

THE ROLE OF THE BURSA OF FABRICIUS IN THE DEVELOPMENT
OF RESISTANCE IN INFECTIOUS LARYNGOTRACHEITIS

by

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D. V. M., Kansas State College
of Agriculture and Applied Science, 1955

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

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INTRODUCTION

Infectious laryngotracheitis is an acute, contagious, respiratory disease of chickens and pheasants. It was first recognized as a major economic poultry problem in 1924 (11). Hinshaw (28) identified the disease as infectious bronchitis. Other investigators (23), (36), (41), used descriptive terms as infectious tracheitis, bronchopneumonia, and tracheo-laryngitis. Transmission was demonstrated by tracheal swabbing by May and Tittsler (41) and was confirmed by other workers (1), (25).

At first, efforts to establish an etiology were unsuccessful. Beach (1) isolated the Pasteurella organism and recovered the fowl pox virus from some affected fowl. The disease was reported by Gwatkin (27) as acute pox, but later observed that recovered birds were susceptible to fowl pox. In 1930, Beaudette and Hudson (9) demonstrated a specific filterable virus as the etiological agent. Evidence of this finding was submitted by other investigators (2), (23), (26).

From this point on, considerable time, work, and patience were spent on the problems connected with the development of resistance against infectious laryngotracheitis. Numerous routes of inoculation and methods of virus treatment were used in an effort to find a satisfactory technique (3), (6), (24), (30), (37), (39). Hudson and Beaudette (31) attempted to modify the virus by serial cloaca to cloaca passage. The fowl inoculated in this manner were demonstrated to be resistant. This method led to the present standard vaccination procedure. A virulent

virus is apparently necessary for establishing immunity.

Gibbs (24) introduced the virus into the bursa of Fabricius. Inoculation of the virus directly into the bursa resulted in resistance which was superior to infection by cloacal vaccination.

Bursal vaccination is limited to birds that still retain the bursa of Fabricius (8). This lymphoid cul-de-sac regresses and involutes near the time of sexual maturity (35). The physiological function of the bursa of Fabricius has not been definitely determined, although it has been associated with immunity, haematopoiesis, nutrition, and fertilization (21), (33), (34), (35).

Beaudette (7) reported that a viremia does not occur in the natural or artificial infection. The virus has been demonstrated in the blood, liver, and spleen, but its presence is believed to be due to injury of the blood vessels in the larynx and trachea (3). The resistance to an infection is attributed to humoral antibodies. It is believed that these antibodies are produced in the trachea or the vaccination site. Burnet and Fenner (19) attribute antibody production to a "complex activity of cells involving lymphocytes, phagocytic cells of the reticule endothelial system, and undifferentiated mesenchymal cells."

Specific neutralization of infectious laryngotracheitis virus has been accomplished by immune serum (4), (12), (17). Virus neutralization is a standard procedure in vivo (29), in vitro (43), and in ovo (17).

The following work was undertaken with a desire to gain a better understanding of the role of the bursa of Fabricius in the development of resistance against infectious laryngo-tracheitis.

REVIEW OF THE LITERATURE

Inactivated Virus

Kernahan (37) attempted to produce immunity by inactivated virus. Suspensions of tracheal exudate were treated as follows: (1) application of heat, 55° C. for one hour, to 10 percent exudate suspensions, after which 0.5 percent phenol was incorporated; (2) addition of 60 cc of 5 percent formalin to 6 g of exudate; (3) suspension of 1 g of exudate in equal quantities of 0.5 percent phenolized saline and glycerine; (4) use of chloroform vapor passed through a suspension of 10 cc of physiological saline and 1 g of exudate. Subcutaneous inoculations were given to three-month-old birds. These treatments did not induce immunity in the fowl.

Gibbs (24) employed liver, spleen, blood, and tracheal exudate as sources of tissue for vaccine preparation. Suspensions of these tissues were treated with 40 percent formalin, 0.5 percent phenol, and 25 percent glycerol. Formalin and phenol destroyed the virus while glycerol preserved the virulence of the virus. These vaccines proved unsatisfactory as immunizing agents against infectious laryngotracheitis.

Immune Serum

Gibbs (24) stated "that repeated smaller doses at 24-hour intervals were more effective than one dose of serum administered the day symptoms appeared." Serum given by the intravenous route 48 hours after the onset of symptoms was of little value.

Brandly (12) demonstrated that immune serum induced temporary protection by the intratracheal route. Birds treated with a non-infectious virus-serum mixture were not resistant to the virus.

Live Virus

Kernahan (37) inoculated fowl intratracheally with ten-fold dilutions of tracheal exudate suspension. All of the birds succumbed to the infection within seven days.

Subcutaneous and intravenous inoculation of virulent virus did not induce immunity (24). Introduction of 0.1 ml of virus directly into the cul-de-sac with a blunt, curved needle fitted to a syringe produced infection of this lymphoid organ. These fowl resisted intratracheal inoculation of virus. Negligible losses occurred in chicks 6, 10, 12, and 14 days of age vaccinated by the intrabursal method and challenged 14 days and 5 months later. It was observed also that bacteria from unimmunized birds produced a reaction similar to the virus inoculation of the bursa of Fabricius. Resistance following intrabursal inoculation was considered superior to that obtained by cloacal vaccination. A larger number of fowl inoculated by way

of the bursa developed inflammatory reactions than those exposed by the cloacal routes. Immunization of birds with virulent virus by intravenous inoculation was attempted (24). Fowl received 1 cc of Seitz (E. K. Schnichten) filtered virus by way of the median vein. Fowl that survived were immune. "Chickens receiving increasingly larger doses of the filtrate over longer periods of time were made immune to laryngotracheitis." Subcutaneous inoculation of 2 cc of tracheal exudate diluted 1-25, and administered in up to three doses did produce immunity. Tracheal exudate diluted with 50 percent glycerol, introduced subcutaneously, imparted negligible resistance. The unsatisfactory results with glycerol tracheal exudate were not understood with chickens under field conditions, although tests with birds in cages were promising.

Active virus was introduced in the upper respiratory tract of birds maintained at an environmental temperature of 40° C. (30), also was administered by the subcutaneous (6) and conjunctiva (6) routes in an attempt to produce active immunity. The results were not sufficiently encouraging to continue further study.

Efforts to modify the virus by passage in a different host were unsuccessful. Kernahan (39) reported an outbreak of the disease among chickens and pheasants. Other species--ducks, domestic mallards, peacocks, pigeons, doves, parakeets, and canaries--in adjacent pens were unaffected. Attempts to transmit laryngotracheitis virus from pheasants to chickens and

pigeons met with failure. Domesticated ducks, sparrows, crows, starlings, doves, pigeons, rabbits, guinea pigs, white rats, and one pig were found to be resistant to the virus (3). The serial passage of the virus in pheasants and in a pheasant-bantam cross, which are susceptible to the disease, did not alter the virulence for chickens (32). Gibbs (24) serially passed the virus in the cloaca of fowl at three-day intervals for 58 days. Modification of the virus did not occur. Brandly (14) probably considered modification when he attempted to grow the virus in duck, guinea fowl, and pigeon eggs. Strain (8090) was propagated in fertile turkey eggs; duck and guinea fowl eggs did not support the growth of strain J (Brandly, 15).

Virus applied to the cloaca by a cotton swab resulted in an inflammatory reaction of the proctodeum portion by the third day (31). This inflammatory reaction or "take" was maintained for five serial cloaca to cloaca transfers at three-to four-day intervals. Fowl inoculated intratracheally with each passage of the cloacal material developed laryngotracheitis. Cloaca infection conferred resistance to intratracheal challenge; tracheal infection resulted in the failure of the cloaca to react to the virus. These results led to the conventional vaccination procedure.

Only 50.5 percent of 1451 chickens vaccinated by Beach et al. (5) by the cloacal method developed "takes". Of the fowl which failed to react, 80.6 percent were susceptible to infection. Cloacal and intrabursal vaccination gave comparable results.

According to Beaudette (8), 1 cc of virus suspension would vaccinate 10 birds by the bursal method while this amount would be sufficient for 50 doses or more by the cloacal procedure. He stated that vaccination by the bursal method could be used only in birds that still retained the bursa of Fabricius.

Molgard and Covett (42) reported that infectious laryngo-tracheitis virus applied to the feather follicles caused a swelling on the second day, developed to its maximum on the fifth day, and decreased by the seventh and eighth day. Birds that were vaccinated by this method resisted cloacal vaccination. The reverse was also true. The percentage of takes were compared in the cloacal and follicle methods of vaccination. No marked difference could be observed in their field trials. These investigators stated that feather follicle vaccination may have an advantage in birds that have wet vents, and further suggested that less virus may be discharged than by the cloacal method.

Beaudette (8) indicated that the follicle method of vaccination requires more time for application and more vaccine. The condition of the cloaca was not considered to be a problem in producing takes in cloacal vaccination, nor was respiratory infection from the dissemination of virus from the cloaca important. Cloacal vaccination at any age after six weeks is the standard method of immunization, although the feather follicle method may have other possibilities.

Neutralization

Cross-neutralization between a New Jersey and a California strain of laryngotracheitis virus was demonstrated by Beach (4). Neutralization of the virus by immune sera as determined by the intratracheal deposit of virus-antiserum mixtures in chicks was dependent on the virus concentration (4) (12).

Burnet (16) first propagated laryngotracheitis virus in the chorio-allantois membrane of the developing chicken egg. From this method, a greater concentration of virus can be obtained. The ectodermal lesions produced by infection are proliferative and necrotic in nature.

Brandly (13) confirmed these findings, and utilized the chorio-allantois propagated virus as vaccines. The titer of chicken embryo propagated virus was appreciably higher than that of tracheal origin. He suggested that the extent of infection of the chorio-allantois membrane depended on the technique of introducing the virus and the environmental factors during incubation. Thirty-five serial passages of virus in fertile eggs did not decrease the virulence (14).

Burnet (17) reported that the number of macroscopic foci which developed on the chorio-allantoic membrane following inoculation with virus suspension was equivalent to the number of active virus particles. Strains of infectious laryngotracheitis virus were classified on the basis of the chorio-allantoic lesions produced. The epizootic strain of New South Wales and an American strain produced irregular, raised lesions with a

characteristic central necrotic zone; the less virulent enzootic Victoria strains produced lesions of uniform texture. The epizootic strains were more easily neutralized by immune serums than the mild enzootic Victorian strains. No qualitative antigenic differences were demonstrated. Burnet concluded that the proportional decrease in virus titer, due to a concentration of immune sera, is independent of the initial virus concentration.

Bursa of Fabricius

The function of the bursa of Fabricius is not definitely known. This cul-de-sac is developed at the time of hatching. Riddle (46) stated that its highest degree of development is related with the growth of the body and thymus. It reaches its maximum growth at four to five months, is approximately 30 mm in length, and weighs 3 g. Involution of the thymus is associated with similar changes in the bursa, and increased growth rate of the gonads. This retrograde evolution, which is usually complete at the time of sexual maturity, occurs from the apex to the base of the bursa (46). Lymphoid follicles separate from the epithelium and are replaced by fibrous connective tissue. By eight months, the structure has lost its ability to function (46). Two months later it is reduced to a cyst. Bursa size, growth, and involution are similar in cockerels and pullets.

Jolly (33) attributed a haematopoietic function to the bursa of Fabricius. The haematopoietic foci in the bursa produce lymphocytes, erythrocytes, and granular leucocytes. This

property, however, is not limited to the bursa. Mesenchyme tissue is also characterized by this function. Starvation is accompanied by rapid degeneration of the bursa of Fabricius (34). He stated further that the structure may have a relation to sexual maturity (35). The appearance of sexual maturity has a similar time sequence to the involution of the bursa. The greatest development of the bursa is reached when spermatogenesis first occurs in the fowl.

Chang, et al. (21) reported that the bursa of Fabricius is important in antibody production. In these trials, two groups of birds were inoculated with a 48-hour broth culture of Salmonella typhimurium antigen. Eight of the 75 birds with the bursa removed demonstrated antibodies, while 60 of 73 controls showed immune bodies. The standard agglutination test was used to measure antibody formation.

MATERIALS AND METHODS

Fifty five-week-old White Rock chicks were used for the experiment. The chicks were vaccinated at two days of age intranasally with the B₁ strain of Newcastle disease vaccine. Twenty-five chicks selected at random were bursectomized; the remainder constituted the control group.

The bursa of Fabricius, which is dorsal to the cloaca, was surgically removed from the chicks in the test group. Sedation was produced by the intravenous administration of 0.1 cc of sodium pentobarbital. The perineal region was cleansed with

0.07 percent roccal. The bursa was exposed by a 4 mm horizontal incision through the skin 8 mm dorsal to cloacal orifice. This structure was ligated with 00 catgut at the cloacal origin. The edges of the skin were approximated with a running mattress suture using 00 catgut. The operative area was covered with a thin layer of flexible collodion.

The control and test groups were vaccinated with infectious laryngotracheitis vaccine¹ three days after the removal of the bursa of Fabricius. The cloacal reaction in each bird was recorded at 24 intervals for 8 days.

Virus Titration

Titration of the virus for challenge was determined by inoculating five-week-old White Rock chicks intratracheally with 0.1 cc of virus suspension.² Seven three-fold serial dilutions were prepared. Three chicks were used for each dilution. The first dilution was 1 to 2400.

Challenge

Three weeks after vaccination, all chicks were challenged with 0.1 cc of 1 to 2400 of virus inoculum introduced intratracheally. Chicks were observed twice daily for symptoms. The chicks were sacrificed 21 days post challenge and the tissues

¹ The vaccine 6205-33 was furnished by Lederle Laboratories, Pearl River, New York.

² Ibid.

carefully observed for gross evidence of disease.

Collection of Blood

Blood was obtained by intracardial puncture from both groups. This was done 24 hours prior to vaccination, and each day thereafter until the ninth day. Samples were also collected the 14th and 19th days post vaccination and two and six days following challenge. The serum was removed, centrifuged for 10 minutes at 2000 rpm in a Servall angle centrifuge, and stored at -30° C.

Neutralization Test

Allantoic Method. A neutralization technique¹ based on embryonic mortality was initially investigated. The lethality of a virulent strain of laryngotracheitis virus² inoculated into the allantoic cavity of nine-day-old chicken embryos formed the basis of this procedure. The allantoic route 50 percent end point of the chorio-allantois membrane propagated virus, as determined by the Reed and Muench method (45) was 1 to 15,625. Subsequent end point virus titrations as well as virus-serum mixtures were not successful. The chorio-allantoic membrane neutralization technique employed by Burnet (17) was then used to obtain the antibody titer of the sera.

¹ F. S. Markham, personal communication.

² This virus was a sterile, lyophilized product from Lederle Laboratories, Pearl River, New York.

Chorio-allantoic Method. Fertile chicken eggs, obtained from the college poultry farm, were incubated at 39° C. for 12 days, candled, and the margin of the air space marked. A rectangular area 12 by 5 mm was marked over the region of maximum development of the chorio-allantois membrane. An electric hand drill attached with an abrasive disc was used to cut the shell rectangle, avoiding injury to the underlying structures. A small opening through the shell and shell membranes of the natural air cell was made. The rectangular section of shell was removed and a small opening produced in the shell membrane by separating the fibers. A drop of sterile saline was placed over the opening and the false air cell was produced by gentle suction, using a two-ounce rubber bulb applied to the opening over the natural air cell. The artificial openings were closed with "Scotch tape" to prevent dehydration of the membranes. The eggs were incubated at 36° C. until the virus-serum mixture could be inoculated.

Preparation of the Inoculum

The stock virus suspension was prepared by inoculating the chorio-allantoic membranes of one dozen 12-day fertile eggs with 0.2 cc of a 1 to 100 dilution of the Vine Mu strain of laryngo-tracheitis virus. On the fifth day of incubation, the inoculated area of the membranes was removed aseptically with a thumb forceps and a small pair of scissors, placed in a sterile petri dish, and weighed. Trituration was accomplished with a modified TenBroeck tube grinder. Sufficient physiological saline

containing 100 units of penicillin and 100 mg of streptomycin was added to give a 50 percent suspension. The suspension was then centrifuged at 2500 rpm in a horizontal centrifuge for 10 minutes to sediment the particulate matter and the supernate stored at -30° C.

The serum was heated at 56° C. for 30 minutes and then centrifuged at 2500 rpm for 10 minutes. To check sterility, two nutrient broth tubes for each serum sample were inoculated with 0.01 cc of the serum and incubated at 36° C. for 24 hours. Seven serial five-fold dilutions of the serum were made and 1 cc from each dilution was added and mixed with an equivalent amount of 1 to 100 suspension of virus. This concentration of virus was based on the number of lesions produced by previous chorio-allantois membrane inoculations. The saline-virus control and the various virus-serum mixtures were incubated at room temperature for one hour, and for an additional hour at 4° C. Five eggs per dilution were inoculated on the chorio-allantois membrane with 0.2 cc of the various mixtures with a 25-gauge needle fitted to an intradermal syringe. The two artificial shell openings were sealed with "Scotch tape", and then the eggs were incubated for five days at 36° C. One virus control series, consisting of 0.1 cc of the virus suspension and an equal quantity of sterile saline, was used for each serum titration.

Pre-vaccinal serum, serums secured four and eight days following vaccination, and two and six day serums after challenge, were tested for virus neutralization capacity.

At the end of the five-day incubation period, the chorio-allantois membrane reactions were observed by removal of that section of the shell covering the artificial air cell. Membrane reactions induced by the inocula, in many instances, were confluent rather than discrete. An arbitrary descriptive method of evaluating the influence of the various serums was devised. Membrane involvement was classified as: slight -- 1; moderate -- 2; and, extensive -- 3.

Chick Neutralization Test

The serums secured 24 hours before vaccination and two days after challenge from the bursectomized and control groups were used to determine the in vivo influence of serums on the infectivity of the virus. The serums had been inactivated and checked for sterility. Two five-fold dilutions of the two-day post challenge serums were made and 1 cc from each dilution was added to an equivalent amount of 1 to 100 suspension of egg propagated Vine Mu strain of virus. One cc of pre-vaccinal serum was added to an equal quantity of a similar virus suspension. The serum-virus mixtures were incubated at room temperature for one hour, and for an additional hour at 4° C.

Three five-week-old White Rock chicks per dilution were inoculated intratracheally with 0.2 cc of the virus-serum mixtures with an avian intradermal syringe fitted to a 26-gauge needle. Five five-week-old Rhode Island Red chicks were administered 0.2 cc of the prevaccinal-virus mixture and three five-week-old

White Rock chicks were inoculated intratracheally with 0.2 cc of the 1 to 200 dilution of the virus. The groups of birds were observed for nine days and then autopsied.

RESULTS AND DISCUSSION

Vaccination and Challenge

Cloacal inflammation, typical of laryngotracheitis vaccination, consisting of edema, hemorrhage, and fibrinous deposition in the mucous membrane was observed on the third, fourth, and fifth days. The reaction had subsided by the seventh day.

To determine the virus titer for challenge, 21 five-week-old White Rock chicks, using seven three-fold dilutions, were inoculated intratracheally with 0.1 cc of virus. The birds in the first two dilutions, 1 to 2400 and 1 to 7200, developed symptoms of the disease on the fourth to the eighth day. One chick in the 1 to 2400 dilutions died on the 12th day of the test. Nine birds, representing the dilutions 1 to 21,600, 1 to 64,800, and 1 to 194,400 were then challenged intratracheally with 0.1 cc of 1 to 2400 dilution to check the viability of the vaccine and the resistance of the birds. The fowl in the dilutions 1 to 64,800 and 1 to 194,400 showed respiratory symptoms on the third day. On autopsy, lesions considered typical of infectious laryngotracheitis were observed.

Three weeks after vaccination the test and control groups of birds were challenged with 0.1 cc of 1 to 2400 dilution of virus intratracheally.

Table 1. The gross pathology of the larynx and trachea on autopsy in the test birds.

Group with bursa removed :	Gross pathology: of the larynx and trachea :	Evidence of bursa	Symptoms or mortality : before autopsy
Bird 1	Marked	-	-
2	Moderate	Scar present	-
3	Moderate	-	-
4	Marked	-	-
5	Marked	-	-
6	Marked	-	-
7	Moderate	-	-
8	Slight	-	-
9	Moderate	-	-
10	Moderate	-	-
11	Moderate	-	-
12			*
13	Slight	Scar present	-
14	Marked	-	-
15	Moderate	-	-
16			*
17	Slight	-	-
18	Moderate	-	Dyspnea on 4th and 5th day
19			*
20	Moderate	-	-
21	Slight	-	-
22	Moderate	-	-
23	Slight	-	-
24	Slight	-	-
25	Marked	-	-

* Birds 12, 16, and 19 died from pendulous crop, suspected chronic respiratory disease, and ruptured air sac, respectively.

Table 2. The gross pathology of the larynx and trachea on autopsy in the control birds.

Group with bursa intact	:	Gross pathology of the larynx and trachea	:	Symptoms or mortality before autopsy
Bird 26		Moderate		-
27		Marked		-
28		Slight		-
29		Marked		-
30		Moderate		-
31		Slight		-
32		Slight		-
33		Marked		-
34		Marked		Dyspnea on 4th day
35		Moderate		-
36		Slight		-
37				*
38		Moderate		-
39		Marked		-
40		Marked		-
41		Marked		-
42		Moderate		-
43		Slight		-
44		Marked		-
45		Slight		-
46		Slight		-
47		Moderate		-
48		Slight		-
49		Normal		-
50				*

* Birds 37 and 50 died from cardiac tamponade and a ruptured air sac, respectively.

In Tables 1 and 2, birds showing numerous petechia, ecchymoses, congestion, and edema of the mucosa with excessive mucus from the anterior larynx to at least the middle third of the trachea, were classified as marked pathology. The mucous membranes of the intranasal sinuses of these birds were congested, edematous, petechiated, and contained a seromucous exudate. The conjunctiva showed several petechia.

The moderately affected group showed sparsely distributed petechia of the laryngeal and tracheal mucosae with less congestion than the marked group. Edema was confined to less than one-third of the upper trachea. The intranasal sinuses were similar in appearance to the more severely affected group except that less exudate was present. The conjunctivae revealed a few petechia.

A mild degree of congestion and edema of the laryngeal and tracheal mucosae characterized the slightly affected group. Petechia were observed in a few fowl. The intranasal sinuses were essentially unchanged. The conjunctiva of a few fowl was petechiated.

Table 3. The percentage of birds evidencing laryngeal and tracheal changes listed in Tables 1 and 2.

Group	Normal	Slight	Moderate	Marked
Bursa removed	0.0	27.2	45.4	27.2
Bursa intact	4.3	34.7	26.0	34.7

Chick Neutralization Results

The purpose of inoculating chicks with virus-serum mixtures was to attempt to compare in ovo and in vivo neutralization results.

Mild respiratory symptoms were observed in one bird in the virus control group on the fourth and fifth days. Abnormalities were not noted in the other chicks. The pathological changes in the symptomatic chick were extensive. The mucous membranes

of the respiratory tract were characterized by petechiation, ecchymoses, and the presence of a sero-mucoid exudate. The other birds in this group showed moderate changes. The cephalic fourth of the tracheal mucous membrane was edematous. A minimal number of petechia were present.

The fowl in the prevaccinal serum-virus group exhibited moderate changes of the respiratory tract which were comparable to those in the two chicks in the virus-control group. The remaining three birds in this group showed edema of the mucous membranes of the larynx, trachea, and intranasal sinuses, with no evidence of hemorrhage.

The lesions observed in the chicks inoculated with serums from the bursectomized and control groups were similar. The pathological changes were characterized by edema affecting the upper one-fourth of the tracheal mucous membrane. Petechiation was minimal or absent.

Since lesions observed were similar in the birds inoculated with two-day challenge serum, no correlation to in ovo neutralization could be made. Apparently, three of the chicks inoculated with the prevaccinal serum-virus mixtures were somewhat resistant to infection since lesions of laryngotracheitis did not develop. The virus control chicks developed more extensive lesions than did the birds inoculated with two-day challenge serum. The results did suggest that neutralizing antibodies were present in the post challenge serum.

EXPLANATION OF PLATE I

Pock lesions on the chorio-allantois membranes of 17-day fertile chicken eggs inoculated with laryngotracheitis virus and serum secured two days after challenge from the bursectomized group of birds. The numbers 20 and 80 on the egg shells refer to the dilutions 1 to 20 and 1 to 80 of the serums, respectively.

PLATE I



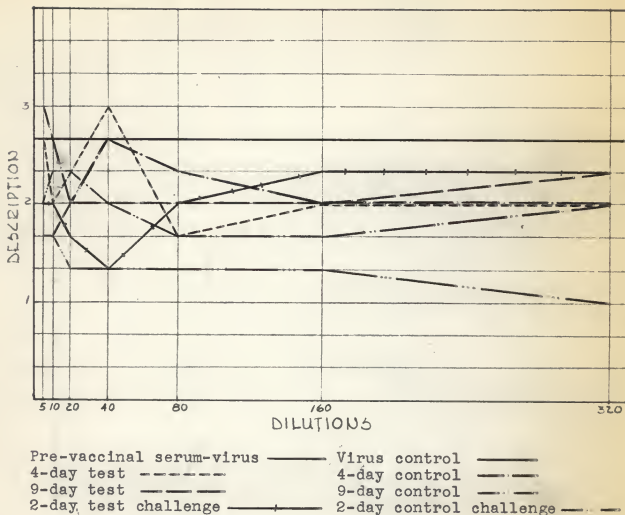


Fig. 1. The description of the pathological changes in the chorio-allantois membranes of developing eggs inoculated with virus alone, and virus-serial five-fold dilutions of serums collected from bursectomized and control birds 24 hours before vaccination, four and nine days following vaccination, and two days after challenge.

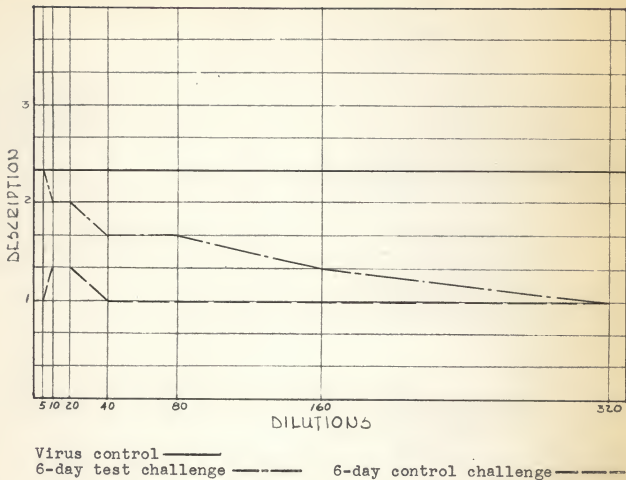


Fig. 2. The description of the pathological changes in the chorio-allantois membranes of developing eggs inoculated with virus and five-fold dilutions of serum collected from the bursectomized and control birds six days following challenge.

The description of pathology expressed numerically in Figs. 1 and 2 was based upon an average of the lesions found in the five eggs inoculated with each serum sample. Extensive lesions were confluent or dendritic opacities involving at least half of the membrane and were given a numerical value of three. Moderate lesions, with a value of two, were those that involved from one-fourth up to one-half of the membrane. A value of one represented lesions that were circumscribed, localized pocks that affected less than one-fourth of the membrane.

Vaccination of bursectomized and control groups of birds produced cloacal inflammatory reactions that were characteristic of the vaccinal reaction considered typical for this disease. The vaccine, on application to the cloacal mucosa, produced over 95 percent readable "takes". It is generally known that the older the bird, the less severe the cloacal reaction, however, older birds are as solidly immune as younger birds (8). The possibility arises that the presence of a well-developed bursa of Fabricius may only be an influence in producing a higher antibody titer following infection of the cloacal membrane.

Apparently, susceptibility to the virus existed in the bursectomized and control birds since the laryngeal and tracheal changes produced by challenge paralleled the gross pathology observed in naturally infected fowl. In this experiment, the immunity produced by vaccination against the disease was insufficient to withstand the intratracheal administration of the virus.

The possibility arises that the magnitude of the virus inoculum on challenge was too severe a test of the immune state, and the larynx and trachea were much more intensely exposed to the virus than would occur in the case of a natural infection. This would suggest that under natural conditions the respiratory epithelium is exposed to a relatively minute amount of virus. Although 0.1 ml of laryngotracheitis virus was introduced intratracheally in each bird, the actual amount retained is not known because a portion of it was exhaled immediately after inoculation. The tissue response in the larynx and trachea of the bursectomized and control birds may suggest that vaccinated birds would become carriers if subjected to the conditions of this experiment.

There appeared to be a correlation between laryngotracheitis and influenza virus infections. It is believed that inhaled influenza virus spreads over the surface of the respiratory epithelium and if significant antibodies are present in the thin film of fluid covering the mucous membrane, infection may be prevented (44). In studies of influenza immunities, attacks have occurred in individuals with high antibody levels, but less frequently than in persons having lower antibody titers (22). Similar observations during the experiment on laryngotracheitis possibly explain the reaction that developed after challenge.

If antibodies on the respiratory epithelium are significant to immunity, the question of the effectiveness of local vaccination should be considered. If an effective accumulation of antibodies in the film of secretions bathing the respiratory

surface had occurred in this experiment, it is probable that less tissue involvement would have followed challenge. Perhaps the difficulty was the incapacity of humoral antibodies to traverse to the surface of the respiratory epithelium. This is a possible correlation to the investigations by Smith, et al. (47) on subcutaneous inoculations of influenza virus in ferrets and their susceptibility on challenge. There is, however, some evidence that some degree of immunity was present in the test and control birds. The disease may have been modified, as evidenced by the presence of minimal symptoms, absence of mortality, and the failure of the infection to influence the feed intake during the three-week period following challenge.

Virus neutralization tests, using serums secured two and six days after challenge, were the only ones that produced isolated foci of infectious laryngotracheitis consistently. The four- and nine-day serum-virus mixtures introduced on the chorio-allantoic membranes produced radiating, confluent lesions. Other workers (4), (17), (20), (23) have noted no immunogenic difference among laryngotracheitis virus strains tested. Although different strains of virus were used in vivo and in ovo studies, there is no reason to suspect that antigenic differences influenced the results obtained. It was observed that partial neutralization of the virus, as compared to the investigations by Burnet, et al. (20), produced small, minute foci in the membranes. The poek counting method has not been extensively used for neutralization tests (10). Vaccinia, herpes, ectromelia,

fowl pox, and myxomatosis of rabbits have been successfully titrated on the chorio-allantois membrane (10).

In evaluating Table 3, more resistance to the virus challenge was observed in the control group than in the bursectomized group. However, a lesser percent of the latter group evidenced marked changes in the larynx and trachea than in the control group. In comparing the two groups, the difference was not significant, but it may suggest that the presence of the bursa of Fabricius was the contributing factor in the development of humoral antibodies. It is generally known that individuals may vary in the ability to form antibodies against a particular antigen (Raffel, 44). This observation is commonly seen in most vaccination experiments or programs. From the standpoint of resistance, certain individual variations in the ability to resist challenge infections may also be seen in actively immunized individuals. The exact nature of the mechanism of the acquired resistance involved in laryngotracheitis is yet to be investigated.

The method for detecting neutralizing antibodies was based on the reduction of membrane involvement in the chorio-allantois of developing eggs inoculated with virus-serum mixtures, as compared to approximate virus-saline inoculated membranes. In Figs. 1 and 2, no appreciable difference was observed in the degree or extent of pathological lesions induced by the various inocula. The amount of virus neutralization in the serums obtained on the second and sixth days following challenge

illustrate a significant difference between the test and control groups. The introduction of virus on challenge in the two groups may have resulted in the production of a greater concentration and a better combining quality of antibodies. The intratracheal route of administration, which is the natural portal of entry for the virus, may have influenced a higher degree of antibody response, in comparison to the cloacal route of vaccination. Lymphoid follicles within the respiratory tract may have been the source of additional antibody production.

The research by Chang, et al. (21) indicates that the bursa of Fabricius is important in antibody production. The results of their research showed that the agglutinating antibody titer for Salmonella typhimurium was lower in the bursectomized group. In comparison, the procedure used in this experiment to detect neutralizing antibodies did not produce unequivocal evidence of the antibody titer in either the bursectomized or the control birds. If an in vitro sensitivity test was available, the serum antibody level for infectious laryngotracheitis could be accurately determined.

SUMMARY AND CONCLUSIONS

The role of the bursa of Fabricius in relation to the immunity of infectious laryngotracheitis was studied by immunological procedures.

It was evident on autopsy that vaccinated fowl in the bursectomized and control groups were not completely immune to

virus challenge. Gross tissue changes in the respiratory tract indicated that an acute infection had occurred after challenge. In comparison, the control group had more resistance to the virus than the bursectomized group.

A significant antibody titer was found in serums from the control birds two and six days after challenge. The chorio-allantois membranes of chicken embryos inoculated with virus and serum, which was secured from the bursectomized group, revealed more extensive lesions than the controls. From these observations, it was apparent that less neutralization had occurred in the bursectomized virus-serum mixtures than in the controls.

Chick neutralization tests with two-day post challenge serums suggested that antibodies were present.

Gross observations of the respiratory tracts on autopsy following challenge and neutralization studies suggested that the bursa of Fabricius contributes to antibody production.

ACKNOWLEDGMENTS

Grateful acknowledgment is given to Dr. J. L. West, Major Instructor, Department of Pathology, who suggested and directed this experimental study; to Dr. Dean S. Folse, Associate Professor of Pathology; and to Dr. M. J. Twiehaus, Head of the Department of Pathology, for their helpful criticism and advice.

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THE ROLE OF THE BURSA OF FABRICIUS IN THE DEVELOPMENT
OF RESISTANCE IN INFECTIOUS LARYNGOTRACHEITIS

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1956

The bursa of Fabricius, a lymphoid structure peculiar to the avian species, may be concerned with the development of resistance to infectious laryngotracheitis. The physiological function of this cul-de-sac is not definitely known although it has been associated with nutrition, haematopoiesis, fertilization, and immunity.

In the experiment, 25 bursectomized and 25 controls of five-week-old White Rocks were vaccinated cloacally with laryngotracheitis virus vaccine, challenged intratracheally with 0.1 cc of 1-2400 dilution of titered virus, and autopsied. In comparison to the bursectomized group, the controls had more protection to the virus challenge as determined by tissue changes on autopsy.

Neutralization tests were conducted with serums obtained before vaccination, four and nine days after vaccination, and two and six days after challenge. Twelve-day-old fertile chicken eggs were inoculated on the chorio-allantois membrane with 0.2 cc of virus-serum mixture. Serums secured two and six days after challenge from the controls revealed a significant antibody titer in comparison to the bursectomized group.

Gross observations of the respiratory tracts after challenge and neutralization tests, using the chorio-allantois technique in fertile eggs and chickens, suggested that the bursa of Fabricius contributes to antibody production.

