV Serotypes

Four different serotypes of IBV were utilized in this experiment and one was used in two embryo passage levels. The serotypes and strains used were Mass serotype, strain 33, 7th embryo passage; Mass serotype, strain

33, 55th embryo passage; Aust T serotype, 11th embryo passage; Conn serotype, strain A5968, 9th embryo passage; and Iowa 609 serotype, 3rd embryo passage.

The isolates of IBV used represented widely different serological types. Strain 33 was originally isolated at New York State Veterinary College, Ithaca, New York in 1944 by Dr. M. S. Hofstad. It is a Mass serotype and both embryo passage levels were available at the Veterinary Medical Research Institute, Iowa State University, Ames, Iowa. The low passage level was used to simulate what would occur under field conditions. The 55th passage was administered to simulate live vaccines currently being used in many parts of the world.

Isolate A5968 is a Conn serotype virus. Dr. R. E. Luginbuhl isolated this strain in 1951 from a flock at the University of Connecticut.

Aust T serotype of virus was isolated in Australia by Dr. R. B. Cumming, University of New England, Armidale, N.S.W. It represents one of the nephrogenic types of IBV.

Iowa 609 serotype was isolated and antigenically characterized by Dr. M. S. Hofstad. This virus was recovered in 1952 from a mild respiratory disease in mature chickens at the Veterinary Medical Research Institute, Iowa State University, and is capable of producing a mild type of infection of short duration.

Experimental Procedure

The chickens were placed in modified Horsfall-Bauer isolation units. The virus was administered to the chickens as an aerosol by a hand-pumped

Peralta vaporizer[1]. The chickens were maintained in the isolation units for 15 minutes and were then removed to brooder batteries, where they remained until the conclusion of the experiment. Each bird received approximately 10,000 chicken infective doses of IBV. The chickens received no therapeutic treatment during any stage of the experiment. A tabular representation of viral serotypes inoculated, age of chickens at exposure and number of chickens in each group is shown in Table 1. Table 2 shows parameters measured in this experiment, tissue collection times post inoculation and the number of chicken replications for each time and parameter. Clinical signs were observed and mortality was recorded. Birds were killed by dislocation of the atlanto-occipital joint and all were examined macroscopically.

Table 1. A tabular representation of viral serotypes inoculated, age of chickens at exposure and number of chickens

Chicken age at exposure days	IBV serotypes				
	Mass 33[a] 7[b]	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
1	70[c]	70	75	70	70
8	60	60	60	60	60
15	60	60	60	60	60
22	60	60	60	60	60
29	60	60	60	60	60

[a]Viral strains.

[b]Chicken embryo passage level.

[c]Number of chickens exposed.

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

Table 2. A tabular representation of parameters measured in this experiment, tissue collection times post inoculation and the number of chickens under observation for each evaluation

Parameters evaluated	Tissue collection times (days) post inoculation				
	3	6	10	20	30
Clinical signs	60[a]				
Mortality	60[b]				
Histopathology	3	3	3	3[c]	5
Viral titration	4	4	4	0	0
Immunofluorescence	3	3	3	0	0
Macroscopic pathology	10[d]	10	10	3	5[e]

[a]All chickens remaining alive, this number declined depending on mortality and time post inoculation.

[b]This number declined with increasing time post inoculation.

[c]Tissues were collected only from chickens exposed to Mass 33, 7th passage and Aust T, 11th passage at this time.

[d]This number represents all chickens necropsied; these tissues were also utilized for one of the other parameters.

[e]Minimum number, all remaining chickens were necropsied at termination of experiment.

Tissues for histopathological examination were collected 3, 6 and 10 days post inoculation and again approximately 30 days post inoculation for chronic lesions. Three birds were killed at each time except at the terminal day when all remaining birds were killed. Additional tissues were collected 20 days post inoculation from the low embryo passage Mass serotype and the Aust T. Table 3 shows a schematic diagram of all tissues collected and stained sections examined. Control tissues were also collected for comparison from uninfected chickens of the same age as the infected groups.

Table 3. Schematic representation of tissues collected and sections stained for histopathological examination

IBV serotypes				
Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
Chicken age at exposure (days)				
1	8	15	22	29
Tissue collection times, post inoculation (days)				
3	6	10	20	30
Number of chickens killed				
3[a]				
Number of equally spaced transverse sections of oviduct examined				
4				

[a]Replications.

The tissues were placed in Carnoy's fluid within five minutes after killing and after 18 hours the tissues were transferred to storage in 70 percent ethyl alcohol. Four representative samples from each oviduct were embedded in Paraplast[1] tissue embedding media. The tissues were sectioned

[1]Scientific Products, Chicago, Illinois.

at 6 μm and mounted on glass slides with Permount[1] mounting medium. Sections were stained with Harris' hematoxylin and counterstained with eosin Y as described in the U.S. Armed Forces Institute of Pathology, Manual of Histological and Special Staining Technics (1969). Four sections were made from each oviduct. The anterior three sections always included the kidney with the oviduct. The kidney was used as a base on which the oviduct lay. The fourth section, or posterior section, included a portion of the bursa of Fabricius which was used as a base for the oviduct when sectioning.

Viral Titration Procedures

Trachea, oviduct and kidney tissues were collected from each chicken. Collections were made 3, 6 and 10 days post inoculation. Tissues from four chickens were harvested at each sampling. These were immediately placed in two pools. One pool was washed in sterile saline solution while the other was not washed. This procedure was performed to consider the question of surface contamination of the oviduct with virus from the air sac membrane. The tissues were ground in a mortar with pestle and alundum and suspended in tryptose phosphate broth[2]. Samples were frozen at -20° C until chicken embryos were available for inoculation.

Ten or 11 day embryonating eggs from the supply flock of the Veterinary Medical Research Institute were used for titration of virus in the tissues. The tissues were diluted in tryptose phosphate broth and 0.1 ml of dilution was inoculated into the allantoic sac. Five eggs were

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

[2]Difco Laboratories, Detroit, Michigan.

inoculated per dilution. Titration end points were based primarily on mortality and typical stunting of the embryos and were calculated mathematically from the formula of Reed and Muench (1938).

Antisera Preparation

The antigen used to immunize the chickens was prepared by inoculating $1x10^3$ embryo infective doses (EID_{50}) into 11-day-old chicken embryos. Twenty-four hours post inoculation the amnioallantoic fluid (AAF) and the chorioallantoic membranes (CAM) were harvested. The CAM was handled and stored in the same way as the tissue samples collected from chickens which will be detailed later. The AAF was stored at -20° C.

Roosters were inoculated intramuscularly with 2 ml of AAF in all cases except for Mass serotype where the roosters were inoculated by tracheal swabbing. Two weeks later all roosters received 1.5 ml each of AAF intramuscularly including the group which was formerly exposed by tracheal swabbing. Three weeks following this inoculation sera was harvested and stored at -20° C until ready to fractionate. A virus neutralization test as detailed in the Methods for the Examination of Poultry Biologics (1963) was performed on the sera of each serotype.

Conjugation Reagents

The following reagents were used in the conjugation of fluorescein isothiocyanate to immunoglobulins:

1) Saturated ammonium sulfate solution. Enough $(NH_4)_2SO_4$ was added to deionized water at room temperature to obtain a saturated solution. The solution was stored at 4° C.

2) Saturated solution of $BaCl_2$ in deionized water.

3) 0.5 M carbonate-bicarbonate buffer. This buffer was prepared by mixing 50 volumes of 0.5 M $NaHCO_3$ and 4.5 volumes of 0.5 M Na_2CO_3 so that a pH 9 was reached.

4) pH 7.7 phosphate buffered saline (PBS). The buffer consisted of 91.5 ml 0.1 M $Na_2HPO_4 \cdot 7H_2O$, 8.5 ml 0.1 ml NaH_2PO_4, 8.5 g NaCl and sufficient distilled water to make a total of one liter.

5) Physiological saline. Distilled water was added to 8.5 g NaCl to make one liter.

Serum Fractionation

Globulins were obtained from hyperimmune chicken serum by dropwise addition of one volume of a saturated solution of ammonium sulfate at 4° C to two volumes of serum at 4° C. The serum was constantly agitated with a magnetic stirrer during the addition of the ammonium sulfate and the mixture was continually agitated for 12 hours at 4° C. Globulins were then sedimented by centrifugation for 15 minutes at 2000 x g. The supernatant fluid was carefully decanted from the sedimented globulins and the precipitate restored to the original serum volume by adding cold physiological saline solution. This fractionation procedure was repeated two additional times but on the last time the precipitate was only restored to one-half the original serum volume.

The redissolved globulins were then freed of residual ammonium sulfate by dialysis against physiological saline. Dialysis tubing[1], average

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[1]Arthur H. Thomas Co., Philadelphia, Pennsylvania.

pore diameter of 4.8 nm, was used for the dialyzing process. To increase the agitation of the fluid outside the dialysis tubing, a magnetic stirrer was used. The sample was dialyzed for 24 hours at 4° C during which time the saline was changed three times. Insoluble material present in the globulin solution after the dialysis was removed by centrifugation for 15 minutes at 2000 x g. The presence of sulfate ions was checked by mixing equal volumes of the dialyzing saline and saturated barium chloride. A precipitate will form in the presence of sulfate ions.

The concentration of protein contained in the globulin fraction was determined by the Lowry modification of the Folin-Ciocalteu test (Lowry et al., 1951), utilizing a Gilford spectrophotometer 2400[1], reading at a wave length of 750 nm.

Conjugation of Globulin

The protein concentration of the globulin solution was adjusted to 10 mg/ml by either dilution with physiological saline or pervaporation to increase concentration. Fluorescein isothiocyanate[2] was dissolved in a volume of carbonate-bicarbonate buffer at pH 9, which represented 10 percent of the volume of the globulin. The dissolved fluorescein isothiocyanate was then added to the globulin. The final solution contained 0.02 mg fluorescein isothiocyanate (FITC) per 1.0 mg protein (1:50). The mixture was placed at 4° C overnight with constant agitation.

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[1]Gilford Instrument Laboratories Incorporated, Oberlin, Ohio.

[2]Baltimore Biological Laboratories, Cockeysville, Maryland.

Unconjugated fluorescein isothiocyanate was removed by dialysis of the conjugate against phosphate buffered (0.16 M) pH 7.7 saline. The dialysis was continued until the dialysate showed no fluorescence with a portable Wood's lamp. Dowex[1] (Dowex 2-x, 20 to 50 mesh anion exchange resin in the chloride form) was added to the buffer to reduce the time required for dialysis. Approximately 10 g of Dowex was added to the buffer in the beaker. This resin absorbs the free fluorescein and fluorescein derivatives as they pass through the membrane, thus providing a continuous maximal gradient for optimal dialysis. The last 12 hours of dialysis was against a Tris buffer of 0.15 M and pH 8.7. This buffer was used in the chromatography procedure. Insoluble material present in the globulin solution after the dialysis was removed by centrifugation for 15 minutes at 2000 x g.

Chromatography

Fifteen grams of diethylaminoethyl cellulose[2] (DEAE cellulose), type 20, was dispersed in distilled water in a two-liter beaker. Ten minutes settling time was allowed and the supernatant was discarded. This procedure reduced the "fines" and was repeated again. "Fines" refer to shortened or broken cellulose fibers. Activation was initiated by stirring the DEAE cellulose into one liter of 0.5 N HCl. After standing for 30 minutes, the supernatant was decanted and the sediment placed in a Buchner funnel. The DEAE cellulose was washed with distilled water using a Buchner

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[1]Bio-Rad Laboratories, Richmond, California.

[2]Schleicher and Schuell Incorporated, Keene, New Hampshire.

funnel on a side arm flask until a pH reading of 4 was reached. The DEAE cellulose was replaced in the flask and one liter of 0.5 N NaOH was added. It was allowed to stand for 30 minutes and the supernatant fluid was decanted. The slurry was washed with distilled water in the Buchner funnel for five minutes. Seventy percent ethyl alcohol was added to the slurry for 10 minutes to reduce the time required to lower the pH and to further aid in reducing "fines." Water washing of the slurry continued until the filtered effluent was nearly neutral. Equilibration buffer (Tris buffered saline at pH 8.7) was added to the DEAE cellulose and was stored at 4° C. This buffer contained 0.14 M NaCl and 0.01 M Tris (Hydroxymethyl) amino methane[1] and was adjusted with HCl to pH 8.7. Prior to packing of the column the buffer wad changed twice over a 12-hour period and allowed to stand at room temperature. The DEAE cellulose was degassed by stirring the slurry in a stoppered Buchner flask connected to a water pump until no bubbles were observed. The DEAE cellulose was packed in 2.5 x 20.0 cm columns and washed with several column volumes of the buffer.

The protein-FITC conjugates were pumped onto the columns. The elution was judged by a LKB Uvicord II ultraviolet absorptiometer detector unit, type 8303A[2], and a permanent graph was plotted by a LKB six channel recorder. The eluates were collected in test tubes as they emerged from the absorptiometer and were stored at -20° C until tests for potency were performed.

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[1]Sigma Chemical Company, St. Louis, Missouri.

[2]LKB, Uppsala, Sweden.

Specimens for Immunofluorescent Study

Specimens for immunofluorescence were collected from chickens 3, 6 and 10 days post inoculation. The oviducts were removed with a minimum of contact with adjoining tissues. Three chickens were killed at each harvest time. The oviducts were divided into three equal lengthwise segments.

Table 4 shows a schematic diagram of all tissues collected for immunofluorescent examination and number of replications. The oviducts were placed in OTC compound[1] in small plastic cups cut from serological plates[2] and frozen in previously prepared depressions in solid carbon dioxide. The frozen specimens were wrapped in aluminum foil and stored at -70° C in screw cap bottles. The oviduct sections for use in the immunofluorescence test were cut at either 6 or 8 µm with a model CTI International Cryostat[3]. Two consecutive sections from each tissue were placed 3 cm apart on a glass slide and allowed to air dry. The specimens were fixed for 60 minutes in acetone at -20° C and stored at -20° C until ready for staining. Control specimens were collected from noninfected chickens and harvested in the same way. Chorioallantoic membranes were also harvested from IBV infected embryonating eggs.

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[1]Ames Company, Elkhart, Indiana.

[2]Model 96-SC, Linbro Chemical Company, Inc., New Haven, Connecticut.

[3]International Equipment Company, Needham Heights, Massachusetts.

Table 4. Schematic representation of tissues collected for immunofluorescent examination

IBV serotypes				
Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
Chicken age at exposure (days)				
1 8 15 22 29				
Tissue collection times, post inoculation (days)				
3	6		10	
Number of chickens killed				
3[a]				
Portions of the oviduct collected				
Anterior	Middle	Posterior		
Number of tissue sections examined				
3[a]				

[a]Replications.

Titration of Conjugates

The infected chorioallantoic membranes were used as standards for testing the conjugates. Two-fold dilutions of the conjugates were made in PBS and each dilution was tested with the homologous serotype of IBV only.

The intensity of fluorescence was rated from 1+ through 4+. The penultimate dilution giving a 4+ reaction was used for examining the specimens. If the undiluted conjugate did not give a 4+ reaction, pervaporation was performed on the conjugate using dialysis tubing to obtain a concentration which when diluted two-fold gave a 4+ reaction (penultimate dilution).

Preparation of specimens

One drop of a protein-FITC conjugate was placed on each specimen and spread evenly over an area previously outlined with the Mark-tex pen point[1]. The slides were placed in a slide box which was placed standing in a beaker in which there was water one cm deep to maintain a moist atmosphere. The specimens were incubated at 37° C for 30 minutes with the conjugate and the conjugate was then poured off the slides. The slides were washed in PBS for 10 minutes and followed by a 10-minute washing in distilled water. The mounting medium consisted of nine parts glycerine and one part PBS. Cover slips were mounted on the slides after applying a small drop of mounting medium to the slide.

Microscopy

Fluorescence was observed through a binocular Leitz Ortholux microscope[2] equipped with a dark field condensor lens and an Osrum HBO 200 mercury vapor lamp. An exciting filter, UG1, a heat absorption filter, BG38, and a yellow barrier filter, K430, were employed throughout this

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[1]Mark-tex Corporation, Englewood, New Jersey.

[2]Ernst Leitz, Wetzlar, Germany.

work. A Leitz Mikas micro-attachment with a Ml camera attachment was employed for photography. Photomicrographs were taken on high speed Anscochrome 200 color transparency film[1].

Controls

The conjugates were tested for specificity and potency against frozen sections of chorioallantoic membranes which were infected with IBV and others which were not infected. Only homologous serotypes of IBV were used. For this test, and for all other staining, the penultimate dilution of conjugate was used.

Oviduct tissues from noninfected chickens were utilized for checking the presence of autofluorescence and nonspecific staining. Finally, the specificity of the conjugates was tested by absorption of the fluorescent staining in both infected chorioallantoic membranes and infected tissues. The conjugates were incubated with homogenized chorioallantoic membranes infected with the homologous serotypes or with uninoculated chorioallantoic membranes.

The chorioallantoic membranes were prepared by homogenization in an Omni-mixer[2] and centrifuged at 1000 x g for 10 minutes. The supernatant fluid was centrifuged at 65,000 x g for 90 minutes at 4° C. The resulting pellet was resuspended in the conjugate when ready to stain. Specimens were incubated for 30 minutes at 37° C and then centrifuged at 2000 x g

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[1]GAF Corporation, 140 West 51 Street, New York, New York.

[2]Ivan Sorvall Incorporated, Norwalk, Connecticut.

for 15 minutes. This centrifugation procedure eliminated course material. The supernatant fluid was used as the immunofluorescent stain.

Four replicate sections were taken from each specimen block. Three were stained with conjugate which had been incubated with noninfected chorioallantoic membrane while the remaining replicate was stained with conjugate which had been incubated with infected chorioallantoic membranes. The slide specimens treated with infected chorioallantoic membranes served as controls.

RESULTS

The results will be presented by evaluating one parameter of the experiment at a time in this section. The interrelationships between parameters will be examined and discussed in the next section.

Clinical Signs

There was wide variation in severity of clinical signs among chickens exposed to different serotypes and at different ages of inoculation. There was no variation in the character of clinical signs between the serotypes.

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 observed. 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 Mass serotype, strain 33, 7th passage produced the most severe clinical signs, followed in order by Aust T; Mass 33, 55th passage; Conn A5968 and Iowa 609.

The five age groups may be reduced to two main categories with regard to clinical signs. The one-day-old chickens had the severest clinical signs with a high proportion of the birds being involved. The remaining four older groups showed a wide variation in severity of clinical signs within groups with some birds appearing normal. Table 5 shows the number of days the clinical signs persisted in each group following inoculation.

Table 5. Persistence of clinical signs in each group of chickens following inoculation with IBV by aerosol

Chicken age at exposure days	IBV serotypes				
	Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
1	14[a]	13	13	10	4
8	14	10	13	10	4
15	14	10	13	10	4
22	10	10	10	10	4
29	10	10	10	10	4

[a]Days.

Table 6 shows the mortality in each group. The group size was 60 chickens, except in groups which were exposed at one day of age, in which there were 70 chickens.

Table 6. Mortality numbers in each group of chickens following inoculation with IBV

Chicken age at exposure days	IBV serotypes				
	Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
1	27[a]	22	36	7	0
8	2[b]	2	2	0	0
15	4	1	4	0	0
22	3	1	0	0	0
29	0	1	2	0	0

[a]Mortality in group of 70 chickens exposed.

[b]Mortality in group of 60 chickens exposed.

Each virus serotype produced a different mortality pattern. This is not evident from Table 6 because Table 6 only contains total mortality figures and makes no reference to when the chickens died. In chickens exposed to both embryo passage levels of Mass and Aust T serotypes a biphasic pattern of mortality was observed. The first phase was 2 to 3 days post incculation while the second phase occurred 7 to 11 days post inoculation. The Mass serotypes had the greatest mortalities during the first phase while mortality from the Aust T virus was greatest during the second phase. Conn serotype caused mortality only during the 7-11 day period while there was no mortality following exposure to Iowa 609 serotype. Tissues from chickens which died during the experiment were not used for any other parameter being evaluated.

The severity of clinical signs following exposure to the different serotypes is in agreement with the finding of other workers (Hofstad, 1945; Hofstad, 1958a; Cumming, 1963). The clinical signs following inoculations with Mass, Conn and Iowa 609 serotypes consisted of difficult breathing, tracheal rales and coughing. There was an initial respiratory embrassement with the Aust serotype and the chickens were depressed for a longer period than with the other serotypes. Iowa 609 produced a very mild respiratory disease. There was even difficulty ascertaining whether all the groups were infected. A mild respiratory embrassement could be heard only at night when there was relative quiet in the isolation units. The 55th embryo passage of the Mass serotype produced a severe infection, especially in the younger chickens, but the infection was milder in all age groups than the 7th embryo passage of the same serotype.

There was variation in the mortality between the groups exposed to different serotypes and at different ages. The mortality was much greater in the chickens exposed at one day of age compared to other age groups. It is unlikely that chickens with parental antibodies against IBV would suffer such high mortality. The vast majority of all chickens in this country will carry some antibodies against IBV after hatching since there is widespread usage of live vaccine in the United States and elsewhere in the world.

Lesions in the Oviduct

It was possible to formulate a gradient of histological changes in the oviduct based on previous work in this area (Crinion, 1970). These were given numerical values which were used in keeping records and subsequent analysis.

The viral serotypes varied greatly in their ability to produce pathological changes in the oviduct. Two serotypes, Conn and Iowa 609, failed to produce any pathological change in the oviduct. The 7th embryo passage level of Mass produced the greatest number of changes followed by Aust T and 55th embryo passage Mass in that order. The character of the lesions was the same in all cases. The lesions were found in the same time sequence for each virus. Photomicrographs of control oviducts are shown in Figures 1, 2, and 3.

The macroscopic and microscopic lesions following exposure to the 7th and 55th embryo passages of the Mass and the Aust T serotypes were as follows. Lymphoid cell infiltration was seen on the third day post inoculation in the oviduct. The cells were scattered in the submucosa but tended

Figure 1. Transverse section of the oviduct from a four-day-old noninfected control chicken. Hematoxylin and eosin. X 130

Figure 2. Transverse section of the oviduct from an 18-day-old noninfected control chicken. Hematoxylin and eosin. X 150

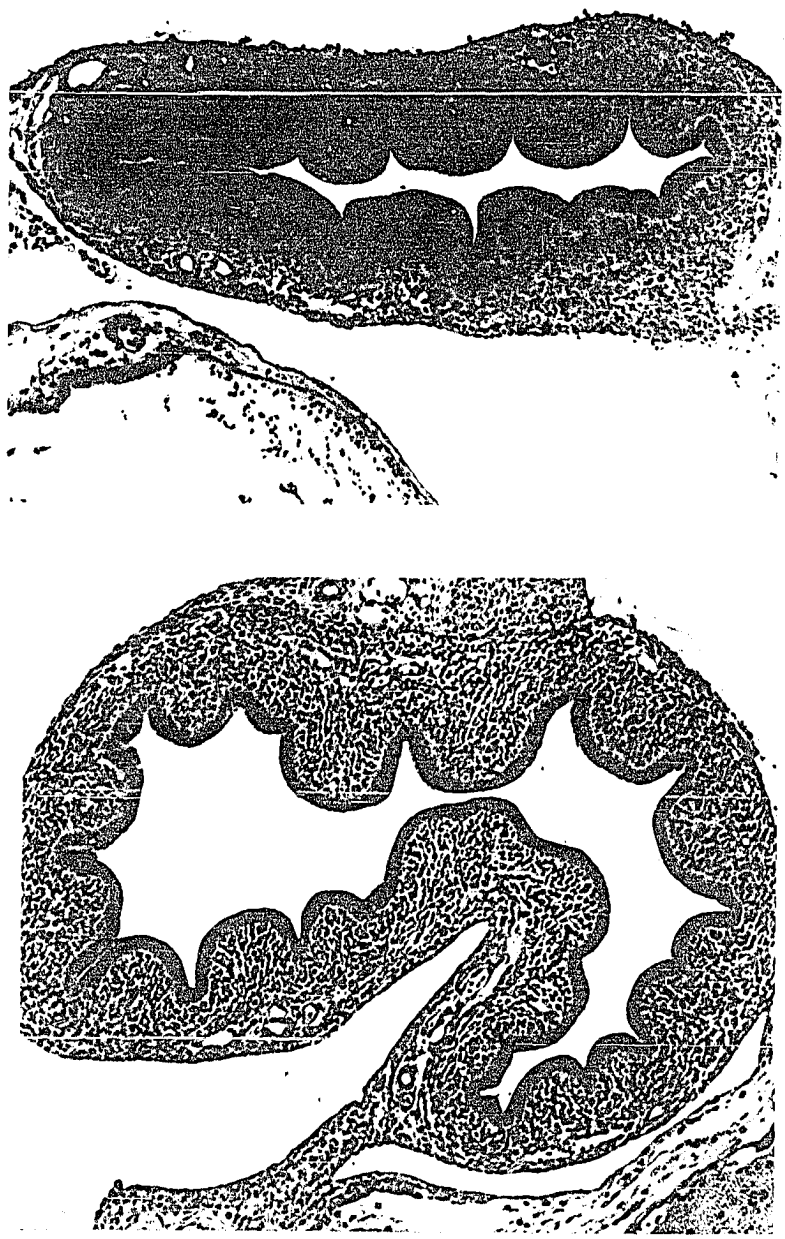
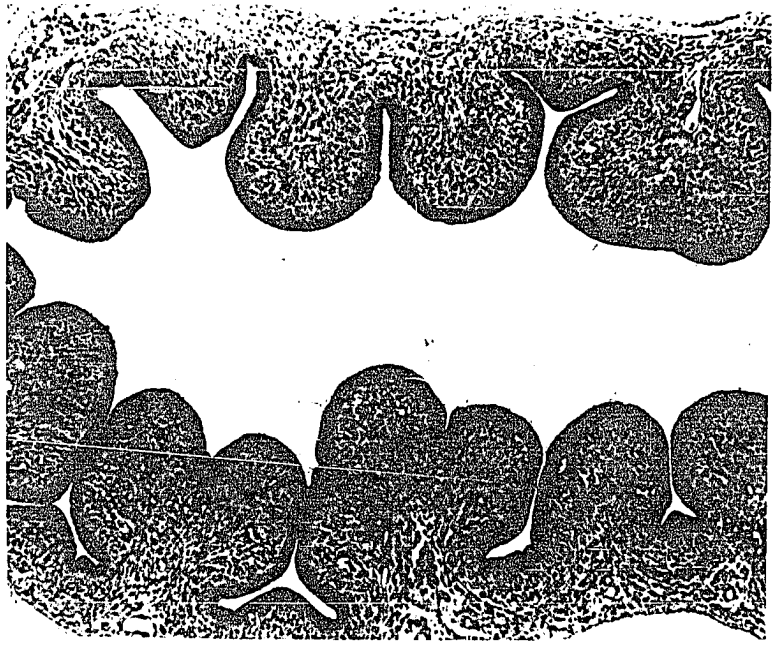


Figure 3. Transverse section of the oviduct from a 69-day-old noninfected control chicken. Hematoxylin and eosin. X 130

Figure 4. Transverse section of the oviduct from a 32-day-old chicken, previously exposed to Mass 33, 7th passage IBV at 22 days of age. Lymphoid cell infiltration is present in the wall together with lymphoid nodules (arrows). Hematoxylin and eosin. X 130

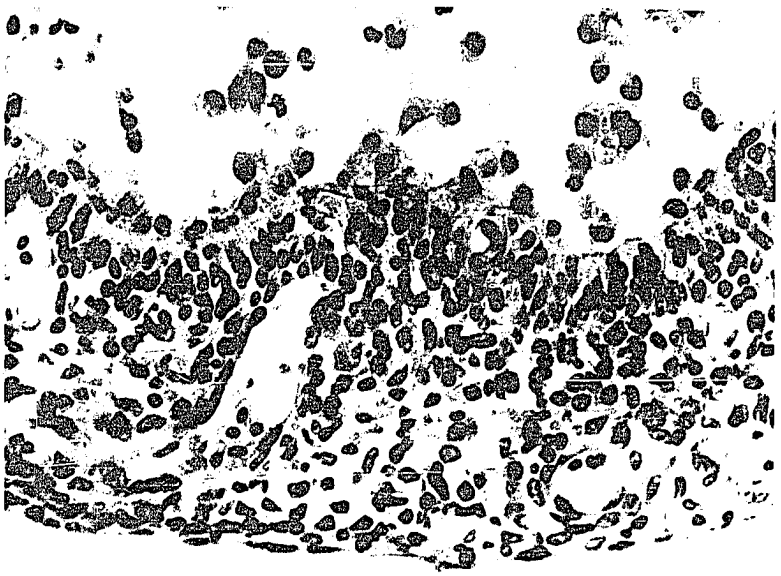


to occur in higher concentrations near blood vessels (Figure 4). Six days post inoculation there was evidence of severe epithelial damage to the oviduct. The lining epithelial cells were sloughed away from the basement membrane and their necrotic remains were visible in the oviduct lumen (Figure 5). Also the cellular infiltration became greater and there was edema in the oviduct wall (Figure 6). Ten days post inoculation lymphoid nodules were found in the oviduct wall (Figure 5). These were located near blood vessels, in similar locations to where lymphoid cell infiltration was first seen. At this time the first oviduct with total loss of lumen was observed (Figure 7). The lining epithelial cells were sloughed away and the lining of the oviduct had coalesced. This occurred in localized areas of the oviduct. Figure 8 shows a transverse section of oviduct caudally to the area of complete blockage. In this section the lumen is present but there is sloughing of epithelial cells into the lumen.

On the 20th and 30th days post inoculation cysts were found in the oviduct (Figure 9). Their incidences are shown in Table 7. The oviducts appeared normal in their cranial portion. Approximately in their mid portions the oviducts came to an abrupt termination. The cysts commenced caudally to the nonpatency and continued caudally to the cloacal region. The cysts were within the oviduct lumen and were filled with a clear serous fluid. The cyst wall was transparent. The area between the apparent normal oviduct and the cyst was connected by a portion of the ligament of the oviduct. The oviduct at these ages still contained numerous lymphoid nodules as well as free lymphoid cells. In the cystic oviducts the anterior intact portion of the oviduct appeared similar microscopically

Figure 5. Transverse section of the oviduct from a 7-day-old chicken, previously exposed to Aust T, 11th passage IBV at one day of age. The epithelial cells have been sloughed into the lumen where their necrotic remains are still evident. Hematoxylin and eosin. X 310

Figure 6. Transverse section of the oviduct from a 39-day-old chicken, previously exposed to Mass 33, 7th passage IBV at 29 days. The epithelium shows loss of staining ability and edema is present in the undifferentiated mesenchymal tissue of the oviduct wall. Hematoxylin and eosin. X 280



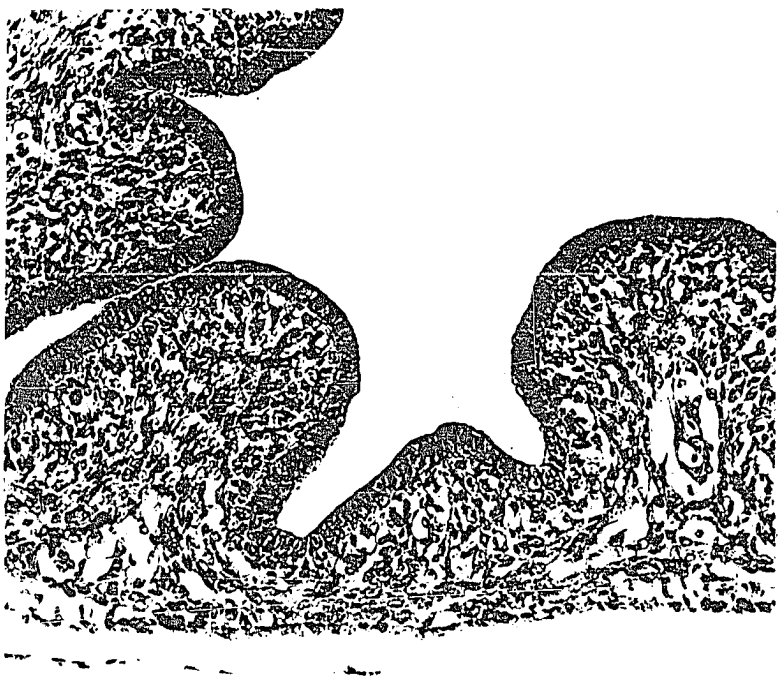
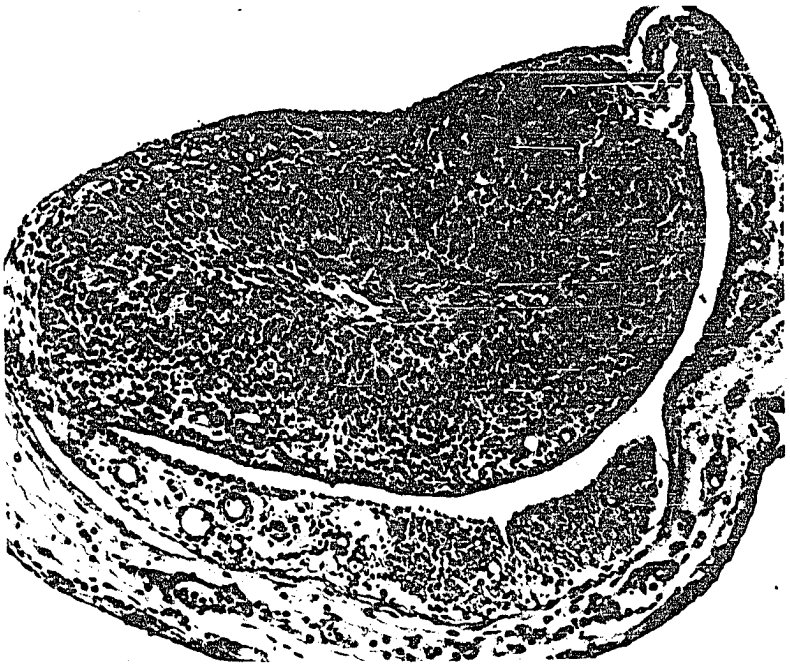


Figure 7. Transverse section of the oviduct from an 11-day-old chicken, previously exposed to Mass 33, 7th passage IBV at one day of age. There is a total loss of luminal epithelium. Hematoxylin and eosin. X 170

Figure 8. Transverse section of the oviduct from an 11-day-old chicken, previously exposed to Mass 33, 7th passage IBV at one day of age. This figure is from the same oviduct as Figure 7 but located 2 cm caudally. The entire epithelial lining has been sloughed but the lumen is still present (arrows). Hematoxylin and eosin. X 160



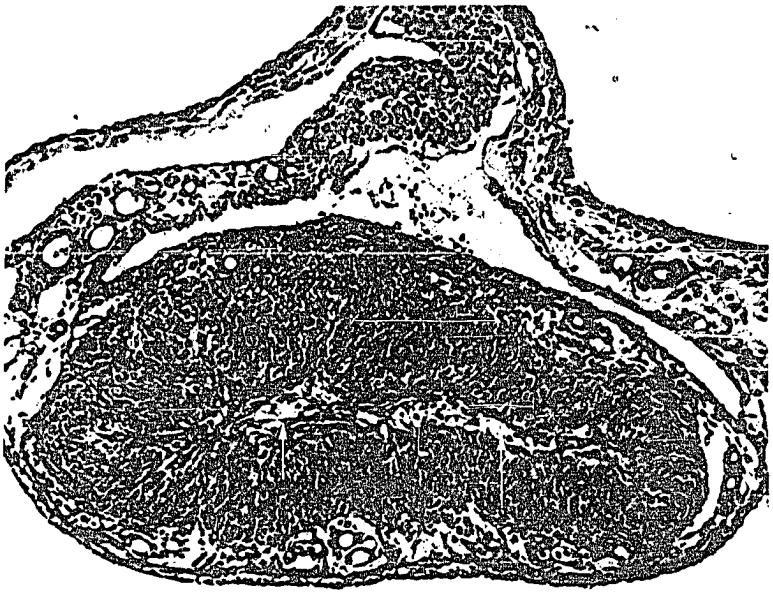
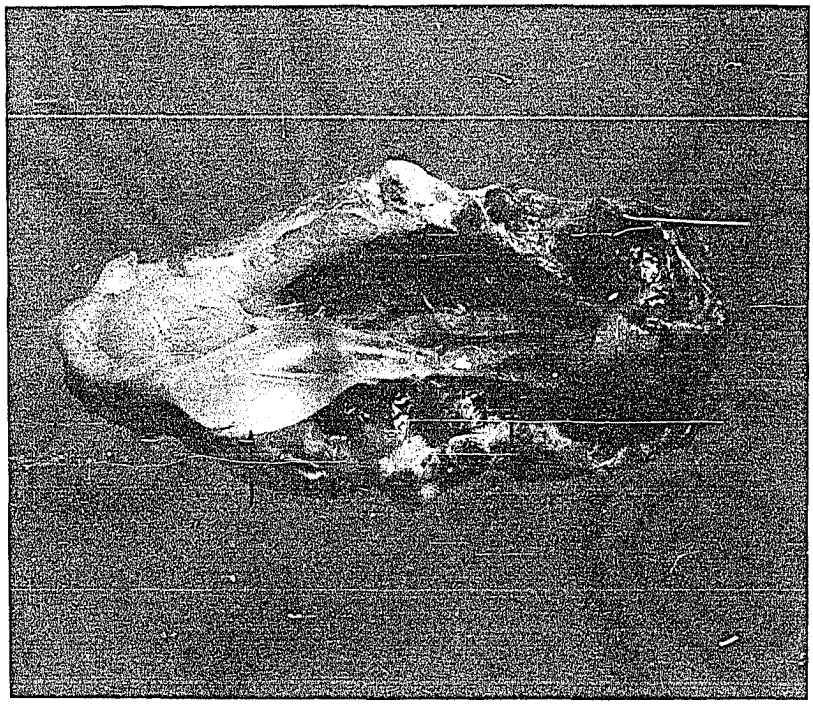
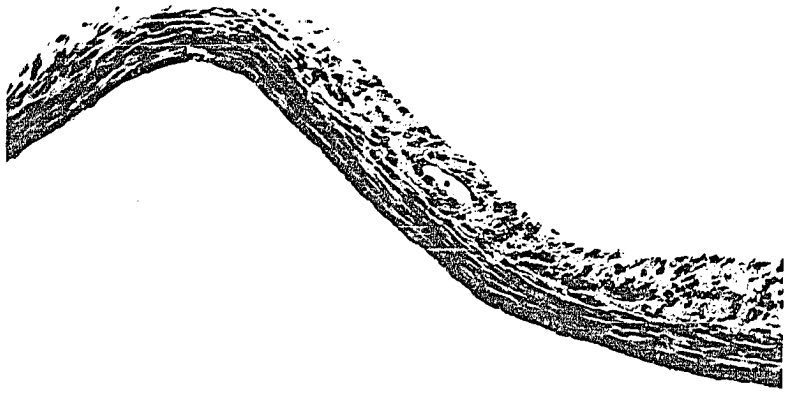


Figure 9. Reproductive organs of a chicken 38 days old, previously exposed to Mass 33, 7th passage IBV at eight days of age. A cyst (arrow) is located in the caudal one-half of the oviduct while the cranial portion of the oviduct (arrow) appears normal

Figure 10. Transverse section of cystic wall of an oviduct from a 22-day-old chicken, previously exposed to Mass 33, 7th passage IBV at one day old. The epithelial cells are intact but the width of the oviduct wall is reduced. Hematoxylin and eosin. X 240





to the oviducts of other exposed chickens. The cyst walls were thin but had an intact internal layer of epithelium (Figure 10).

Table 7. The incidence of macroscopic cysts in the oviduct of chickens following inoculation with IBV

Chicken age at exposure days	IBV serotypes				
	Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
1	1[a]	2	0	0	0
8	2	0	0	0	0
15	1	0	0	0	0
22	0	0	0	0	0
29	0	0	0	0	0

[a]Number of cysts in average of eight chickens examined over 20 days post inoculation.

Tables 8 and 9 show the incidence of histopathological lesions in the oviduct. The lesions were divided into two categories: 1) lesions involving the epithelial lining cells of the oviduct and 2) lesions characterized by infiltration and edema of the oviduct wall. The data given in Tables 8 and 9 do not signify the severity of the lesions. There were major differences in groups exposed at different ages as is seen in Tables 8 and 9.

The lesions which involve the epithelial cells will eventually lead to permanent damage to the oviduct and are thus more serious than the infiltrative type lesions. There was a positive correlation between infiltrative type lesions and epithelial cell damage among the different serotypes of IBV. However, the infiltrative type lesions occurred equally in chickens of all age groups while the younger birds showed a higher

Table 8. Histological lesions involving the epithelial lining cells of the oviduct lumen following inoculation with IBV

Chicken age at exposure days	IBV serotypes				
	Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
1	3[a]	2	3	0	0
8	2	0	1	0	0
15	2	0	0	0	0
22	1	0	0	0	0
29	0	0	0	0	0

[a]Number of chickens with lesions from 15 chickens.

Table 9. Histological lesions found in the oviduct, characterized by cellular infiltration and edema in the oviduct wall

Chicken age at exposure days	IBV serotypes				
	Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
1	5[a]	2	4	0	0
8	3	0	2	0	0
15	6	0	1	0	0
22	4	0	1	0	0
29	7	1	2	0	0

[a]Number of chickens with lesions from 15 chickens.

incidence of epithelial cell damage. The 7th passage of the Mass serotype was able to cause epithelial damage up to three weeks of age while the same virus at the 55th passage was only capable of causing damage in chickens inoculated at one day of age. The Aust T virus produced epithelial damage when chickens were inoculated at one day or eight days of age.

The incidence of macroscopic cysts appeared similar to the microscopic lesions involving the epithelial cells. However, no cysts were found in oviducts of chickens exposed to the Aust T serotype. It is most probable that the number of cysts found would have been greater had the birds been allowed to live longer. Crinion et al. (1971a) found cysts first at 44 days post inoculation using the same virus and source of chickens.

The pathogenesis of lesions produced in the oviduct were similar for chickens following either Mass or Aust T inoculation. The first observed reaction was the infiltration of lymphoid cells into the oviduct wall together with edema. This was closely followed by degeneration and sloughing of the lining epithelial cells of the oviduct into the lumen. The oviduct lumen became obliterated by the apposition of the oviduct walls and the organization of connective tissue. The oviduct lumen caudal to the blockage became a space with no exit. All secretions into it collected and a cyst developed. This procedure took only 20 days in some cases.

These results indicate that two serotypes are capable of producing severe lesions in the oviduct. The number of birds affected by such lesions depends on age of bird at inoculation and the embryo passage level of the virus. The younger the birds are exposed the greater the incidence

of lesions in the oviduct. This is in agreement with Broadfoot et al. (1956). The results of Mass 33, 7th passage on the oviduct agree with the finding of Jones and Jordan (1970), Crinion et al. (1971a) and Duff et al. (1971). The results of inoculation with Conn and Iowa 609 contrasted sharply with those of Mass and Aust T. There were no macroscopic or microscopic lesions found following inoculation with either Conn or Iowa 609 serotypes.

It is difficult to give percentages for any lesion since there was a continually changing population number. The experimental groups were designated primarily for pathogenesis studies and by the end of the experiment there were only a small number of birds still alive. This reduced considerably the chances of getting large numbers of any particular type lesion. The majority of the epithelial damage occurred in the mid section of the oviduct. The lesions found in this study were at earlier times post inoculation than in previous work (Crinion, 1971a). This is most probably due to larger sampling during the first 10 days post inoculation which increased the chances of finding a particular type lesion.

Finne and Vike (1951) reported cystic oviducts in chickens and 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 other 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 1 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 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.

The histological reaction during the acute stage of the disease in the oviduct wall 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) following IBV infection. However, this cell type was not prominent in this study. Sevoian and Levine also observed lesions in the epithelial cells. Thus, the virus has the same primary target cells (epithelial cells) in both day-old and sexually mature chickens.

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 mammalian embryo, the one-day-old avian oviduct is very close to an embryonic tissue and may possibly have some characteristics in common with those of intra-uterine embryos.

The immunological status of 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 these experiments. However, under field conditions there are flocks in isolated areas where parental antibodies are absent. In such flocks early exposure to infectious bronchitis virus would make the flock relatively unprofitable.

The 52nd embryo passage level of a Mass serotype virus is now being used for vaccine purposes in Great Britain. Duff et al. (1971) showed that the vaccine strain Mass "H" strain will cause cysts on the oviduct of chickens which are exposed at an early age. This experiment showed that a similar passage level is capable of causing epithelial damage only in chickens exposed prior to eight days of age. This loss of pathogenicity with increasing embryo passage level concurs with Hofstad and Yoder (1966), who stated that there was a gradual loss of invasiveness for nonrespiratory tissue as the embryo passage levels increased.

Viral Titrations

The viral titers found in the oviduct are presented in tabular form. Table 10 portrays the virus distribution in the oviduct following inoculations with the different serotypes and ages at inoculation. All serotypes of IBV were recovered from the oviduct but Iowa 609 was present only at low levels. Three days post inoculation the oviducts contained high titers of virus except following inoculation with Iowa 609. At six days post inoculation titer levels were similar to those seen at three days post inoculation. However, at 10 days post inoculation, the titers of Aust T had remained at a high level while titers of both Mass and Conn were considerably reduced, especially in the older age groups. There was an

Table 10. Titration of IBV in the oviduct following inoculation of chickens with IBV

Collection post inoculation days	Chicken age at exposure days	IBV serotypes				
		Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
3	1	4.9[a]	3.1	4.2	4.5	2.0
3	8	4.9[b]	3.5	2.9	4.3	0.3
3	15	4.1	3.2	2.8	3.7	0.0
3	22	4.2	3.0	4.1	3.3	0.0
3	29	4.0	3.4	4.0	4.5	0.3
6	1	4.5	4.3	4.0	4.5	1.1
6	8	3.9	3.1	4.9	3.7	0.8
6	15	2.8	3.1	4.9	3.6	0.8
6	22	4.5	4.2	4.3	3.8	0.0
6	29	3.8	4.4	4.1	4.2	0.0
10	1	3.7	3.6	4.0	1.6	0.0
10	8	1.8	1.6	2.8	2.2	0.0
10	15	1.0	0.9	2.4	0.6	0.3
10	22	0.4	1.8	1.1	1.1	0.0
10	29	0.0	0.0	3.5	0.0	1.7

[a] Log_{10} titer of virus per 0.1 ml tissue.

[b] Average in tissue suspensions from four chickens.

interesting relationship between the two embryo passage levels of Mass. At three days post inoculation the 7th embryo passage levels were consistently higher than the high embryo passage level, but on the following two collection times there was no significant difference between them. The levels of Iowa 609 were so low it was difficult to find any pattern.

Titers were initially high in all age groups after inoculation except for Iowa 609, but they persisted at higher levels in the younger birds at 10 days post inoculation. Both Mass embryo passage levels and Conn follow similar overall patterns but Aust T and Iowa 609 were different as Aust T

persisted in the oviduct while Iowa 609 titers were too low to ascertain any pattern.

The viral titers found in the trachea are shown in Table 11. Both the absolute levels and relative relationships are rather similar to titers found in the oviduct with some exceptions. In the trachea the Aust T titers drop at 10 days in older birds, similar to Mass and Conn, but titers persisted in the oviduct. While the titers found following Iowa 609 were the lowest, they were relatively higher in the trachea compared to the oviduct. Both embryo passage levels of Mass had similar titers.

Table 11. Titration of IBV in the trachea following inoculation of chickens with IBV

Collection post inoculation days	Chicken age at exposure days	IBV serotypes				
		Mass 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
3	1	3.9	4.1	4.9	4.0	2.9
3	8	4.1	2.6	3.8	4.1	0.0
3	15	4.9	3.7	4.0	2.8	0.0
3	22	3.0	3.3	3.7	3.6	3.3
3	29	2.6	4.2	3.7	4.6	3.0
6	1	4.4	3.7	3.4	3.9	1.1
6	8	4.9	4.1	4.0	3.0	1.3
6	15	3.6	1.4	4.1	2.5	0.3
6	22	4.0	3.6	4.1	2.2	1.5
6	29	4.3	3.5	3.9	0.9	1.5
10	1	3.8	3.9	4.4	0.9	0.4
10	8	0.8	1.5	3.5	0.0	0.0
10	15	0.0	1.6	2.0	0.0	0.6
10	22	0.0	1.1	0.7	0.0	0.0
10	29	0.0	0.6	0.4	0.0	0.0

IBV of each serotype was isolated from the kidney and the titers are shown in Table 12. The viral titers are lower than found in the previous two tissues except for Aust T which showed a marked increase. The Aust T serotype also persisted at higher levels in the kidney than in the trachea. The other serotypes, while having lower titers, had the same general relationship to one another as found in other tissues.

Table 12. Titration of IBV in the kidney following inoculation of chickens with IBV

Collection post inoculation days	Chicken age at exposure days	IBV serotypes				
		Mass[a] 33 7	Mass 33 55	Aust T 11	Conn A5968 9	Iowa 609 3
3	1		0.5	3.9	2.6	2.1
3	8		1.7	3.7	2.5	0.6
3	15		2.4	2.8	2.6	0.0
3	22		2.3	2.9	2.5	0.0
3	29		2.5	1.6	2.5	0.3
6	1		3.2	5.5	3.4	1.6
6	8		2.6	5.4	2.6	0.4
6	15		1.6	5.0	2.2	0.4
6	22		2.8	5.0	2.5	0.0
6	29		2.3	4.5	2.7	0.0
10	1		0.8	3.9	1.7	0.8
10	8		0.4	3.8	0.7	0.6
10	15		0.7	3.6	0.4	0.0
10	22		0.3	3.3	0.0	0.0
10	29		0.7	3.2	0.0	1.1

[a]Tissue not harvested.

The viral titration results indicate the presence of the virus in the oviduct. The titers found suggest that the virus is actually replicating in the oviduct rather than being passively present following a viremia or direct contact from the abdominal air sacs. All the serotypes showed the same affinity for the oviduct as for the trachea except Aust, in which

case the titers compared to the kidney titers. The reproductive lesions are not due, therefore, to enhanced tropism of any serotype of IBV for the oviduct tissue. The viral titers in the trachea and kidney are in agreement with the findings of Hofstad and Yoder (1966) and Doherty (1967).

The problem of viral contamination of the oviduct is difficult to evaluate. One-half of the tissues harvested were washed in saline to reduce surface contamination from the abdominal air sacs during removal of the oviduct if it were present. There was no difference in titers between washed and unwashed**; thus surface contamination was not present. Another type of error in titers concerns the inability of oviduct removal from young chickens without the simultaneous removal of air sac or ligament of the oviduct. If this were the situation the ligaments and/or the air sacs would have been titered with the oviduct and thus reduce the accuracy of the final titers. The results from the immunofluorescent studies detailed later in this chapter will help solve this problem. Viral antigen was localized in the ligament and air sac as determined by immunofluorescent staining in addition to the oviduct.

Immunofluorescent Staining

Fluorescent antibody staining was utilized to identify sites in the oviduct where viral antigen was present. IBV antigen was localized in the oviduct and adjoining ligaments and air sacs in this experiment. The specific fluorescence was evaluated within the range 0 through 4+. For comparisons all positive values were equally weighted. Each positive

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**$P \leq 0.01$. This notation will be followed throughout the text.

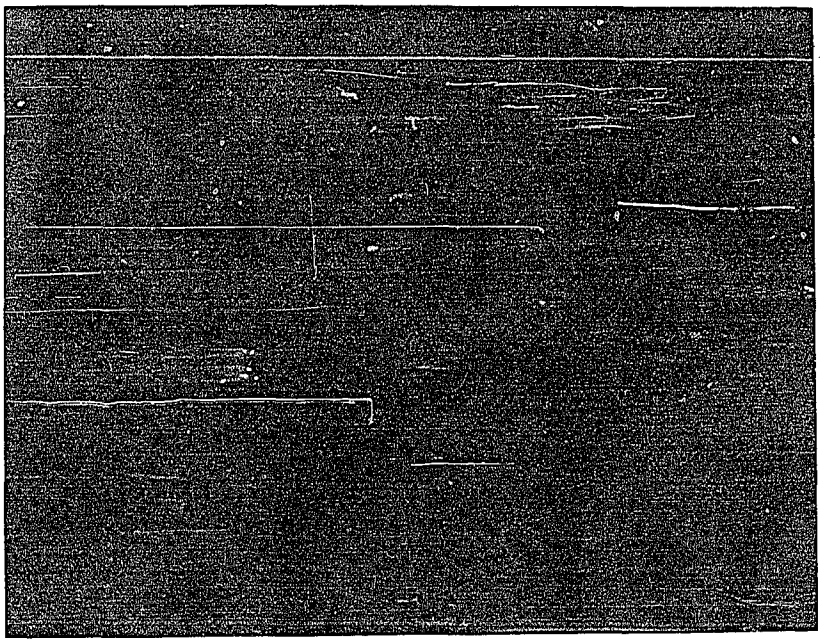
section represents localization of antigen in that particular tissue replication.

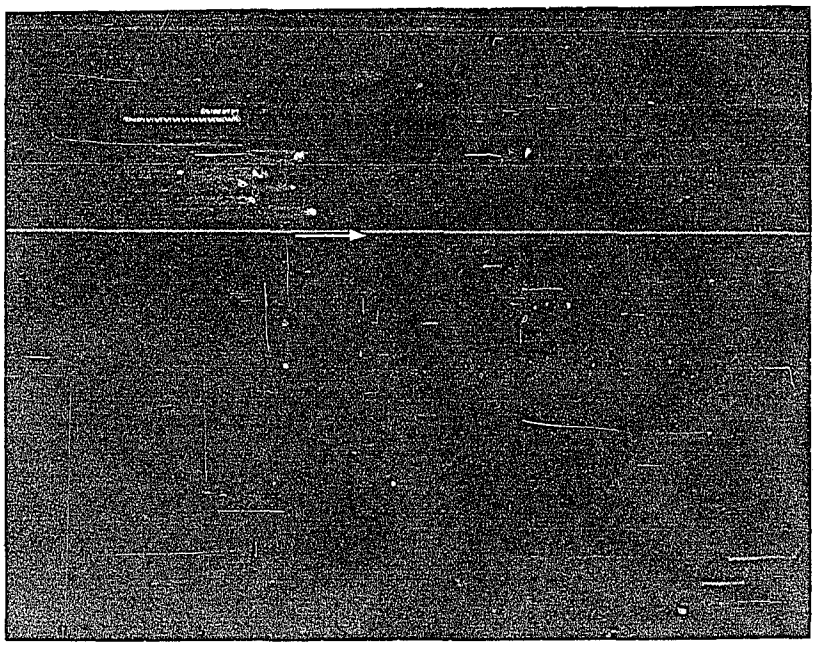
Serum neutralization tests showed that all the hyperimmune sera were capable of neutralizing the homologous viruses at least or at higher dilutions than 1/400 of sera in chicken embryos which received 100 embryo infective doses of IBV. The conjugates were tested on infected and noninfected chorioallantoic membranes prior to oviduct staining for specificity of staining. Homologous conjugates were used for each serotype of virus being localized.

The oviduct autofluoresced a blue color which did not interfere with the reading of specific activity which had an apple green color. Viral antigen was confined to the epithelial lining cells of the oviduct (Figures 11 and 12). There was also some viral antigen in the serosa of the oviduct but it was often difficult to ascertain whether this specific staining was from surrounding tissue or closely adhering air sac. For the comparison of viral antigen only that involving the lining epithelial cells was evaluated. No specific staining was ever seen in the undifferentiated cells beneath the epithelial lining cells. The number of cells showing specific staining characteristics varied greatly as is shown in Figures 11 and 12. In Figure 12 only a few isolated epithelial cells show viral antigen while in Figure 11 the entire luminal epithelial lining of the oviduct shows specific activity. The cellular localization of the antigen was entirely intracytoplasmic. The nuclei could be seen as dark staining bodies in the green staining cytoplasm. There was no difference in the character of cellular localization between the different viral serotypes and different ages at inoculation.

Figure 11. Fluorescent antibody staining of transverse sections of oviduct six days post inoculation from chickens exposed at one day of age to Mass 33, 55th passage IBV. The entire epithelial cellular lining of the oviduct shows specific activity. X 240

Figure 12. Fluorescent antibody staining of transverse sections of oviduct six days post inoculation from chickens exposed at one day of age to Mass 33, 55th passage IBV. Specific activity is localized in the cytoplasm of individual epithelial cells of the oviduct (arrows). X 240





Tables 13, 14, 15, 16 and 17 summarize all the collected data from the immunofluorescent staining. Each table represents the viral antigen localization following exposure to one serotype of virus. There was great variation in the incidence of specific staining in the oviduct following different serotypes and also among chickens receiving the same serotype but at different ages at inoculation. Iowa 609 antigen could not be localized in the oviduct in any age group of chickens, while Conn antigen was localized only on a few occasions. The Aust T antigen was found in all age groups as was also the Mass 33, 7th embryo passage level. However, Mass 33, 55th embryo passage level, was found only in the younger two groups of inoculation. In all age groups the Mass, 55th passage, showed much lower incidences compared to the same serotype of IBV but at lower passage level. This concurs with the finding of Hofstad and Yoder (1966) who reported a decrease in invasiveness of virus in nonrespiratory tissue with increasing embryo passage levels. Chickens inoculated at an older age showed a lower incidence of viral antigen compared to younger chickens. Viral antigen was observed in the three segments of the oviduct. The numerical order of total viral antigenic localizations was Mass, 7th embryo passage; Aust T; Mass, 55th passage; and Conn, respectively. The number of specific areas of fluorescence was twice as great with Mass 33, 7th passage, compared to Aust T. The pattern of localization within the oviduct differed with the serotype of IBV but not with the age of chicken at inoculation. Both embryo passage levels of Mass showed no selective pattern of localization in any particular segment of the oviduct. Viral antigen of Aust T was found in higher amounts in the anterior and posterior

Table 13. The IBV antigen localizations in the oviduct and surrounding tissue using immunofluorescent staining following inoculation of chickens with Mass 33, 7th embryo passage

Collection post inoculation days	Chicken age at exposure days	Tissue segment					
		Anterior		Middle		Posterior	
		Oviduct	Ligament[a]	Oviduct	Ligament	Oviduct	Ligament
3	1	0	3[b]	2	3	3	6
3	8	3	9	3	7	0	9
3	15	4	7	2	9	0	7
3	22	0	9	2	9	0	9
3	29	2	6	0	4	1	6
6	1	5	4	6	4	8	6
6	8	7	8	6	8	5	6
6	15	4	3	5	7	5	5
6	22	6	9	5	8	4	5
6	29	2	7	0	8	2	8
10	1	3	9	3	8	3	5
10	8	0	0	3	0	3	2
10	15	0	0	0	0	0	0
10	22	0	0	2	0	0	0
10	29	0	0	0	0	0	0

[a]Ligament evaluation also includes air sac if it was present.

[b]Number of viral antigen localizations per nine observations (three tissue replications x three chicken replications).

Table 14. The IBV antigen localizations in the oviduct and surrounding tissue using immunofluorescent staining following inoculation of chickens with Mass 33, 55th embryo passage

Collection post inoculation days	Chicken age at exposure days	Tissue segment					
		Anterior		Middle		Posterior	
		Oviduct	Ligament[a]	Oviduct	Ligament	Oviduct	Ligament
3	1	2	5[b]	0	3	0	1
3	8	0	3	0	0	0	0
3	15	0	3	0	3	0	1
3	22	0	3	0	3	0	3
3	29	0	8	0	5	0	6
6	1	3	8	3	9	3	5
6	8	1	6	1	6	0	6
6	15	0	7	0	3	0	2
6	22	0	9	0	9	0	7
6	29	0	7	0	3	0	3
10	1	0	3	0	0	0	0
10	8	0	0	0	0	0	0
10	15	0	0	0	0	0	0
10	22	0	0	0	0	0	0
10	29	0	0	0	0	0	0

[a]Ligament evaluation also includes air sac if it was present.

[b]Number of viral antigen localizations per nine observations (three tissue replications x three chicken replications).

Table 15. The IBV antigen localizations in the oviduct and surrounding tissue using immunofluorescent staining following inoculation of chickens with Aust T, 11th embryo passage

Collection post inoculation days	Chicken age at exposure days	Tissue segment					
		Anterior		Middle		Posterior	
		Oviduct	Ligament[a]	Oviduct	Ligament	Oviduct	Ligament
3	1	4	9[b]	0	5	0	1
3	8	0	0	0	0	0	0
3	15	0	1	0	0	0	3
3	22	6	9	0	4	0	0
3	29	0	0	0	0	0	2
6	1	6	9	3	3	1	4
6	8	3	7	2	9	6	9
6	15	3	9	0	9	3	7
6	22	3	9	0	9	3	6
6	29	3	7	0	9	3	9
10	1	0	7	0	3	0	0
10	8	0	1	0	0	0	0
10	15	0	0	0	0	0	0
10	22	5	0	0	0	0	0
10	29	0	2	0	3	0	0

[a]Ligament evaluation also includes air sac if it was present.

[b]Number of viral antigen localizations per nine observations (three tissue replications x three chicken replications).

Table 16. The IBV antigen localizations in the oviduct and surrounding tissue using immunofluorescent staining following inoculation of chickens with Conn A5968, 9th embryo passage

Collection post inoculation days	Chicken age at exposure days	Tissue segment					
		Anterior		Middle		Posterior	
		Oviduct	Ligament[a]	Oviduct	Ligament	Oviduct	Ligament
3	1	0	3[b]	0	3	0	0
3	8	0	0	0	3	0	3
3	15	0	2	0	3	0	1
3	22	0	3	0	0	0	0
3	29	0	8	0	4	0	6
6	1	0	9	2	6	0	3
6	8	0	2	0	2	0	0
6	15	1	4	0	0	1	2
6	22	0	1	0	2	0	3
6	29	0	6	0	4	0	0
10	1	0	2	0	1	0	0
10	8	0	1	0	1	0	0
10	15	0	0	0	0	0	0
10	22	0	0	0	2	0	1
10	29	0	1	0	0	0	0

[a]Ligament evaluation also includes air sac if it was present.

[b]Number of viral antigen localizations per nine observations (three tissue replications x three chicken replications).

Table 17. The IBV antigen localizations in the oviduct and surrounding tissue using immunofluorescent staining following inoculation of chickens with Iowa 609, 3rd embryo passage

Collection post inoculation days	Chicken age at exposure days	Tissue segment					
		Anterior		Middle		Posterior	
		Oviduct	Ligament[a]	Oviduct	Ligament	Oviduct	Ligament
3	1	0	3[b]	0	3	0	0
3	8	0	0	0	0	0	0
3	15	0	0	0	0	0	0
3	22	0	0	0	0	0	0
3	29	0	0	0	0	0	0
6	1	0	3	0	0	0	0
6	8	0	0	0	0	0	0
6	15	0	0	0	0	0	0
6	22	0	0	0	0	0	0
6	29	0	0	0	0	0	0
10	1	0	0	0	0	0	0
10	8	0	0	0	0	0	0
10	15	0	0	0	0	0	0
10	22	0	3	0	0	0	0
10	29	0	0	0	0	0	0

[a]Ligament evaluation also includes air sac if it was present.

[b]Number of viral antigen localizations per nine observations (three tissue replications x three chicken replications).

sections of the oviduct while the middle segment had a lower level*. There were too few antigenic localizations in the oviduct following inoculation with Conn to determine any pattern.

The largest amount of viral antigen was found six days post inoculation. There was a sharp drop by the 10th day post inoculation, especially in those birds inoculated at an older age. The viral antigen persists longer in the oviducts of chickens exposed at younger age compared to older chickens at exposure. The viral antigen was found in the oviduct three days post inoculation. A higher incidence of viral antigen was observed in the anterior segment of the oviduct at this particular period post inoculation for all serotypes except Mass, 7th embryo passage level, in which case it was equally present in the middle segment of the oviduct. By six days post inoculation the viral antigen was localized in equal or higher amounts lower down the oviduct. This applied to all age groups irrespective of age at inoculation.

It was often impossible to tell whether specific antigenic fluorescence was localized in the ligament of the oviduct or abdominal air sac. In both tissues only mesothelial cells showed intracytoplasmic staining. For comparison both these tissues were grouped together and evaluated as one. Viral antigen was found following inoculation with each serotype of IBV. However, the incidence varied greatly with only a few viral antigenic localizations following inoculation with Iowa 609. The same order of incidence was found in the ligament-air sac tissues as in the oviduct.

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*$P \leq 0.05$. This notation will be followed throughout the text.

The initial incidence of viral antigenic localizations was not related to age at inoculation but the younger the chickens at inoculation the greater the persistence of viral antigen in the tissues. The viral antigen was localized from all three sections but except for Mass, 7th passage, there was a gradient in incidences. Specific fluorescence was observed in largest amounts in the anterior portion while the posterior portion had the lowest level. Mass, 7th passage, had the same incidence of specific fluorescence in all three portions. The relative levels were higher by three days post inoculation in the ligament-air sac tissues compared to the oviduct. Mass, 7th passage, Conn and Iowa serotypes reached their highest incidence three days post inoculation while Aust T and Mass, 55th passage, had their highest levels at six days post inoculation. There was a sharp reduction in all serotypes by the 10th day post inoculation.

The intracytoplasmic localization of viral antigen agrees with the finding of Corstvet and Sadler (1964) and Braune and Gentry (1965) who also found specific staining to be confined to the cell cytoplasm. The pattern of viral antigenic localization in the ligament-air sac is of interest in that three low passage serotypes of IBV all reached their peak incidence three days post inoculation while the Aust T, which was the only nephrogenic serotype of IBV inoculated, and the high embryo passage level of Mass reached their peak six days post inoculation. Chickens exposed to Aust serotype did not show significant differences between ages of inoculation and final number of antigenic localizations in the oviduct, while in chickens exposed to other serotypes there was a reduction in incidence with increasing age at inoculation*.

These results do not agree with the finding of Lucio and Hitchner (1970) who failed to show localization of viral antigen in the oviduct following inoculation with IBV. The conjugation procedures followed in both experiments were similar but they were using adult hens in their studies. It is apparent from this experiment that the older the chickens at inoculation, the more difficult it is to localize the viral antigen. Aust serotype was an exception to this rule but Lucio and Hitchner (1970) did not work with Aust serotype. The total number of viral antigenic localizations was higher in the ligament-air sac tissues compared to the oviduct. The same order of incidence was observed in both types of tissues. There was a positive correlation between the presence of antigen in both tissues for the low passage serotypes but the 55th passage Mass showed a reduction in antigenic localizations in the oviduct.

There was no significant difference between the total number of localizations in each portion of the oviduce except following Aust T. However, in the ligament-air sac tissues the anterior segment had the greatest number. This could possibly be due to the reduction of air sac in the more posterior aspects of the abdominal cavity. The viral antigen oc rred earlier in the ligament-air sac tissues than in the oviduct. Th s may point to the method of spread of infection to the oviduct. The anterior segment of the oviduct also had the highest incidence in the oviduct at three days post inoculation. There has been difficulty in the past in efforts to isolate virus from the blood (Hofstad and Yoder, 1966; Doherty, 1967). Based on immunofluorescence it would appear the virus first enters the abdominal air sacs and then invades the anterior portion of the oviduct which is open at the infundibulum and spreads down the

oviduct. All serotypes behave in the same way with regard to invasiveness for the oviduct in different age groups. The older the chicken at inoculation the less invasive the virus is for the oviduct. This is different than in the ligament-air sac tissues where there is little difference between the incidences regardless of when they were inoculated.

DISCUSSION

Since 1939 it has been recognized that the greatest economic losses associated with IB in mature flocks concerned a reduction in egg production, quality, both external and internal, and reduced hatchability (Van Roekel et al., 1939). Broadfoot et al. (1954) reported field cases where young chickens suffering from IB subsequently failed to reach their expected egg laying capacity. Broadfoot et al. (1956) showed experimentally that exposure of young chickens to IBV resulted in the development of cysts in the oviduct. The younger the chicken at exposure the more severe the lesions.

There was no other work performed on young chickens until the late 1960's when work was carried out at the Veterinary Medical Research Institute, Iowa State University, by Crinion (1970), Crinion et al. (1971a) and Crinion et al. (1971b). Their work considered exposure of one-day-old chickens to Mass serotype IBV strain 33, 7th embryo passage level, and the pathogenicity of IBV for the oviduct. It was decided to expand this investigation and evaluate the pathogenicity of different serotypes of IBV for the oviduct and concurrently to evaluate the effect of chicken age at inoculation upon subsequent development of lesions in the oviduct.

The pathogenicity of IBV serotypes had been reported for the respiratory system and the kidney (Hofstad, 1958a; Cumming, 1963). The only known serotype to cause damage to the oviduct was Mass. In some reports (Broadfoot et al., 1956) the IBV was not classified into any serotype. It was decided to use serotypes of virus which represented the widest range of pathogenicity for the other tissues. Mass serotype represented the

severest pathogenic virus while Iowa 609 represented the mildest. Conn serotype had an intermediate degree of severity while Aust T serotype caused its major pathological changes in the kidney. Mass 33, 55th embryo passage level, was used to simulate the vaccines now being used in many parts of the world.

Various ages of chickens at inoculation have been reported in the literature. Broadfoot et al. (1956) inoculated chickens at intervals up to 18 days of age. Urban and Goodwin (1953) and Box (1964) showed that egg production was reduced significantly when chickens were exposed after sexual maturity while exposure at earlier ages had reduced effects and at six weeks of age had minimal effects on subsequent egg laying. The results of these experiments and the literature review indicate that there are two age periods in the chicken, very young and again after sexual maturity, when the oviduct is very sensitive to exposure with IBV. It was decided in this experiment to cover the age range one day to 29 days of age with each group seven days apart.

In the previous work by Crinion (1970) the chickens were maintained for one year following inoculation but this was impossible in this experiment because of the large numbers of chickens involved. For practical reasons one month was the limit of time chickens were kept post inoculation. During this month all available information was collected so that an evaluation of the pathogenicity of each IBV serotype for the particular age of inoculation could be calculated. Four parameters were utilized for evaluating the effects of IBV on the oviduct, namely, gross lesion, microscopic pathology, virus titration of IBV in the oviduct and viral antigenic localization by immunofluorescence within the oviduct.

The results of each of these parameters were evaluated in the previous section. The interrelationship of each of these parameters will now be evaluated.

The clinical signs observed were in agreement with the finding of Hofstad (1958a) and Cumming (1963). The persistence of clinical signs differed little in the different age groups. However, the severity of the disease was very much dependent on the age at inoculation. The chickens exposed at one day of age had more severe clinical signs and a greater mortality than older chickens. The chickens which were exposed from one through four weeks showed little difference in severity of clinical signs and mortality.

There was a positive correlation between damage to the oviduct epithelial cells and the presence of viral antigen in the epithelium of the oviduct. This correlation applied to all serotypes and ages of chickens used. However, Aust serotype was an exception as it was invasive in older chickens whereas epithelial damage was absent in older chickens, but in the older birds the invasiveness positively correlated with cellular infiltration of the oviduct. The viral titer results did not correlate as well with the other three parameters. This may be explained by the collection of ligament and air sac as contaminants while harvesting the oviduct. Thus the actual oviduct viral titration results also include some virus from the ligaments and air sacs. This was demonstrated in two ways. The oviducts collected for immunofluorescent studies were harvested in the same manner as the oviducts for viral titration. On subsequent examination with dark field microscopy, ligaments and air sacs were seen adhering to the oviduct and these additional tissues contained viral

antigen which was demonstrated by immunofluorescent staining. Further, if the incidence of specific viral fluorescence from both the oviduct and other tissue is added, there is a higher positive correlation between them and the viral titers found in the oviduct than between the fluorescence in the oviduct alone and the oviduct titers. This contamination problem has been discussed by Hofstad and Yoder (1966) and Crinion (1970). They suspected the presence of air sac contamination of other tissues during harvesting but they had no method of demonstrating it. Therefore, there is strong evidence in this experiment that viral titers reported in the oviduct actually represent the titers found in the oviduct plus the additional tissues which were closely associated with the oviduct. It would be very difficult to overcome this problem since the oviduct size in young chickens is very small and difficult to remove without the simultaneous removal of some other tissues.

Coria (1970) reported that there was no correlation between immunofluorescent localization of viral antigen and viral titers. However, virus was always isolated in tissues in which viral antigen was localized. This suggested that titration was a more sensitive technique for the identification of virus in the tissue. This was also suggested by Lucio and Hitchner (1970). In this experiment the same findings were observed.

There was a positive correlation between infiltrative type lesions and epithelial cell damage among the different serotypes of IBV. However, the infiltrative type lesion occurred equally in chickens of all age groups while the epithelial cell damage varied with age of chicken at inoculation. The younger birds showed a higher incidence of epithelial cell damage. The epithelial cell damage was dependent on age at the time

of inoculation while the infiltrative lesions were independent of age. 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 number of lymphoid nodules and plasma cells in the oviduct. The same type of response seems to apply to the oviduct of chickens exposed to IBV. While the infiltrative type lesion may signify the pathogenicity of particular serotypes, the actual degeneration of the epithelial cells of the oviduct probably gives a better parameter since it is a more direct measurement of pathogenicity. There is no direct evidence that infiltration of the wall will cause any permanent pathological lesion in the oviduct while degeneration of the epithelial cells and their subsequent sloughing may lead to organization and permanent blockage of the lumen.

The presence of macroscopic cysts in the oviduct was positively correlated with the presence of microscopic lesions involving the epithelial cells of the oviduct in both embryo passage levels of Mass serotype. There were no cysts observed following inoculation with Aust serotype. From the histological evidence it would seem most probable that cysts would develop at a later time, in addition to those already observed. Therefore, for a more accurate comparison of oviduct cyst incidence, observations would probably have to be recorded at a later time post inoculation than 30 days as done in this experiment.

Crinion (1970) reported the presence of localized hypoglandular areas in the oviducts of laying chickens which had previously been exposed to IBV at one day of age. There was no glandular development in the oviducts of birds in this experiment since they were killed prior to such

development. It is possible that oviducts which had epithelial degeneration and organization but not sufficient to cause complete blockage of lumen represent such birds because glandular development normally would commence as the epithelial lining cell invaginate into the oviduct wall (Figure 3). If there were no epithelial cells present, glands could not develop in such areas.

Pathogenicity evaluations were made primarily on microscopic lesions involving the epithelial cells and viral invasiveness of the oviduct as shown by immunofluorescent staining. There was a positive correlation between both of these parameters. The findings using low passage Mass inoculated at one day of age were in agreement with Crinion (1970). The 7th embryo passage level of Mass serotype was found to be the most pathogenic. It was most pathogenic for chickens exposed at one day of age while the pathogenicity was gradually reduced for chickens of increasing age at inoculation. Two serotypes (Conn and Iowa 609) failed to produce any pathological change in the oviduct. Iowa 609 inoculation did not result in any viral antigenic localizations in the oviduct while Conn was localized on only four occasions in the oviduct from over 400 sections examined. Aust T, which represented the only nephrogenic serotype used in this experiment, produced epithelial damage and was invasive to the oviduct. The pathogenicity was reduced as the chicken age of inoculation increased. The comparison of the two embryo passage levels of Mass serotype is very interesting. Both passage levels were invasive for the oviduct and produced epithelial cell damage in the oviduct. However, the high passage level Mass was always less pathogenic compared to the low passage virus and, further, the high passage virus was only pathogenic for

very young chickens while the low passage caused epithelial damage in chickens inoculated at three weeks of age. Mass, 7th embryo passage level, was the most pathogenic serotype followed in order by Aust T and Mass, 55th embryo passage level. Conn and Iowa were nonpathogenic for the oviduct.

There was a positive correlation between the pathogenicity for the oviduct and mortality of chickens during the acute stage of the disease, with the exception of the Aust strain which caused the greatest mortality. The pathogenicity was also related to severity of clinical signs but not to their persistence. This may be a useful correlation for the field veterinarian who has meager laboratory facilities to ascertain the future potential of a flock who suffered an outbreak of IBV at an early age. At a later date the presence of macroscopic cysts in the oviducts may serve the same purpose.

The pathogenicity is positively correlated with the identification of antigen in the oviduct for the serotypes and ages of chickens at inoculation. The immunofluorescent technique could be used in a diagnostic laboratory for the rapid evaluation of potential oviduct damage. However, tissue would have to be collected during the acute stage of the disease since very little antigen was found 10 days post inoculation compared to earlier collection times.

The classification of serotypes into respiratory and nephrogenic does not relate to the oviduct since representatives of both types are pathogenic for the oviduct and at least two respiratory classified serotypes are nonpathogenic. This experiment was performed using four different serotypes but it must be kept in mind that Mass serotype represents by

far the most common serotype observed in the field in the United States and represents the only serotype found in Great Britain. Aust T is representative of the serotype most commonly observed in Australia. Thus the pathogenic serotypes for the oviduct represent the most commonly isolated field viruses.

The same pathogenesis was observed following Aust and both embryo passage levels of Mass and also for all age groups. It appears from the evidence in this study that the infundibulum becomes infected from the air sacs and the infection then extends caudally towards the cloaca.

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.

The epithelial cells are the only cells in the oviduct which show viral antigenic localization and also the only ones which degenerate and become necrotic. From this evidence it seems the epithelial lining cells are the primary cells for viral attack. Garside (1965) and MacDonald and McMartin (1970) have reported desquamation of tracheal epithelium following exposure to IBV. All chickens did not demonstrate this lesion and they associated the desquamation with either virulent strains of the virus or extremely susceptible birds.

The cellular pathogenesis is not known, but electron microscopic observations of alterations in the rough surfaced endoplasmic reticulum with disturbances in synthesis of protein might very well disrupt the

normal function and development of the affected cell (Crinion et al., 1971a). However, many agents produce this type of injury so that it must be regarded as relatively nonspecific. 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. In general the cytopathogenic effect produced by infectious bronchitis virus has been described as degenerative 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).

The evidence in this experiment suggests a similar type of effect may occur in the oviduct. Six days post inoculation necrotic cells (pyknotic, karyolytic and karyorhetic) were observed in the lumen of the oviduct and at the same time post inoculation viral antigen was localized in epithelial cells and there was a positive correlation between the number of viral localizations observed and the number of oviducts showing epithelial cell damage. When the epithelial cells were sloughed away organization will occur, producing permanent blockage of the oviduct lumen. Once the oviduct becomes occluded any secretion of the oviduct caudal to the area of blockage will have no means of escaping. This will lead to cyst formation and the first cyst was seen 20 days post inoculation in this experiment.

It is unfortunate that Mass serotype vaccines are potentially the most dangerous serotype for the oviduct in young chickens, since they are also the most immunogenic (Hofstad, 1961; Winterfield, 1968). The reason

why this problem has become more noticeable in the poultry industry probably is that young chickens generally have parental antibodies in this country because of the widespread vaccination. However, Hofstad and Kenzy (1950) demonstrated active IB infections in chickens four, six, seven and ten days of age which had been hatched from eggs laid by bronchitis-recovered hens. These chickens had high antibodies against IBV in their sera. It is difficult to explain this type of outbreak. It is unlikely that a viremia would occur in the presence of high serum antibodies. Hofstad (1961) suggested a local tissue immunity of the respiratory system may be involved in the immunological protection afforded following this disease. Hofstad and Kenzy (1950) also suggested that complete immunity to IBV may depend on an interaction between tissue immunity and circulating antibodies. The recent report of Cook (1971) on the distribution of virus in day-old chickens from hatching eggs should warrant investigation into possible pathological effects on the oviduct in such chickens.

Raggi and Lee (1957) demonstrated that there is no correlation between serum neutralization antibodies and immunological protection. This has been confirmed by Hofstad (1961) and Hitchner et al. (1964). If the virus reaches the oviduct via the air sacs, which evidence in this study supports, there is a good probability that the virus would be capable of invading the oviduct even in the presence of serum antibodies. Experimental proof would be required to show oviduct infection in the presence of humoral antibodies.

The major objectives of this experiment have been achieved but this experiment has only touched on the area concerning the potential hazards

capable of being caused by vaccines now in use in this country and elsewhere. Duff et al. (1971) recently reported that a vaccine currently being used in Great Britain (Mass, H strain, 52nd embryo passage level) is capable of producing severe pathological lesions in the oviducts of chickens one day of age which were housed with older chickens which had received the vaccine. It is apparent from this work and the literature that there is little leeway in the vaccination procedures which are now available to the poultry industry.

The pathogenicity of IBV for oviduct is closely associated with at least three independent factors: 1) IBV serotype, 2) age of chicken at inoculation, and 3) embryo passage level of virus. There have been many different isolates of Mass serotype reported and there are antigenic differences among them (Berry and Stokes, 1968). It is unknown whether all these isolates would have the same pathogenicity for the oviduct since they do not all produce identical clinical signs and mortality. The strains inoculated in this experiment were used as representative samples of their respective serotypes.

The IBV serotypes found in this country have the same relative pathogenic relationships for both the respiratory system and the oviduct. The immunological protection by each of them is also in the same order (Hofstad, 1961). It appears, therefore, that the poultry industry must continue using a serotype for vaccination which is potentially dangerous and capable of causing significant pathological lesions in the oviduct until a better vaccine is available. Winterfield (1968) reported that the JMK serotype of IBV produced good immunological protection and this serotype if attenuated may in the future serve as a safe vaccine. The ideal situation

would evolve if an inactivated viral vaccine could be developed which would afford immunological protection to chickens. Coria (1970) has reported encouraging results using β-propiolactone inactivated IBV Mass, strain 33, via two routes: aerosol exposure and subcutaneous inoculation. He suggested that the aerosol exposure is sufficient to sensitize and initiate an immune response in tracheal epithelium. Inoculation and the slow release of antigen from the IBV inactivated vaccine are sufficient to enhance the immunological process initiated by the aerosoled antigen. Inoculation by aerosol is the optimal method of infecting chickens with IBV and constitutes the severest challenging procedure. The vaccination procedure utilizing drinking water would probably constitute a milder challenge to the chickens compared to aerosol.

SUMMARY

The pathogenicity of different serotypes of infectious bronchitis virus (IBV) for the oviduct was evaluated and the susceptibility of chickens exposed at different ages to the virus was ascertained. Massachusetts (Mass) serotype, strain 33, was evaluated at two chicken embryo passage levels, 7th and 55th. The other serotypes, Connecticut (Conn) A5968, Australian T (Aust) and Iowa 609, were evaluated at embryo passage levels 9th, 11th and 3rd, respectively. The methodology involved the evaluation of four parameters: gross lesions, microscopic pathology, virus titration for the presence of virus in the oviduct and finally the antigenic localization of specific activity by immunofluorescence. The chicken ages at inoculation were 1, 8, 15, 22 and 29 days.

Data were collected for one month post inoculation. The clinical signs and mortality were in agreement with previous work. The viral serotypes varied in their ability to produce pathological changes in the oviduct. Two serotypes, Conn and Iowa 609, failed to produce any pathological change in the oviduct. The 7th embryo passage level of Mass produced the greatest number of changes, followed by Aust and 55th embryo passage Mass in that order. The character and pathogenesis of the disease were the same following each serotype and age of inoculation. The first observed lesions consisted of infiltration of the oviduct wall with lymphoid cells. This was followed by degeneration of the epithelial lining cells in localized areas and resulted in their sloughing into the lumen. Organization occurred in these areas resulting in luminal blockage and cyst

development in the oviduct. The younger the chickens were when exposed the more severe the resulting lesions.

Viral antigen was localized only in the epithelial lining cells of the oviduct as determined by immunofluorescent staining. The number of cells involved varied from a small number to the entire epithelial lining. Antigenic localizations were not seen following Iowa 609 inoculation while it was found in nonsignificant numbers ($P \leq 0.01$) following Conn serotype. The 7th embryo passage level of Mass produced the greatest number of antigenic localizations followed by Aust and 55th embryo passage level Mass in that order. The younger the chickens were inoculated the longer the viral antigen persisted in the oviduct.

Portions of ligaments and air sacs were collected with the oviduct while tissue was being harvested for titration evaluation. The calculated titers were thus the sum of the oviduct titer and the closely adhering ligaments and air sacs. All serotypes of IBV were recovered but Iowa 609 was present in low levels only. The virus persisted longer in chickens exposed at an early age. The Aust serotype persisted longer in the oviduct than the other serotypes, especially in chickens exposed at an older age.

There was a positive correlation between all parameters but due to contamination, virus titer results were not used in evaluating overall pathogenicity. Pathogenicity was based primarily on histological lesions and immunofluorescent localization of antigen in the epithelial lining cells of the oviduct. There was a positive correlation between antigenic localization and epithelial damage in the oviduct. The 7th embryo passage level of Mass serotype was found to be the most pathogenic. It was most pathogenic for chickens exposed at one day of age and the pathogenicity

was reduced for chickens of increasing age at inoculation. This serotype was followed in pathogenicity by Aust and the 55th passage Mass serotype. The same age relationship was observed with these two serotypes also. The other two serotypes, Conn and Iowa 609, failed to produce any pathological changes in the oviduct and neither showed viral antigen in the epithelial cells of the oviduct at a significant level.

Both embryo passage levels of Mass serotype were pathogenic for the oviduct and they had the following relationship: the high passage virus was always less pathogenic than the low passage, and the high passage virus was only pathogenic for very young chickens while the low passage was also pathogenic for older chickens.

The pathogenicity of IBV for the oviduct is associated with at least three independent factors: 1) IBV serotype, 2) age of chicken at inoculation, and 3) embryo passage level of virus.

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