Pathologic responses of turkeys to Mycoplasma meleagridis infection

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Pathologic responses of turkeys to *Mycoplasma meleagridis* infection

by

Keith Ray Rhoades

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Ames, Iowa
1971
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INTRODUCTION

Mycoplasma meleagridis is one of the few pathogens included among the many recognized serotypes of avian Mycoplasmata. This organism is apparently pathogenic only for turkeys, and has wide geographic distribution. Mature turkey reproductive tracts are often infected and an important sequela of this infection is transmission of organisms, through eggs, to embryos and subsequently to poults. Air sac lesions occur in infected poults and presently available information indicates that production of these air sac lesions is the most important pathogenic characteristic of M. meleagridis infection.

Relatively little of the research on this disease has been concerned with pathologic responses of either turkeys or turkey embryos to infection. Information on pathologic responses is an important part of an understanding of the pathogenesis of any disease.

The purpose of this study was to provide information on the pathologic responses of turkeys to M. meleagridis infection and consequently to contribute toward an understanding of the pathogenesis of this disease. The study was primarily concerned with responses of those turkey tissues involved in the sequence of transmission of organisms indicated above. Microscopic comparisons of infected and uninfected tissues included: mature turkey reproductive tracts, embryos, and poult air sacs.
LITERATURE REVIEW

History

Early work with avian Mycoplasmata established M. gallisepticum as a major cause of airsacculitis in turkeys (1). Air sac lesions, however, were also often observed in young poult s free of M. gallisepticum infection (2,3,4). Mycoplasmas were usually isolated from these air sacs and the correlation between isolation and air sac lesions suggested that additional Mycoplasma spp. could cause airsacculitis in turkeys (2). Investigation of isolates of a particular type recovered from young poult air sacs resulted in identification and partial characterization of a Mycoplasma sp. which was later to be named M. meleagridis (2,5,6).

The first reported isolation of M. meleagridis was in 1958 (2). Culled turkey poult s from 10 breeding flocks from 4 states were examined for lesions and cultured for mycoplasmas. Some were examined 1 day after hatching; others were examined at 3 weeks. Lesions were found in air sacs of poult s from each of the 10 flocks. The lesions were described as accumulations of yellow caseous exudate which varied from barely detectable foci to masses involving the entire air sac. Mycoplasmata were isolated from poult s of 9 of the 10 flocks represented. Mycoplasma gallisepticum was recovered from poult s of one flock. Mycoplasmas from the other 8 flocks were antigenically similar to each other, but different than
M. gallisepticum. This new antigenic type, M. meleagridis, was designated "N" for purposes of identification. Observations indicated that the incidence of air sac lesions in the pouls correlated with recovery of Mycoplasmata, and that the infection was transmitted through the egg.

Naming

The Mycoplasma sp. designated as "N" strain was included in studies of characteristics of a group of 7 isolates of avian origin (5,6). These isolates were separated into 5 groups, I through V, with the "N" strain being group V.

In a later study (7), 15 isolates of avian origin were serologically compared using agglutination and cross-agglutination tests. The results of this study indicated 8 serotypes which were designated alphabetically, A through H. Strain "N" in this system was serotype H.

Further studies characterizing avian Mycoplasmata increased the number of reported serotypes to 12 (8) and later to 19 (9). Both studies continued the alphabetical system of designating serotypes and further documented the serologic distinctness of serotype H.

The name Mycoplasma meleagridis was proposed in 1965 (10), and consequently has been used to designate this particular serotype of avian Mycoplasmata. The proposed species designation was based on the organism's pathogenic specificity for the turkey, Meleagris gallopavo.
Characteristics

*Mycoplasma meleagridis* is a facultative anaerobe which can be readily isolated from infected tissue using either agar or broth media (10). Giemsa stained organisms are characteristically coccoid (8,10), and approximately 0.4 micron in diameter (8). When cultivated on agar medium the organism forms colonies which are small (8,9,10), with a diameter of 0.04 to 0.2 millimeters (8,10). Studies on growth and survival in broth medium at various pH values indicated that *M. meleagridis* would not grow at initial pH values of 6.4 or 9.3, but would grow at pH values from 7.0 to 8.7. Cultures initiated at the higher pH values of this range remained viable at 37C for longer periods than those at lower pH values (11). Studies of the survival of *M. meleagridis* in an artificially created aerosol indicated that viable organisms could be recovered in gradually decreasing amounts during a 6-hour period (12). Several trials resulted in an average recovery of 0.1% of the original recovery 6 hours after the aerosol was created.

The results of studies (6,7,8,9,13) of the ability of *M. meleagridis* to ferment various carbohydrates have been uniformly negative. The following carbohydrates were included in these studies:

<table>
<thead>
<tr>
<th>Carbohydrate</th>
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<tr>
<td>adonitol</td>
<td>fructose</td>
<td>lactose</td>
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<td>arabinose</td>
<td>galactose</td>
<td>levulose</td>
<td>rhamnose</td>
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<tr>
<td>dextrin</td>
<td>glycerol</td>
<td>maltose</td>
<td>salacin</td>
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<tr>
<td>dextrose</td>
<td>inositol</td>
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<td>sorbitol</td>
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<tr>
<td>dulcitol</td>
<td>inulin</td>
<td>mannose</td>
<td>starch</td>
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</table>
The organism does not reduce tetrazolium (6,8). In studies of ability to cause hemolysis, chicken and turkey erythrocytes were not lysed (6), but horse erythrocytes were lysed (8). Although many isolates have been examined for ability to cause hemagglutination (6,8,9,10), there are reports of only 2 isolates which have had this characteristic (14,15).

Sensitivity of *M. meleagridis* to certain antibiotics has been determined *in vitro* and *in ovo* (16). Tylocin was consistently active *in ovo*; tetracycline, chlortetracycline, and streptomycin were variable; and erythromycin was inactive. When evaluated *in vitro*, tylocin, chloromycetin, tetracycline, and oxytetracycline were active; novobiocin and neomycin were variable; and kanamycin, streptomycin, penicillin, and erythromycin were inactive.

Identification

Although some cultural, biochemical, and biological characteristics can be utilized in presumptive identification of *M. meleagridis*, definitive identification has been dependent on serologic examination (10). Agglutination (2,7,8,9,13), antiglobulin (17), fluorescent antibody (14,18), growth inhibition (9,13), and complement fixation (19) tests have been successfully used for this purpose.

A method which does not utilize serologic examination has been described for identifying mycoplasmas, and presum-
ably could be used to identify *M. meleagridis*. A comparison of cell proteins of several *Mycoplasma spp.*, including *M. gallisepticum*, was made using polyacrylamide-gel electrophoresis (20). The electrophoretic patterns which resulted were described as suitable for identification and classification of *Mycoplasma*.

Distribution

*Mycoplasma meleagridis* is apparently widely distributed among turkey flocks (10), indeed a 1968 report stated that all commercial turkey flocks were infected (21).

This organism has been isolated from a variety of anatomic sites in turkeys, primarily from tissues of the respiratory and reproductive tracts. Isolations have been made from infraorbital sinuses, tracheae, lungs, air sacs, infundibula, magnums, isthmuses, uteri, vaginae, cloacae, semen samples, and excretory papillae of the vasa deferens (15,21,22,23). It has also been recovered from hock joints, lungs, air sacs, and bursae of Fabricius of poults experimentally infected by intravenous inoculation (24).

Yolk and albumen of eggs found in the uterus of infected reproductive tracts have been found to be infected (25), as have yolk sacs of naturally and experimentally infected embryos (14,25,26,27). Isolations have also been made from broken, softshelled, infertile, and dead-germ eggs laid by experimentally infected turkey hens (22). Embryos experi-
mentally infected by way of the yolk sacs have been examined using both immunofluorescent microscopy and isolation techniques to determine the distribution of infection (28). In embryos of 18-day-incubation and older, organisms were found extracellularly attached to yolk sac membranes, tracheae, small bronchi, alveoli, and air sacs. They were also found in the contents of the lumina and along the villi of the lower intestines and in the parenchyma of the bursae of Fabricius.

*Mycoplasma meleagridis* has been isolated from the air sacs of chickens 21 days following exposure by way of the air sacs (29). Infection also occurred in the air sacs and lungs of young chickens, and in pipped embryos and dead-in-shell embryos following yolk sac exposure as 7-day-old embryos (30).

**Transmission**

Transmission of *M. meleagridis* from mature turkeys to progeny through infected eggs occurs commonly (22,25,31). Egg transmission from naturally infected turkeys resulted in a 23.6% incidence of infection in air sacs of pouls examined at the time of hatching (25). Lesions were observed in air sacs of 12% of the pouls. All pouls hatched over a 3 week period were included in this study. Fertile eggs which did not hatch had an infection rate of 34% during this same period.

Another study of 2 groups of naturally infected turkeys revealed isolation rates of 32.6 and 24.2% from progeny (31).
These breeding turkeys had been selected as infected on the basis of serologic examination and microbiological examination of either oviducts or semen.

The pattern of egg transmission in naturally and experimentally infected turkey flocks has been investigated (22). The organism was not readily transmitted in the early part of the laying cycle, but a high level of transmission was rapidly reached. After a peak level of transmission was reached, the level decreased slowly and was low at the end of the laying cycle. So far as egg transmission of this organism by individual infected hens is concerned, there is no consistent pattern (25,31).

Although the manner in which eggs and embryos become infected has not been determined, there have been some suggestions. The localization of infections in uteri was described as perhaps affording a good chance for infection of eggs, since each egg remains longer in the uterus than in other parts of the oviduct after ovulation (25). Semen could perhaps act as a vehicle for carrying M. meleagrisis to the upper part of the oviduct, resulting in infection of the yolk sac before albumen and shell membranes were formed. As spermatozoa fertilize the ovum, the organisms might enter the yolk sac, thereby establishing infection (22).

Attempts to obtain uninfected pouls from infected breeding flocks include selection of breeding turkeys which are free of infection as determined by antemortem microbiological and serological examination (26,32,33), and treating eggs
Reproductive tract infections in hens result readily from insemination with *M. meleagridis* contaminated semen (22,23,25). Once established, reproductive tract infections, involving either oviducts or phalli, persist for long periods (23). Intravaginal exposure resulted in infections which persisted for at least 8 weeks, and experimentally induced phallic infections persisted for at least 49 days, the lengths of the test periods. The phallic infections of the 2 toms included in this study did not result in infection of either the vasa deferens or testicles. Infection in reproductive tracts of unmated turkeys of both sexes has also been reported (21,32), indicating that reproductive tract infections do not result only from venereal transmission.

In a recent study (35) female turkeys exposed via cloacae and bursae of Fabricius developed oviducal infections at a time which was closely correlated with perforation of cloacal-vaginal occluding membranes. Infection ascended the reproductive tracts of both inseminated and uninseminated hens, indicating that spermatozoa were not responsible for spread of infection.

Nonvenereal contact between turkeys may also result in transmission of this organism (22,35). Usually this type of transmission results in respiratory tract infections, but reproductive tract infections also have resulted (22,36). A study in which 19-day-old embryos were experimentally
inoculated with *M. meleagridis* and incubated and hatched with uninoculated embryos resulted in intra-incubator contact transmission (22). The incidence of recovery of the organism from the uninoculated pouls was low, and no air sac lesions were found. The results of a prior similar study did not indicate intra-hatcher transmission, but contact transmission did occur between pouls following hatching (36).

**Serology**

Serologic tests to determine the presence of antibodies against *M. meleagridis* have been used to evaluate immune responses and as an aid in diagnosing infection. The plate and tube agglutination tests have been used most commonly (21,25,31,36,37), although hemagglutination-inhibition tests have also been used (14,38).

Studies (38) of serologic responses of turkeys to experimentally induced infection have indicated that antibodies of high molecular weight, IgM, were produced within 4 to 6 days after exposure. Low molecular weight antibodies, IgG, were not detected until 10 to 14 days after exposure. Circulating antibodies at 3 to 4 weeks after exposure consisted primarily of IgG, although detectable levels of IgM persisted. Fractionation of selected serums and treatment with 2-mercapto-ethanol indicated that the plate agglutination test is more efficient in detection of IgM than in detection of IgG antibodies. Tube agglutination tests measured both types of
antibodies, whereas the hemagglutination-inhibition test detected only IgG.

Poults infected as 19-day-old embryos had detectable plate agglutination titers at 1 week of age, but tube agglutination titers could not be detected until 3 weeks of age. Hemagglutination-inhibition and 2-mercaptoethanol-resistant tube agglutination titers did not appear until 6 weeks of age. Thus, poults infected as embryos had an early but depressed IgM response, and the IgG response was delayed until 6 weeks of age.

Serologic examination of poults, infected as 14-day-old embryos, first revealed agglutination 3 weeks after hatching (35). Poults exposed by contact with these experimentally infected poults first developed agglutinins 5 weeks after hatching. Both plate and tube agglutination tests were employed at 3, 5, 7, 9, and 10 weeks.

Serologic responses of experimentally infected turkeys have been determined at various intervals after exposure (37). The tube agglutination test was used to determine and compare antibody titers of birds exposed by either intravenous inoculation, air sac inoculation, combined inoculation of trachea and sinus, or by a combination of all these exposure methods. Each group consisted of 3 turkeys. Maximum antibody titers occurred in all groups 4 weeks after exposure, and titers of all groups were high. Of the 4 groups, titers were highest in those exposed intravenously and lowest in those exposed
by way of the air sacs. Turkeys experimentally infected by reproductive tract exposure and naturally infected poults had much lower antibody titers than those described above. The serum plate test was described as useful for screening purposes, but not as sensitive as the tube agglutination test.

Young chickens experimentally exposed to *M. meleagridis* at 1 and 5 days after hatching had specific serum agglutinins at 21 days (29). However, the number of chicks which developed demonstrable antibody was small, and the antigen-antibody reactions which occurred were described as weak.

Passive agglutinins against *M. meleagridis* have been reported in turkey poults hatched from infected breeding flocks (31,33,37). Presence of this antibody was not believed to interfere with infection or lesion development (37).

Pathogenicity

In early studies of *M. meleagridis* infection in poults, the correlation between isolation of the organism and the occurrence of air sac lesions was presumptive evidence that *M. meleagridis* was pathogenic (2,31). Later the development of air sac lesions in experimentally exposed poults substantiated the validity of this conclusion (8,9,33,39).

Lesions in poults experimentally infected by air sac inoculation were macroscopically identical to those of natural infections (39). The affected air sacs were thickened and contained yellow purulent to caseous exudate. Microscopic
examination indicated that the air sacs were 8 to 20 times thicker than normal. Generalized lymphocytic infiltration and many large lymphofollicular areas were observed, as were increased vascularity and increased amount of connective tissue.

Air sac lesions also occurred in pouls following exposure to *M. meleagris* during embryonic development (33). Varying concentrations of organisms were inoculated into yolk sacs of turkey embryos, and pouls which developed from these embryos were examined for lesions and mycoplasmas. The incidence of air sac lesions in pouls from 3 hatches varied from 37 to 100%, with a mean of 72.4%.

The organism was generally isolated from lesions. There was no significant difference in incidence of air sac lesions between pouls from embryos inoculated after 7 or 14 days incubation. Inoculation of embryos with as few as 0.685 colony forming units resulted in air sac lesions, indicating that very few organisms are necessary to produce infection.

Pouls hatched from naturally infected eggs were included in this study, and the incidence of air sac lesions under these circumstances ranged from 7 to 28%, with a mean of 18%. Hatchability of fertile eggs, both experimentally and naturally infected, was apparently not affected. Although air sac lesions were found in pouls and pipped embryos, they were rarely found in dead embryos, and only 1 poult in the study developed clinical signs of respiratory involvement.
Gross lesions were found more frequently in clavicular and thoracic air sacs than in abdominal air sacs. The air sacs were thickened and had yellow caseous material attached to the walls and free in the lumina. Microscopically there was thickening of the air sac walls with heavy lymphocytic infiltration and multiple areas of lymphofollicular reactions which were described as resembling encapsulated lymph nodes.

Poults intravenously inoculated with *M. meleagridis* developed air sac and hock joint lesions (24). Affected air sacs were cloudy and contained creamy exudate. Examination of hock joints at various intervals after inoculation revealed lesions in 8 of 16 inoculated poults. Mild infiltration of heterophils and mononuclear cells was present in subcutaneous intertendonal and periarticular tissue in 3 of 4 poults examined 1 week after exposure. The mononuclear cells were primarily lymphocytes, with a few plasma cells and macrophages. In some instances the lymphocytes had accumulated around arteries and in periarticular tissues. In 1 of 4 poults killed 2 weeks after inoculation there was infiltration, primarily in the synovial area, with heterophils, lymphocytes, and plasma cells. Two birds at both 4 and 6 weeks after exposure had lymphofollicular foci in the periarticular tissue and at the crypt of the synovial membrane. In the hock joint there were synovial cell hypertrophy and hyperplasia, with infiltration by lymphocytes, plasma cells, and macrophages. In addition, a poult examined 6 weeks after exposure had a
mild proliferation of granulation tissue around the synovial membrane and articular cartilage.

Air sac lesions resulting from *M. meleagridis* infection eventually become resolved. In a flock of naturally infected pouls, lesions were present in 20% of those pouls examined 1 day after hatching, 44% of those examined at 6 weeks, but in only 0.07% of those examined at 20 1/2 weeks (40).

The histologic response of turkey oviducts to certain pathogenic agents, including *M. meleagridis*, have been described (41). Small lymphoid foci were found in 11 of 13 oviducts from *M. meleagridis* infected hens included in the study. The foci were most frequently found in vaginae and infundibula, and were accompanied by slight to moderate increases in numbers of plasma cells. Perivascular cuffing with lymphocytes, plasma cells, and heterophils was fairly common.

Mature turkey hens with infected reproductive tracts have been studied to determine the effect of fertility (42). No relationship was found between infection and level of fertility, and no consistent relationship was found between infection and lymphofollicles in the lower sections of the oviducts. There was a significant relationship between the presence of lymphofollicles in the vaginae and lowered fertility.

Histologic examination of phallic structures and ampulla of the ductus deferens of experimentally infected male turkeys
has revealed only slight changes (43).

Lesions apparently are not produced in infraorbital sinuses, tracheae, and lungs of turkeys infected with *M. meleagridis*, even though the organism has been isolated from these tissues (22,23,24), and sinuses (44,7) and tracheae (7) have been used as sites for experimental exposure. Reports of a small firm reddish area in the posterior lobe of a poult lung following intravenous exposure (24), and a small amount of exudate in an infraorbital sinus of 1 poult of a group of 20 exposed by way of infraorbital sinuses (44) are the only available descriptions of lesions involving these tissues in *M. meleagridis* infected turkeys.

Young chickens inoculated by way of the air sacs with *M. meleagridis* did not develop grossly observable lesions (29). However, young chickens which were experimentally exposed as embryos developed air sac lesions which were grossly similar to those observed in naturally infected turkey poult.s (30). These lesions were believed to result because the naturally resistant chicken host was exposed at a stage when the defense mechanism was not yet operative. The greater susceptibility of the turkey, regardless of the stage of development, was considered to suggest that it was the natural host for *M. meleagridis*. 
MATERIALS AND METHODS

Turkeys

Toms

Mature, Broad-Breasted-Bronze toms were obtained from the Mycoplasmata-free flock of the Veterinary Medical Research Institute (VMRI), Iowa State University, Ames, Iowa. Twelve were exposed to M. meleagridis via the surface of the phallus, while 4 remained unexposed and served as noninfected controls. Exposed and unexposed groups were maintained in separate isolation rooms.

Tracheae and phalli of the exposed toms were cultured for mycoplasmas and blood samples were taken for serologic examination at 2-week intervals.

At 4, 8, and 12 weeks after exposure, 4 exposed toms were killed by exsanguination. Reproductive and respiratory tracts were cultured for mycoplasmas and examined for gross lesions. Reproductive tract tissues were fixed in 10% formalin and processed for microscopic examination. Those tissues examined microscopically were: testicle, epididymus, anterior vas deferens, posterior vas deferens, and phallic structures including the excretory papilla. The unexposed controls were treated in a similar manner at the termination of the 12 week study.
Hens

The mature Broad-Breasted-Bronze hens used in the study were also obtained from the VMRI flock. They were randomly separated into two groups and each group housed in a separate isolation room. Each hen was maintained in a separate cage, but the cages were in close apposition. One group of 20 hens was exposed intravaginally with a broth culture of *M. meleagridis*. The 10 hens of the other group were maintained as unexposed controls.

Tracheae and vaginae of the exposed hens were cultured for mycoplasmas and blood samples were taken for serologic examination prior to exposure and at 2-week intervals thereafter. The unexposed hens were examined in a similar manner at 4-week intervals.

All hens were artificially inseminated every other week with semen obtained from toms maintained in the VMRI flock. Semen samples were cultured for mycoplasmas each time the birds were inseminated.

At the end of the laying period, 10 to 12 weeks after exposure, the hens were killed by exsanguination, examined for gross lesions, and cultured for mycoplasmas. Reproductive tracts were fixed in 10% formalin and processed for microscopic examination. Those tissues examined were: ovary, infundibulum, anterior magnum, middle magnum, posterior magnum, isthmus, uterus, and vagina.
**Embryos and eggs**

Eggs from both the exposed and unexposed hens were collected twice daily and identified with the number of the hen which laid them and the date. They were stored at 25°C until incubation at weekly intervals.

Separate incubators were used for incubating eggs from each of the 2 groups of hens. Eggs laid during the first 2 weeks after exposure were incubated for 24 days, and cultured for mycoplasmas. After the first 2 weeks, the eggs laid each week were randomly divided into 4 groups, incubated for 1, 2, 3, or 4 weeks, opened, examined for fertility and viability, and cultured for mycoplasmas.

Embryos viable at the time of examination were preserved for possible later study. Approximately half were fixed in 10% formalin, the others were wrapped in aluminum foil, frozen at -65°C, placed in plastic bags, and stored at -65°C. Following examination for mycoplasmas, the infected embryos and a similar number of uninfected control-embryos were sectioned, stained and observed histologically. Tissue sections from the formalin fixed embryos were examined to determine if lesions resulted from infection, while sections from the frozen embryos were observed, using immunofluorescent microscopy, to determine the distribution of infection. Sections of control-embryo tissues were examined for comparative purposes.

Turkey embryos from the Small-Beltsville-White breeding
flock of the National Animal Disease Laboratory (NADL), Ames, Iowa, were also used to determine the lesions resulting from infection. The breeding flock from which they were obtained was serologically free of antibodies against *M. meleagridis* and *M. gallisepticum*, and was apparently disease free. Embryos were inoculated via the yolk sac, with either broth culture of *M. meleagridis* or sterile broth. Those inoculated with sterile broth served as unexposed controls.

The schedule for inoculation and examination of these embryos and the number examined are indicated in Table 1.

Table 1. Schedule of inoculation and examination of turkey embryos

<table>
<thead>
<tr>
<th>Number of embryos</th>
<th>Inoculum</th>
<th>Age when inoculated</th>
<th>Age when examined</th>
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<tr>
<td>5</td>
<td><em>M. meleagridis</em></td>
<td>1 week</td>
<td>3 weeks</td>
</tr>
<tr>
<td>5</td>
<td><em>M. meleagridis</em></td>
<td>1 week</td>
<td>4 weeks</td>
</tr>
<tr>
<td>5</td>
<td><em>M. meleagridis</em></td>
<td>2 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>5</td>
<td>Sterile broth</td>
<td>1 week</td>
<td>3 weeks</td>
</tr>
<tr>
<td>5</td>
<td>Sterile broth</td>
<td>1 week</td>
<td>4 weeks</td>
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</table>

At the appropriate time, the embryos were killed by exsanguination, allantoic fluid and yolk material were cultured for mycoplasmas, and the embryos were fixed in 10% formalin and processed for microscopic examination.
Experimentally infected embryos from the NADL flock were used to compare distribution of infection with the naturally infected embryos. Five embryos, incubated for 1 week, were inoculated via the yolk sac with *M. meleagridis*. When the embryos had been incubated for 3 weeks, they were killed by exsanguination, cultured for mycoplasmas, and frozen at -65°C as previously described. Frozen-sections from the embryos were examined for distribution of infection using immunofluorescent techniques. An equal number of non-exposed embryos were examined in the same manner for comparative purposes.

**Poults**

Poults from the NADL flock were used to study air sac lesions resulting from *M. meleagridis* infection. Twenty-eight poults, 7 weeks old, were inoculated via the left lesser abdominal air sac with 0.1 ml of a broth culture of *M. meleagridis*. Fourteen poults were inoculated in the same manner with 0.1 ml of sterile broth and served as noninfected controls.

At intervals of 1, 2, 4, 8, 16, 24, and 32 days after inoculation, 4 exposed and 2 control poults were killed, cultured for mycoplasmas and other bacteria, and examined for gross lesions. One portion of each lesser abdominal air sac was fixed in 10% formalin, another was frozen and
stored at -65°C, and a third portion was fixed in 2.5% gluteraldehyde. A segment of left lung including diaphragm and adjacent lesser abdominal air sac tissue was also fixed in formalin. Formalin fixed tissues were processed and examined for lesions using light microscopy, frozen tissues were processed and examined using immunofluorescent microscopy, and the gluteraldehyde fixed tissues were processed and selected sections examined using transmission electron microscopy.

The names used in this study to indicate various air sacs are those previously described for turkeys (45). They are the aggregate air sac and paired lesser and greater abdominal air sacs. The aggregate air sac is a single compartment which includes the single anterior thoracic and paired posterior thoracic and thoraco-cervical air sacs.

Inoculum and Exposure Techniques

Mycoplasma meleagridis, isolate 8M92, was obtained from Dr. M. L. Frey (VMRI). The isolate was cultivated in broth, a sample was transferred to agar, incubated, and a single colony selected and cultivated in broth. This process of selection and subsequent cultivation was accomplished 3 times to obtain a pure culture. The culture was dispensed in 1 ml amounts in small vials and frozen and stored at -65°C until needed. At appropriate times the frozen cultures were thawed at room temperature and used to inoculate 10 ml of broth. After incubation for 24 hours, 1 ml of broth culture was
transferred to 10 ml of broth which was incubated for 24 hours and used as inoculum.

The exposed hens each received 0.25 ml of broth culture containing approximately $3.5 \times 10^8$ organisms per ml. The exposed toms each received 0.25 ml of broth culture containing approximately $5.4 \times 10^8$ organisms per ml. Each of the experimentally exposed embryos received 0.1 ml of broth culture. Those inoculated after 1 and 2 weeks incubation and used to evaluate lesion development received broth cultures which contained approximately $2.0 \times 10^7$ and $6.5 \times 10^6$ per ml, respectively. Those embryos used to evaluate distribution of infection received broth containing approximately $1.4 \times 10^7$ per ml. The poult's were exposed by inoculating 0.1 ml of broth containing approximately $3.5 \times 10^7$ organisms per ml into the left lesser abdominal air sac.

The vagina or phallus to be inoculated was exposed by applying pressure to the abdomen and partially everting the cloaca. The inoculations were made with 1 ml syringes without needles. Embryo yolk sacs were inoculated by directing a 23 gauge 1½ inch needle through the air space into the yolk sac (46). The left lesser abdominal air sacs were inoculated using 1 ml syringes and 26-gauge 3/8-inch needles. The needles were directed perpendicularly between the 5th and 6th ribs at the point of vertebral and sternal articulation.

A broth culture of *M. meleagridis*, isolate 8M92, was also examined by electron microscopy to determine morphology. The techniques used are included in the section on electron microscopy.
Media

The broth medium used for cultivating Mycoplasma was slightly modified from that previously described (13). It consisted of: distilled water, 100 ml; yeast autolysate, 2 gm.; proteose peptone no. 3, 2 gm.; heart infusion broth, 2 gm.; thallium acetate, 1:2000; and sufficient sodium hydroxide (1N) to adjust the pH to 7.8. This material was mixed and autoclaved to sterilize. After it cooled, 10 ml of filter-sterilized horse serum and 50,000 units of penicillin were added. Generally this medium was dispensed in 10 ml amounts in screw capped test tubes, but 1 liter volumes were used for cultivation of Mycoplasma for antigen production.

The agar medium used for Mycoplasma was described previously (29). It consisted of: PPLO agar, 3.4 gm.; distilled water, 100 ml; yeast autolysate, 1 gm.; thallium acetate, 1:2000; and sufficient sodium hydroxide (1N) to adjust the pH to 7.8. This material was autoclaved, cooled to 45°C, horse serum and penicillin were added as indicated above, and the warm medium poured into Petri dishes. The plates of agar medium were stored at 5°C in containers to prevent drying. All plates were used within a week of being prepared.

Blood agar plates were prepared using blood agar base, 2

1 Albimi Laboratories, 35-22 Linden Place, Flushing 54, New York.
2 Difco Laboratories, Inc., Detroit, Michigan.
as recommended by the manufacturer, and 5% defibrinated bovine blood.

Isolation Techniques

**Mature turkeys**

**Antemortem**  
Sterile, moist, cotton swabs were used to obtain material for antemortem examination for mycoplasmas. The swabs were inserted into the anterior trachea, everted vagina, or phallic area, and the mucosa swabbed. Each swab was then used to streak agar media and was twirled in broth. The agar plates were incubated in candle-jars at 37°C for 6 days and examined for mycoplasmal colonies. After the broth had been incubated for 6 days, 0.1 ml was transferred to fresh agar medium which was incubated for 6 days and examined. When growth of mycoplasmas occurred on agar, single colonies were selected and transferred to fresh broth. This broth was then incubated for 10 days, frozen, and maintained at -65°C for later study.

**Postmortem**  
Postmortem examination of turkey tissues for mycoplasmas involved essentially the same techniques as described above. The tissue to be examined was seared with a small heated spatula, then an opening made with a heat-sterilized scalpel blade. A swab was introduced into the tissue and used to inoculate media. The following tissues from each turkey were examined: infraorbital sinus, anterior trachea, middle trachea, posterior trachea, lung, inter-
clavicular air sac, thoracic air sac, and abdominal air sac. Additional hen tissues examined included yolk material from 2 mature ovarian follicles, infundibulum, anterior magnum, middle magnum, posterior magnum, isthmus, uterus, vagina, cloaca, and hock joint.

Two areas of each hen's peritoneal cavity were examined, the posterior ventral abdominal area and the ovarian surface. The testicular serosa and parenchyma, anterior vas deferens, posterior vas deferens, and phallus of each tom were also examined.

**Embryos**

All unbroken and uncracked eggs from both exposed and unexposed hens were incubated and examined later for mycoplasmas. Egg shell surfaces were wiped with 70% ethyl alcohol and allowed to air dry. The air space end of the shell was opened with sterile forceps and scissors. The yolk of infertile eggs, tissues and fluids of dead embryos, and allantoic fluid and yolk material of viable embryos were cultured by introducing sterile cotton swabs into the tissue or fluids and using the cultivation techniques described above.

Allantoic fluid and yolk material of each of the experimentally infected and noninfected control embryos were examined for mycoplasmas as described above, except that only agar medium was used in isolation attempts.
Poults

Postmortem examination of the poults for Mycoplasmata was conducted as described for postmortem examination of the mature turkeys. Those tissues examined were: left infraorbital sinuses, hocks, middle tracheae, left lungs, and left and right lesser abdominal air sacs. Left lesser abdominal air sacs were also examined for bacteria other than mycoplasmas, using blood agar medium and aerobic incubation.

Serologic Techniques

Serum samples from the mature turkeys were examined for antibodies against *M. meleagridis* using a tube agglutination test. Antigen was prepared using *M. meleagridis*, isolate 529, obtained from Dr. R. Yamamoto, University of California, Davis, California. Previously described techniques for preparing antigen and conducting the test (8) were employed.

Serum samples from the poults were examined for *M. meleagridis* antibodies using a hemagglutination-inhibition test. Antigen was prepared from *M. meleagridis*, isolate E-2. Antigen was prepared and the test conducted as previously described (14).

Identification of Isolates

Tube agglutination tests and immunofluorescent microscopy were used to identify the Mycoplasmata isolated during the study. Antigens prepared from selected isolates were used
in tube agglutination tests with both anti-*M. meleagris*dis and normal rabbit serum. Agglutination titers obtained were compared with those obtained using antigens prepared with *M. meleagris* isolates 8M92 and 529. If the titers were similar the isolates were considered to be *M. meleagris*. The techniques for preparing antigen and conducting the tests were the same as those used for serologic examination. The immunofluorescent techniques used in examining the isolates are included in the description of immunofluorescent microscopy.

Histologic Techniques

**Light microscopy**

Most tissues examined using light microscopy were fixed in 10% formalin at 5C. The exceptions were hematoxylin and eosin stained frozen-sections which were used in determining the location of areas of specific fluorescence in the immunofluorescent studies. After fixation in formalin the tissues were trimmed to approximately 5 mm thick. Reproductive tracts of both hens and toms were, in general, trimmed transversely. The phallic area was cut obliquely to include one ejaculatory duct and adjacent phallic structures.

Embryos were also trimmed transversely, generally into 5 or 6 segments. Air sac tissues from the poult's were not cut into segments, because of the small amount of tissue involved. A portion of the posterior surface of the left lung
of each poult was trimmed to obtain a piece of tissue which included lung, thoraco-abdominal diaphragm, and lesser abdominal air sac.

The trimmed tissues were dehydrated, infiltrated, embedded, and sectioned using conventional techniques. Tissue sections were routinely stained with hematoxylin and eosin, and selected sections were stained with Gomori's trichrome and methyl green pyronin stains using previously described staining procedures (47).

**Immunofluorescent microscopy**

All immunofluorescent studies of tissues were made with frozen sections. The frozen unfixed tissues to be sectioned were trimmed as described for the formalin fixed tissues and embedded in chicken egg albumen. The technique used for embedding the tissues was similar to that previously described (48). The albumen from several chicken eggs was thoroughly mixed using a magnetic stirrer, filtered through 2 layers of cheese-cloth, and stored at 5°C. Frozen tissue to be embedded was placed in a small aluminum-foil boat, which was then filled with albumen and frozen at -65°C. Sections were cut from the frozen albumen-embedded tissues using a cryostat at approximately -20°C. Sections of most air sac tissues were cut 6 microns thick, while sections of embryos varied from 6 to 10 microns. At least 5 sections were cut from each block of tissue. The frozen sections were picked up from the cryo-
stat microtome blade with warm slides, allowed to thaw, and were dried for a few minutes at 37°C. Sections to be stained with fluorescent antibody (FA) were fixed in acetone for 2 minutes, allowed to dry, and were stored at 5°C until used. Sections from the same block of tissue were also fixed in either 10% formalin or absolute alcohol and were stained with hematoxylin and eosin. Following FA staining and examination, some sections were also restained with hematoxylin and eosin. These hematoxylin and eosin stained frozen-sections were used to verify the anatomic location of fluorescence observed in immunofluorescent examination.

Immunofluorescent techniques were also used to identify cultures of *Mycoplasma*. Smears of mycoplasmas, either colonies from agar cultures or sedimented organisms from broth cultures, were examined using FA against *M. meleagridis*. This procedure was to verify that those organisms isolated following exposure to *M. meleagridis* were also *M. meleagridis*. Techniques used to fix and stain the smears for FA examination were the same as those used on the tissue sections.

To verify immunological specificity of the FA against *M. meleagridis*, 3 control measures were used: (1) fluorescein conjugated normal turkey gamma globulin was used to stain selected tissue sections and smears; (2) tissue sections from unexposed control poultts and embryos were examined with FA against *M. meleagridis*; and (3) a one-step blocking technique (49) was used. Using the one-step blocking technique unlabeled
turkey serum with antibodies against *M. meleagris* should
block the specific fluorescence of FA against *M. meleagris*,
while unlabeled normal turkey serum should not affect this
specific fluorescence. Previous studies (14,18) using immuno-
fluorescent techniques indicated that *M. meleagris* was dis­
tinct from other avian *Mycoplasma*.

Staining of tissue sections with FA was accomplished by
flooding the tissue with an appropriate concentration of FA
in phosphate buffered saline (PBS) at a pH of 7.2. The appro­
priate concentration was determined by staining the antigen
against which the antibody was prepared with varying dilutions
of FA and selecting the highest dilution at which maximum flu­
orescence was observed. The FA flooded tissue was incubated
in a moist chamber at 37°C for 1 hour. It was then washed 3
times in PBS, washed once in distilled water, and air-dried at
37°C. Cover slips were applied using a solution of 50% glycerin
in PBS as mounting medium. Sections were examined using con­
ventional immunofluorescent microscopy techniques.

Fractionation of serum gamma globulins and subsequent
conjugation of the globulins with fluorescein isothiocyanate
was accomplished as previously described (50,51). Pooled
serums from 4 turkeys with high antibody titers against *M.
meleagris* were used to prepare FA against *M. meleagris*.
Serum from an apparently normal turkey with no evidence of
circulating antibody against *M. meleagris* was used to pre­
pare fluorescein conjugated normal turkey gamma globulin.
The FA against *M. meleagris* served to determine sites of
infection in tissue sections and to identify mycoplasmal isolates. The FA prepared from normal turkey globulin served as a control measure in determining if the FA against *M. meleagris* was specific.

**Electron microscopy**

Tissues to be examined with the electron microscope were trimmed to approximately 1 mm size, fixed 1 hour in 2.5% gluteraldehyde, and washed 3 times in sodium cacodylate buffer at pH 7.4. This was followed by post fixation in 2% osmium tetroxide and 2 more washings in buffer. They were then dehydrated in ethanol and embedded in epon (52).

Sections of epon embedded tissue were cut approximately 1 micron thick and stained with basic fuchsin and methylene blue (53). These stained sections, still in epon, were examined with a conventional light microscope. From this examination certain tissues were selected for further study. Thin sections, cut from these selected tissue samples, were stained with lead citrate and uranyl acetate (54), and examined with an electron microscope.

Thin sections of *M. meleagris*, isolate 8M92, were also examined with an electron microscope. A sufficient quantity of 25% gluteraldehyde was added to a 24-hour broth culture to result in a 2.5% concentration. The culture was fixed for 1 hour at 4°C, then centrifuged. The resulting pellet of organisms was washed, post fixed, rewashed, dehydrated, embedded, and examined as described above.
RESULTS

Mature Turkeys

Clinical signs

No clinical signs of infection were observed during the study.

Microbiological examination

*Mycoplasma meleagridis* was isolated, at biweekly intervals, from the phalli of all inoculated toms. No isolations were made from phalli of the unexposed controls. Examination of tracheae resulted in isolation of *M. meleagridis* from 3 toms. Table 2 indicates the time of isolation after exposure. No mycoplasmas were obtained from pre-exposure isolation attempts or from the unexposed controls.

Table 2. Isolation of *Mycoplasma meleagridis* from tracheae of tom turkeys

<table>
<thead>
<tr>
<th>Turkey number</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
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<tbody>
<tr>
<td>31362</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3463</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3464</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>nc^a</td>
<td>nc</td>
</tr>
</tbody>
</table>

a Not cultured. Turkey 3464 was killed and examined 8 weeks postexposure.
At postmortem examination, *M. meleagridis* was isolated from phalli of all inoculated toms. No isolations were made from testicles, anterior vasa deferens, or posterior vasa deferens of any of the toms. The only other isolations were from the respiratory tracts of the 3 toms from which ante-mortem tracheal isolations had been made. The isolations were from the tracheae of 2 toms, the sinuses of 1, and the interclavicular air sac of 1. Mycoplasmas were not isolated from the unexposed toms.

The results of antemortem examination of tracheae and vaginae of the hens from mycoplasmas are presented in Table 3.

<table>
<thead>
<tr>
<th>Weeks after exposure</th>
<th>Exposed Tracheae</th>
<th>Vaginae</th>
<th>Control Tracheae</th>
<th>Vaginae</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3/20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20/20</td>
<td>nc&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nc</td>
</tr>
<tr>
<td>4</td>
<td>3/20</td>
<td>20/20</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>6</td>
<td>3/20</td>
<td>20/20</td>
<td>nc</td>
<td>nc</td>
</tr>
<tr>
<td>8</td>
<td>3/20</td>
<td>16/20</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>10</td>
<td>0/20</td>
<td>13/20</td>
<td>nc</td>
<td>nc</td>
</tr>
<tr>
<td>12</td>
<td>0/13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12/13</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of isolations/number of hens examined.

<sup>b</sup>Not cultured.

<sup>c</sup>Seven hens were killed and examined before 12 weeks postexposure.
No mycoplasmas were isolated from any of the hens prior to the time of exposure. The 12 isolations of *M. meleagridis* from tracheae indicated in Table 3 were made from 7 exposed hens. Table 4 indicates the hens from which *M. meleagridis* was isolated and the time of isolation. Mycoplasmas were not isolated from any of the semen samples used during the study.

Table 4. Distribution and time of isolation of *Mycoplasma meleagridis* from turkey hen tracheae

<table>
<thead>
<tr>
<th>Turkey number</th>
<th>Weeks after exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>3407</td>
<td>+a</td>
</tr>
<tr>
<td>3413</td>
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<tr>
<td>3414</td>
<td>-</td>
</tr>
<tr>
<td>3416</td>
<td>-</td>
</tr>
<tr>
<td>3417</td>
<td>-</td>
</tr>
<tr>
<td>3419</td>
<td>+</td>
</tr>
<tr>
<td>3421</td>
<td>+</td>
</tr>
</tbody>
</table>

*aMycoplasmas isolated.*

*bNo mycoplasmas isolated.*
Postmortem examination of tissues from the exposed hens resulted in isolation of *M. meleagridis* from the mid-tracheal tissue of one hen (no. 3416), an abdominal air sac of another (no. 3414), and infraorbital sinus of a third (no. 3421). With these exceptions, no isolations were made from the respiratory tracts and associated structures, or from the hock joints. *Mycoplasma meleagridis* was isolated from the peritoneal cavities of 7 of the exposed hens. It was isolated from the ventral abdominal areas of 2 hens, the ovarian surfaces of 2, and from both areas of 3 hens. The results of examination of reproductive tracts are represented in Figure 1. No isolations of mycoplasmas were made from the unexposed hens.

**Identification of Mycoplasmata isolates**

Using a tube agglutination test, 46 isolates from the mature turkeys were examined. Twenty were from hen reproductive tracts, 12 were from phalli, and 14 were from respiratory tracts of both hens and toms. All 46 were identified as *M. meleagridis*.

**Serologic examination**

Antibodies against *M. meleagridis* could be demonstrated with the tube agglutination test in serum samples from only 2 of the inoculated toms. Table 5 presents the reciprocals of antibody titers of these 2 toms. Antibodies could not be demonstrated in pre-inoculation serum samples or in samples
Table 5. Reciprocals of *Mycoplasma meleagridis* antibody titers in turkey toms

<table>
<thead>
<tr>
<th>Turkey number</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>3462</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>3463</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>40</td>
<td>20</td>
</tr>
</tbody>
</table>

*No evidence of antibody at the lowest serum dilution (1:10) used.*

from the unexposed toms.

Antibodies against *M. meleagridis* were demonstrated in serum samples from all exposed hens. No evidence of specific antibody was found in the samples from the unexposed hens, or in pre-exposure serum samples from all hens. Average antibody titers of the exposed hens are represented in Figure 2.

**Gross lesions**

No gross lesions attributable to *M. meleagridis* infection were observed in either hens or toms. Several of the hens, both exposed and unexposed, had pieces of yellowish caseous material in peritoneal cavities and lumina of reproductive
Figure 1. Postmortem isolations of *Mycoplasma meleagridis* from the reproductive tracts of 20 experimentally exposed turkey hens

Figure 2. Average *Mycoplasma meleagridis* antibody titers in experimentally exposed turkey hens
ISOLATIONS OF MYCOPLASMAS

0 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

Yolk Material
Surface of Ovary
Infundibulum
Anterior Magnum
Middle Magnum
Posterior Magnum
Isthmus
Uterus
Vagina

WEEKS AFTER EXPOSURE

AVERAGE ANTIBODY TITER

320
160
80
40
20
10

2 4 6 8 10 12
tracts. Sizes of these particles varied from a few millimeters to approximately 1 centimeter.

**Microscopic lesions**

Examination and comparison of infected and noninfected hen reproductive tracts indicated that accumulations of lymphocytes were the most significant histologic response to infection. Three types of accumulations were observed: focal encapsulated (Figures 3 and 4), focal nonencapsulated (Figures 5 and 6), and diffuse (Figures 7 and 8).

The encapsulated accumulations, lymphoid follicles, were found only in infected reproductive tracts (Figures 3, 4, 9, 10, 11, 12, 13). Their occurrence in different areas of the tracts was as follows: ovaries 20%, infundibula 70%, anterior magnums 5%, middle magnums 15%, posterior magnums 30%, isthmuses 40%, uteri 80%, and vaginae 70%. The focal nonencapsulated aggregates were found, in similar frequency, in both infected and noninfected reproductive tracts. The diffuse accumulations were also found in both, but the incidence in infected tracts was 4 times that of noninfected tracts.

The encapsulated accumulations were predominantly medium sized lymphocytes with small amounts of pyroninophilic cytoplasm. The cells of the diffuse areas varied in size with a moderate number being pyroninophilic, while those of the nonencapsulated aggregates were predominantly small, dense, and nonpyroninophilic.
Figure 3. Lymphoid follicles in a section of infundibulum from a *Mycoplasma meleagridis* infected hen. 

x 125

Figure 4. Higher magnification of a portion of Figure 3. 

x 312.5
Figure 5. Focal nonencapsulated aggregate of lymphocytes in a section of uterus from an uninfected hen. x 125

Figure 6. Higher magnification of a portion of Figure 5. x 312.5
Figure 7. Diffuse accumulation of lymphocytes in a section of infundibulum from an uninfected hen. x 125

Figure 8. Higher magnification of a portion of Figure 7. x 312.5
Figure 9. Numerous lymphoid follicles in a section of ovary from a *Mycoplasma meleagridis* infected hen. x 50

Figure 10. Lymphoid follicles in a section of posterior magnum from a *Mycoplasma meleagridis* infected hen. x 50
Figure 11. Lymphoid follicle in a section of isthmus from a *Mycoplasma meleagridis* infected hen.  x 50

Figure 12. Lymphoid follicles in a section of uterus from a *Mycoplasma meleagridis* infected hen.  x 50
Figure 13. Lymphoid follicles in a section of vagina from a Mycoplasma meleagridis infected hen. x 50

Figure 14. A section of yolk material within the lumen of the isthmus of an uninfected hen. x 50
Plasma cells and heterophils were observed in the lamina propria of both infected and noninfected reproductive tracts, but appeared to be more numerous in the infected tracts. Variation in numbers among turkeys, within each tract, and in different areas of the same tissue section made evaluation of their significance difficult.

Microscopic examination of the caseous material which had been observed grossly in hen peritoneal cavities and lumina of reproductive tracts indicated that it was inspissated yolk (Figure 14) rather than exudate.

Microscopic examination of reproductive tracts of the male turkeys did not reveal lesions attributable to *M. meleagridis* infection. Lymphocytic accumulations of all 3 types were found in both infected and noninfected male reproductive tracts. Lymphoid follicle type accumulations (Figure 15) were found in phallic areas of 6 of 12 infected and 1 of 4 noninfected reproductive tracts. They were not found in vas deferens, epididymis, or testicular tissue.

**Embryos and Eggs**

**Microbiological examination**

*Mycoplasma meleagridis* was isolated from 67 of 258 (26%) incubated eggs from the exposed hens. Infected eggs were laid by 14 of the 20 exposed hens. Each hen produced eggs during the study, but, even though eggs were collected twice daily, many were either cracked or broken and not used. Of
Figure 15. Lymphoid follicle and diffuse accumulation of lymphocytes near a phallic excretory papilla of a *Mycoplasma meleagridis* infected male turkey. x 50
the 15 hens from which 5 or more eggs were examined, 14 laid infected eggs. No isolations were made from the 162 eggs examined from the unexposed hens. Table 6 indicates the number of isolations per week from eggs laid 3 to 12 weeks after exposure. Table 7 presents the number of isolations relative to the length of incubation of eggs prior to examination. Table 8 indicates the distribution of isolations from infertile eggs, dead embryos, and embryos alive immediately prior to examination.

*Mycoplasma meleagridis* was isolated from the extra-embryonic membranes of each of the experimentally exposed embryos, but not from the unexposed control-embryos.

**Identification of Mycoplasmata isolates**

Examination of isolates from 20 of the naturally infected embryos, using the tube agglutination test, indicated that all were *M. meleagridis*. Isolates from 8 of the 20 experimentally infected embryos examined using immunofluorescent microscopy, were also *M. meleagridis*.

**Immunofluorescent examination**

Examination of FA stained sections from 2 naturally infected embryos incubated for 7 days did not reveal fluorescence.

Examination of 2 similar embryos after 14 days incubation revealed fluorescence only on the skin and developing feathers. The fluorescence was observed only in a few areas
Table 6. Isolations of *Mycoplasma meleagridis* from incubated turkey eggs

<table>
<thead>
<tr>
<th>Weeks after exposure</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed hens</td>
<td>5/37^a</td>
<td>19/33</td>
<td>7/22</td>
<td>12/29</td>
<td>9/12</td>
<td>0/2</td>
<td>2/8</td>
<td>4/16</td>
<td>4/17</td>
<td>1/12</td>
<td>0/6</td>
</tr>
<tr>
<td>Control hens</td>
<td>0/16</td>
<td>0/17</td>
<td>0/4</td>
<td>0/12</td>
<td>0/13</td>
<td>0/13</td>
<td>0/12</td>
<td>0/12</td>
<td>0/11</td>
<td>0/16</td>
<td>0/8</td>
</tr>
</tbody>
</table>

^a Number infected/number examined.
Table 7. Isolation of *Mycoplasma meleagridis* from turkey eggs incubated for varying lengths of time

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Eggs from exposed hens</th>
<th>Eggs from unexposed hens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12/47&lt;sup&gt;a&lt;/sup&gt; (25%)</td>
<td>0/36</td>
</tr>
<tr>
<td>7</td>
<td>8/49 (16%)</td>
<td>0/32</td>
</tr>
<tr>
<td>14</td>
<td>23/46 (50%)</td>
<td>0/32</td>
</tr>
<tr>
<td>21</td>
<td>20/51 (39%)</td>
<td>0/34</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number infected/number examined.

Table 8. Isolation of *Mycoplasma meleagridis* from infertile eggs, dead embryos, and live embryos

<table>
<thead>
<tr>
<th></th>
<th>From exposed hens</th>
<th>From unexposed hens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertile eggs</td>
<td>5/45&lt;sup&gt;a&lt;/sup&gt; (11%)</td>
<td>0/45</td>
</tr>
<tr>
<td>Dead embryos</td>
<td>32/69 (46%)</td>
<td>0/47</td>
</tr>
<tr>
<td>Live embryos</td>
<td>26/79 (33%)</td>
<td>0/42</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number infected/number examined.
and appeared as small particles on the surface of the skin or developing feathers.

Fluorescence was widely distributed in the 5 naturally infected embryos examined after 21 days incubation. Specific fluorescence was observed in the esophagus, trachea, bronchi, lungs, and air sacs of each embryo. Skin and feathers were involved as described above. Fluorescence was also observed in each crop and nasal cavity examined. Sections from 3 embryos included crop tissue, while those from 2 embryos included the nasal cavity.

Examination of a single naturally infected embryo incubated for 28 days revealed fluorescence in the same areas as described above, in the lumen of the intestine, and in intra-abdominal yolk.

The fluorescence observed within the embryos almost invariably was found along epithelial surfaces. Figures 16, 17, 18, 19, and 20 demonstrate the location of the fluorescence within the trachea, esophagus, air sac, and lung. Figures 21 and 22 show fluorescent particles on developing feathers and skin. Fluorescent particles, not closely associated with epithelial surfaces, were observed in the lumina of crop and intestine and within yolk material (Figure 23).

Distribution of infection, as demonstrated by immunofluorescent microscopy, in experimentally infected 21-day-old embryos was similar to that in naturally infected 21-day-old
Figure 16. Section of trachea from a naturally infected embryo after 21-days-incubation. Fluorescing areas indicate the presence of *Mycoplasma meleagridis* organisms along epithelial cells. x 200

Figure 17. Section of esophagus from a naturally infected 21-day-old embryo. Fluorescence indicates the location of *Mycoplasma meleagridis* organisms along epithelium. x 100
Figure 18. Section of tissue from a naturally infected 21-day-old embryo. Fluorescing areas indicate the location of *Mycoplasma meleagridis* organisms along air sac epithelium (A) adjacent to liver (B). x 100

Figure 19. Section of lung from a naturally infected embryo incubated for 21 days. Fluorescence indicates the location of *Mycoplasma meleagridis* along the epithelium of a secondary bronchus. x 100
Figure 20. Section of lung tissue from a naturally infected 21-day-old embryo. Fluorescence indicates the presence of Mycoplasma meleagridis organisms on mesobronchial epithelium. The inset is the fluorescing area stained with hematoxylin and eosin. x 100

Figure 21. Tissue section from a naturally infected 14-day-old embryo. Fluorescence indicates the presence of Mycoplasma meleagridis along the surface of a developing feather. x 100
Figure 22. Section of skin from a naturally infected 21-day-old embryo. Fluorescent areas indicate the presence of Mycoplasma meleagridis along the surface. x 200

Figure 23. Section of fluorescing Mycoplasma meleagridis in intra-abdominal yolk material of a naturally infected 28-day-old embryo. x 100
embryos. Nasal cavities, tracheae, bronchi, lungs, air sacs, esophagi, and crops had fluorescence distributed along epithelial surfaces. These fluorescent areas included more of the epithelial surface of involved tissue and generally were thicker than those of the naturally infected embryos. Fluorescent particles were found in the lumina of gizzards and proventriculi of several embryos. In 3 embryos a very few, small, fluorescing particles were observed in a small segment of intestine. The particles were found both in material in the lumen and adjacent to epithelial cells. In all 3 the involved segment was adjacent to pancreatic tissue suggesting that it was duodenum, and consequently the anterior part of the intestinal tract.

The control measures used indicated that the fluorescence described above was specific. Examination of an equal number of noninfected embryos at the same stage of development did not reveal fluorescence and the use of conjugated normal turkey gamma globulin did not result in fluorescence in infected embryo tissues (Figure 24). The one-step blocking technique also indicated specific fluorescence. Unlabeled turkey antiserum against M. meleagridis blocked fluorescence, while unlabeled normal turkey serum did not affect fluorescence (Figure 24).
Figure 24. Fluorescent antibody (FA) stained sections of esophagus from an infected embryo used to verify the specificity of the immunofluorescent technique for demonstrating Mycoplasma meleagridis. Section A was stained with FA prepared from normal turkey serum. Sections B, C, and D were stained with FA against M. meleagridis. The FA used for sections B, C, and D was diluted in buffer, turkey antiserum against M. meleagridis (blocking antibody), normal turkey serum respectively. x 100
Microscopic lesions

No lesions were found in tissue sections of 7 naturally infected and 5 experimentally infected 21-day-old embryos.

Only 1 naturally infected embryo at the 28-day stage of development was available for examination. Lesions were observed in air sacs of this embryo, but other tissues were normal.

Examination of 10 experimentally infected 28-day-old embryos, 5 exposed after 7 days incubation and 5 exposed after 14 days incubation, revealed that all had air sac lesions similar to those observed in the naturally infected 28-day-old embryo. In addition to air sac lesions, lung and liver lesions were observed in many. Table 9 indicates the distribution of lesions among these embryos.

Air sac lesions included both exudative and infiltrative inflammatory reactions. The predominant lesion was the presence of highly cellular exudate in air sac lumina (Figures 25, 26, 27). Most of the cells were heterophils, although mononuclear cells were also present. Other constituents of the exudate were varying amounts of fibrin and cell debris. In the most severely affected air sacs there were large areas of epithelial necrosis.

Subepithelial heterophilic and mononuclear cell infiltration occurred often. Air sac lesions usually affected all air sacs, but were most extensive in the aggregate air sacs. Sometimes the entire air sac was involved, but in most
Table 9. Distribution of lesions in 28-day-old embryos experimentally infected with *Mycoplasma meleagris*is

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Air sacs</th>
<th>Lung</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed at 7 days</td>
<td>1</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Exposed at 14 days</td>
<td>6</td>
<td>+</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>Lesions present.
<sup>b</sup>Not examined. Sections from this embryo did not contain lung tissue.
<sup>c</sup>No lesions observed.

Cases only small areas were affected.

Exudative pneumonia which involved air capillaries, atria, and bronchi was the predominant lung lesion in the embryos (Figures 28, 29, 30). When the extent of the pneumonia was slight, the air tubules and atria were more often affected than bronchi, and lung tissue adjacent to air sacs was more severely affected than other areas. The exudate was similar...
Figure 25. Mycoplasma meleagridis infected (A and B) and uninfected (C and D) air sac tissues from 28-day-old turkey embryos. Photographs B and D are higher magnifications of portions of A and C. Air sac lumina (a), livers (b), ribs (c), exudate (d), and air sac epithelium (e) are included. x 40 and 450
Figure 26. Exudate (A) in a lesser abdominal air sac of a 28-day-old turkey embryo experimentally infected with *Mycoplasma meleagridis*. x 125

Figure 27. Higher magnification of a portion of Figure 25. Exudate (A), air sac epithelium (arrows), and an area denuded of epithelium (B) are included. x 450
Figure 28. Exudative pneumonia in a 28-day-old turkey embryo experimentally infected with *Mycoplasma meleagris*. An arrow indicates the location of an exudate filled tertiary bronchus. x 5
Figure 29. Higher magnification of a portion of Figure 28. An arrow indicates the location of the same exudate filled tertiary bronchus. x 40

Figure 30. Higher magnification of a portion of the tertiary bronchus indicated in Figure 29. Cells in the exudate (A) are primarily heterophils
to that in air sacs, but contained more mononuclear cells and less fibrin. The mononuclear cells appeared to be either macrophages or epithelial cells. In addition to the exudative response there were increased numbers of mononuclear cells in interstitial tissue, especially around the air tubules.

Liver lesions observed in the embryos consisted of perivascular aggregations of predominantly immature heterophils (Figures 31 and 32). A few cells of the same type were found perivascularly in liver sections of uninfected embryos (Figures 33 and 34), but livers of infected embryos were much more extensively involved. These cellular aggregates were considered to be sites of granulocytopenesis and not an infiltrative inflammatory reaction.

Poults

**Microbiological examination**

Microbiological examination of the exposed poults resulted in the consistent isolation of *M. meleagridis* from the left lesser abdominal air sacs and tracheae. It was recovered from lung tissue of 23 of the 28 exposed poults, and from the right lesser abdominal air sacs of most poults examined 8 or more days after exposure. The organism was isolated infrequently from the infraorbital sinuses; with only 6 isolations during the course of the study. No isolations were made from hock joints.

No *Mycoplasma* were isolated from the tissues of the
Figure 31. Section of liver tissue from a 28-day-old turkey embryo experimentally infected with *Mycoplasma meleagris*. Arrows indicate the perivascular location of heterophils. x 125

Figure 32. Higher magnification of Figure 31, showing immature heterophils (A) around blood vessel (B). x 450
Figure 33. Section of liver tissue from an uninfected 28-day-old turkey embryo. x 125

Figure 34. Higher magnification of a portion of Figure 33 which includes a blood vessel. x 450
unexposed control poults, and microbiological examination of the left lesser abdominal air sacs using blood agar media did not indicate bacterial infection in either the infected or control poults.

Identification of Mycoplasma isolates

Isolates from each of the 28 exposed poults were examined using immunofluorescent microscopy, and all were identified as *M. meleagridis*.

Serologic examination

The hemagglutination-inhibition test did not reveal evidence of antibodies against *M. meleagridis* in serum samples from infected poults examined 1, 2, 4, or 8 days following exposure, but antibodies were demonstrated in serums from poults 16, 24, and 32 days after exposure. The average antibody titers of the 4 poults killed and examined at each of these periods were 1:120 at 16 days, 1:280 at 24 days, and 1:320 at 32 days. No evidence of antibody against *M. meleagridis* was found in serum samples from the unexposed poults.

Immunofluorescent examination

The results of fluorescent antibody studies to demonstrate the presence of *M. meleagridis* are indicated in Table 10. No fluorescence was observed in air sac tissues from the unexposed poults.

Relatively little variation in the appearance of the
Table 10. Fluorescent antibody demonstration of Mycoplasma meleagridis in experimentally exposed turkey air sacs

<table>
<thead>
<tr>
<th>Days after exposure</th>
<th>Turkey air sacs</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number examined</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Number with fluorescence</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

fluorescent areas was observed at various intervals after exposure. Bright lines of fluorescence were found along the epithelium of the air sacs (Figures 35, 36, 37, 38).

Gross lesions

No gross lesions were found in left lesser abdominal air sacs of poultls examined 1 day after exposure. Yellowish exudate was observed adhering to thickened air sac walls 2 days after exposure. Exudate usually covered the entire surface of the lumina of the air sacs by 4 days after exposure, and air sac walls were more thickened and opaque than at 2 days. Lesions observed 8 days after exposure (Figure 39) were similar to those at 4 days. By 16 days the exudate was fairly well organized with strands of tissue extending from
Figure 35. Fluorescent antibody stained *Mycoplasma meleagridis* in air sac tissue 2 days following exposure. x 100

Figure 36. Fluorescent antibody stained *Mycoplasma meleagridis* in air sac tissue 8 days following exposure. x 100
Figure 37. Fluorescent antibody stained Mycoplasma meleagridis in air sac tissue taken 8 days following exposure. x 200

Figure 38. Hematoxylin and eosin stained frozen-section of air sac tissue adjacent to that shown in Figure 37. Both air sac tissue (A) and exudate (B) are included. x 200
Figure 39. Airsacculitis in a poult 8 days following exposure to Mycoplasma meleagris. Both affected (A) and normal (B) air sacs are shown.

Figure 40. Organized exudate in an air sac 16 days after exposure to Mycoplasma meleagris. An arrow indicates the location of exudate.
one area of the air sac wall to another (Figure 40). At 24 days these adhesions were more evident (Figure 41), and by 32 days the adhesions (Figure 42) had contracted and caused the lumina of the affected air sacs to be reduced in size.

In addition to the inoculated left lesser abdominal air sacs, lesions were also observed in other air sacs. Table 11 indicates the incidence of gross lesions in air sacs of the exposed poult at various intervals. In general, exudate observed in affected uninoculated air sacs was more liquid than that observed in the inoculated air sacs, and consequently contained more froth (Figure 43). The walls of these air sacs were often less thickened and opaque than those which had been inoculated. Although the lesions of the uninoculated air sacs were somewhat less severe than those of the inoculated air sacs, they still were obvious and severely affected the air sacs involved. Except for the air sac lesions, no gross lesions were observed in the infected poult. Gross lesions were not found in the unexposed poult.

**Microscopic lesions**

Microscopic examination of left lesser abdominal air sacs from exposed poult 1 day following inoculation did not reveal lesions (Figure 44). At 2 days after inoculation exposed air sacs were thickened and contained large numbers of lymphocytes which were heavily concentrated around blood vessels (Figure 45). Increased numbers of heterophils.
Figure 41. Adhesions (arrow) in a Mycoplasma meleagridis infected air sac 24 days after exposure

Figure 42. Adhesions (arrow) in a Mycoplasma meleagridis infected air sac 32 days after exposure
Figure 43. Frothy exudate in a *Mycoplasma meleagridis* infected greater abdominal air sac (A), and adhesions (B) in a lesser abdominal air sac 24 days following inoculation.
Figure 44. Section of apparently normal air sac from a Mycoplasma meleagridis infected poult 1 day following exposure. Arrows indicate an area of adipose tissue. x 100

Figure 45. Thickened air sac tissue from a Mycoplasma meleagridis infected poult 2 days following exposure. Exudate (A), epithelial cells (B), and perivascular accumulations of lymphocytes (arrows) are included. x 100
Table 11. Incidence of gross air sac lesions in *Mycoplasma meleagrisidis* infected pouls at various intervals after exposure

<table>
<thead>
<tr>
<th>Air sacs</th>
<th>Days after exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Left lesser abdominal</td>
<td>0/4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Left greater abdominal</td>
<td>0/4</td>
</tr>
<tr>
<td>Aggregate</td>
<td>0/4</td>
</tr>
<tr>
<td>Right lesser abdominal</td>
<td>0/4</td>
</tr>
<tr>
<td>Right greater abdominal</td>
<td>0/4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number with lesions/number examined.

and macrophages were found in subepithelial tissue. In many areas the normally simple squamous epithelial cells had become elongated, and many were spindle shaped (Figure 46). There were a few areas where epithelial cells could not be distinguished, apparently a result of necrosis (Figure 47). Lymphocytes were sometimes found within the layer of epithelial cells, presumably migrating toward the exudate (Figure 46). Much of the epithelium was covered with a layer of exudate (Figures 45, 46, 47) that contained fibrin, heterophils, and
Figure 46. Air sac tissue from a *Mycoplasma meleagrindis* infected poult 2 days following exposure. Elongated epithelial cells (A), exudate (B), and lymphocytes within the epithelium (arrows) are shown. x 200

Figure 47. Air sac tissue from a *Mycoplasma meleagrindis* infected poult 2 days following exposure. Epithelial cells cannot be distinguished at the air sac surface (arrows) which is covered by exudate (A). Fibrin strands, lymphocytes, and heterophils are present in the exudate. x 450
lymphocytes (Figure 47). Lymphocytes were the predominant cell type in the exudate, although heterophils were also numerous.

By 4 days after exposure the accumulations of lymphocytes were larger and involved much of the air sac tissue (Figures 48 and 49). Areas of epithelial necrosis were larger than at 2 days, and in some areas epithelial hyperplasia resulted in formation of acinar-like structures (Figures 50 and 51). Most of the epithelium was covered with exudate. Heterophils in the exudate were most evident adjacent to epithelium.

Large accumulations of lymphocytes were also present in air sac tissues from poults which were killed 8 days following exposure. These accumulations often caused the mucosa of the air sacs to be irregular. This irregularity was especially evident in air sac tissue adjacent to the lung, where folding of the surface and columnar epithelial cells resulted in a gland-like appearance (Figure 52) which varied markedly from similar areas of uninfected air sacs (Figure 53). Many of the acinar-like epithelial structures had coalesced (Figures 54 and 55), and in some areas these coalesced structures allowed separation of exudate and air sac with epithelial cells on both surfaces. Fibroblasts were present in the exudate.

Most of the lymphocytes in the air sacs of poults 16 days after exposure were circumscribed, forming lymphoid
Figure 48. Air sac tissue from a *Mycoplasma meleagridis* infected poult 4 days after exposure. Large masses of lymphocytes are present. x 35

Figure 49. Higher magnification of tissue similar to that in Figure 48. x 200
Figure 50. Air sac tissue from a *Mycoplasma meleagridis* infected poult 4 days after exposure. Exudate (A) covers hyperplastic epithelial cells which have an acinar-like arrangement (arrows). x 200

Figure 51. Same type of tissue as in Figure 50, but from a different poult. The acinar-like structures (arrows) are more distended than in Figure 50. x 100
Figure 52. Air sac tissue from a *Mycoplasma meleagridis* infected poult 8 days after exposure. Masses of lymphocytes cause folding and a gland-like appearance of the epithelium (arrows). The air sac tissue is adjacent to the thoraco-abdominal diaphragm (A). x 100

Figure 53. Section of uninfected poult tissue from the same area as in Figure 52. Air sac (A), diaphragm (B), and lung (C) are included. x 100
Figure 54. Coalescing acinar-like structures (arrows) in air sac epithelium of a poult 8 days after exposure to Mycoplasma meleagridis. Exudate (A) is included. x 100

Figure 55. Air sac tissue from a Mycoplasma meleagridis infected poult 8 days following exposure. Epithelial acinar-like structures (arrows) are evident underlying exudate (A). x 100
follicles. Connective tissue was apparent in both air sac tissue and exudate. There was more separation of exudate from air sac tissue than at 8 days following exposure. Masses of heterophils were observed in many areas at the surface of the exudate and in coalesced acinar-like structures of epithelium.

At 24 and 32 days following exposure, lymphoid follicles were evident (Figures 56 and 57). Air sacs were less thickened and connective tissue in both air sacs and exudate was more dense. Generally the exudate was attached to air sac tissue only at small areas, with the major portion extending into the lumen (Figure 57). The exudate was well organized and contained lymphoid follicles and heterophils.

Electron microscopy was intended to demonstrate the location of infecting mycoplasmas in relation to air sac epithelial cells, and to determine the nature of the membrane or membrane-like material, observed with light microscopy, which surrounds and apparently confines cells within lymphoid follicles.

Air sacs from poult's in early and active stages of infection, 1 and 8 days after exposure, and from an uninfected poult were examined and compared. A few structures resembling *M. meleagris* were found along the free surface of air sac epithelium taken 8 days after exposure (Figure 58). They were morphologically similar to *M. meleagris* cultivated in broth medium (Figure 58), and were not observed in either
Figure 56. Lymphoid follicles in air sac tissue from a *Mycoplasma meleagridis* infected poult 24 days following exposure. x 100

Figure 57. Lymphoid follicles in air sac tissue and organized exudate from a *Mycoplasma meleagridis* infected poult 32 days after exposure. x 60
Figure 58. Structure (arrow) resembling a *Mycoplasma meleagrisides* organism at the surface of an air sac epithelial cell. Broth cultivated *M. meleagrisides* organisms are shown in the inset at the same magnification. x 50,700
uninfected air sac tissue or infected tissue taken 1 day following exposure. No changes were evident in the epithelial cells.

Examination of lymphoid follicles in air sac tissue 32 days after exposure revealed that the follicles were surrounded by bundles of collagen and a few fibroblasts (Figure 59). The collagen appeared to be contiguous with cells of the follicles. Nearly all the cells within the follicles were hemocytoblasts (Figure 60). They had moderate numbers of mitochondria, clumped chromatin, small Golgi apparatus, and large numbers of polyribosomes distributed throughout the cytoplasm. A few macrophages were also found in the follicles.
Figure 59. Periphery of a lymphoid follicle from a *Mycoplasma meleagridis* infected air sac 32 days following exposure. Collagen (arrows) is located along cells of the lymphoid follicle. x 15,300
Figure 60. Hemocytoblasts within a lymphoid follicle of a *Mycoplasma meleagridis* infected air sac 32 days following exposure. The inset contains an area of cytoplasm at greater magnification to show polyribosomes more clearly. Collagen (A) and a fibroblast (B) which surround the follicle are included. x 10,800 and 19,400
DISCUSSION

Experimental exposure of mature turkey reproductive tracts with *M. meleagridis* resulted in infections which persisted in most turkeys throughout the study. Infections in hen reproductive tracts were more widely distributed than those of previous reports (15,25,55). In those studies, as in this study, organisms were most often isolated from lower oviducts. In this study, however, the incidence of recovery from anterior oviducts was also high and isolations were made from the peritoneal cavities of several hens. It seems unlikely that these organisms would be confined to the lower oviducts of laying hens. Sperm transport, within the female reproductive tracts of all animals in which this phenomenon has been studied, is due to contractions by the oviduct (56). Since these contractions also transport inert particles (56), organisms in the uterus and vagina would probably be carried anteriorly as well.

Antibody response resulting from infection varied considerably between hens and toms. Each of the infected hens produced antibody; the maximum average antibody titers occurred 4 weeks after exposure; and the titers were reasonably high. In the toms only 2 of 12 developed antibody; antibody could not be demonstrated until 6 weeks after exposure; and the titers were low. This variation is probably related to the type of tissue exposed to the organism. While *M. melea-
gridis remained in the cloaca of the toms, it involved the entire oviduct of most hens and the peritoneal cavities of a few. This wide distribution could provide more contact with cells involved in antibody production.

The effect of circulating antibody on infection is difficult to evaluate. Tracheal infections in the hens were, in general, short lived, but reproductive tract infections persisted. Since circulating antibody is transported to yolk material in laying hens, recovery of M. meleagridis from infected embryos and eggs may have been adversely affected. The highest incidence of recovery, however, was during a period when circulating antibody titers were high. The limited antibody response of the toms is significant because of the diagnostic importance of serologic tests. Although M. meleagridis was isolated consistently from all exposed males throughout the study, only 2 developed antibodies. Consequently, most toms were serologically-negative carriers of M. meleagridis.

Reproductive tract lesions were mild, consisting primarily of lymphoid follicles, and were limited to female turkeys. Since these hens were examined 10 to 12 weeks after exposure it is possible that more extensive lesions were present at an earlier stage of infection. However, a previous report (41) describes similar microscopic lesions and there apparently is no evidence of either egg production or fertility being affected by infection with M. meleagridis. These
observations also suggest that reproductive tract response to this infection is mild.

Inspissated yolk material was found in reproductive tracts and peritoneal cavities of both infected and uninfected hens. This material grossly resembled and could easily be mistaken for the caseous exudate which often results from avian Mycoplasmata infections. After contact with infected tissue the inspissated yolk would become contaminated and subsequent isolation of mycoplasmas would add to the erroneous impression that it was exudate.

Lack of lesions in male reproductive tracts is not surprising since, with the exception of phallic structures in infected cloaca, the reproductive tracts were not infected with M. meleagridis. Accumulations of lymphocytes, including lymphoid follicles, were found in phallic areas of both infected and noninfected toms, and consequently their relationship to infection could not be determined.

The incidence of egg transmission of M. meleagridis was similar to those of previous reports in which naturally and experimentally infected turkeys were studied (15,23,25). Recovery of the organism from oviducts and ovarian surfaces, but not from yolk material of mature follicles, suggests that infected eggs resulted from contamination after ovulation rather than from transovarian shedding.

Immunofluorescent examination did not reveal evidence of infection in 7-day-old embryos from infected eggs. Organ-
isms were found on the skin and developing feathers of embryos incubated 14 days and were widely distributed in respiratory and anterior digestive tracts by 21 days. Initial appearance of *M. meleagridis* on the skin and feathers and not in other areas suggests that between 7 and 14 days incubation, organisms occur in amniotic fluid surrounding the embryo. Presence of organisms in amniotic fluid at this stage of incubation could result from contact with albumen containing *M. meleagridis*. In chicken embryos after incubation for approximately 10 days and presumably at about this time in turkey embryos, the sero-amniotic connection which separates amniotic fluid and albumen ruptures and allows albumen to enter the amnion (57). The presence of *M. meleagridis* in respiratory and upper digestive tracts following appearance of these organisms in amniotic fluid suggests ingestion or inhalation as a route of infection. At a stage of incubation following the addition of albumen to amniotic fluid there is a decrease in the volume of this fluid which is believed to be almost entirely due to ingestion by the embryo (57). In the chicken embryo this begins at approximately 14 days (57). Material injected into amniotic fluid has been found later in the stomach, intestines, trachea, and primary bronchi (57). This distribution is similar to the distribution of *M. meleagridis* in 21-day-old turkey embryos, and is additional evidence that infected amniotic fluid was responsible for infecting the embryos.

Lesions were found only in 28-day-old embryos even though
infection was widely distributed after 21 days incubation. Experimentally infected embryos, regardless of whether exposed after 7 or 14 days incubation, had similar lesions. This indicates that lesion formation was related to the stage of development of the embryo, rather than to the duration of infection. Inflammatory cells in the lesions were primarily heterophils. There are few circulating leucocytes in avian embryos until near the time of hatching, and at that time most are heterophils (57). The development of lesions late in the incubation period may be related to the occurrence of inflammatory cells in the circulation.

The lack of lymphocytes in affected air sacs of these embryos contrasted with the extensive lymphocytic infiltration of infected poult air sacs. This variation was considered to be due to the absence of circulating lymphocytes in avian embryos. These cells normally do not appear until after hatching (57).

Studies of respiratory tract lesions in *M. gallisepticum* infected avian embryos indicated that they also developed late in the incubation period (58,59,60). Lesions described in these reports and in an additional report (61) vary considerably, and consequently it is difficult to compare lesions resulting from infection with this organism with those of *M. meleagridis* infection. In general, the lung and air sac lesions described in 2 of these reports (58,60) are similar to those found in this study.
In a previous study of M. gallisepticum infected chicken embryos, a widely distributed heterophilic perivasculitis was observed (59). This perivasculitis was most evident in hepatic tissue. The heterophils involved were immature when first observed in 13-day-old embryos, and increased in number and maturity with continued incubation. In the present study, immature heterophils were also found surrounding hepatic blood vessels. They were much more numerous in infected than in noninfected 28-day-old embryos. However, because of their immaturity and adventitial location, their occurrence was considered to indicate granulocytopoiesis rather than perivasculitis. Granulocytopoiesis in hepatic perivascular connective tissue of chicken embryos reaches a maximum after approximately 14 days incubation and then gradually declines (57). The marked response in these turkey embryos near the time of hatching probably was the result of an increased demand for heterophils brought about by inflammatory responses to infection.

Immunofluorescent microscopy of inoculated air sacs of poults indicated the presence of M. meleagridis along epithelial surfaces as long as 16 days following exposure. At 24 and 32 days after exposure, specific fluorescence was not observed even though cultural techniques indicated the air sacs were still infected. Circulating antibody was first demonstrated 16 days after exposure and perhaps blocked immunofluorescence by reacting with the infecting organisms.
Another possible explanation is that insufficient numbers of organisms were present at this stage of infection to be demonstrated by immunofluorescent microscopy.

Air sac epithelial hyperplasia in the exposed poult resulted in acinar-like structures which coalesced and eventually allowed separation of air sac tissue and exudate in many areas. Similar epithelial structures have been observed in *M. gallisepticum* infected chickens and turkeys. They were described as a netlike formation of epithelial projections (58) and as tubular architecture resulting from hyperplasia of epithelial cells (62). These alterations were attributed to reaction of the epithelium to exudative detrital masses (62), and they were described as promoting separation of exudate from the air sac membrane (58).

Lymphoid follicles were prominent microscopic lesions in infected hen reproductive tracts and poult air sacs. These structures are histologically similar to splenic germinal centers which are an important part of the avian lymphoid system. The lymphoid system of the chicken has been described as consisting of bursa of Fabricius and thymus dependent components (63). The thymus dependent portion is represented histologically by small lymphocytes and functionally by the development of cellular immunity. The bursal dependent portion is represented histologically by larger lymphocytes of germinal centers and plasma cells and functionally by the formation of circulating antibody. Presumably the lymphoid
system of the turkey is similar to that of the chicken. The histologic similarity between lymphoid follicles and splenic germinal centers suggested that they might be functionally similar, and consequently that lymphoid follicles observed in *M. meleagridis* infected tissues were involved in the formation of circulating antibody.

Electron microscopic examination of lymphoid follicles from infected poult air sacs also indicated their similarity with splenic germinal centers and furthermore indicated that cells of the lymphoid follicles were bursal dependent. The fine structure of bursal and thymic dependent cells has been described (64). Bursal dependent cells had an abundance of polyribosomes widely distributed in the cytoplasm. They differed from thymic dependent lymphocytes in which ribosomes were usually single units with occasional clusters of 2 or 3, and were not uniformly distributed in the cytoplasm. Splenic germinal centers were composed primarily of bursal dependent cells identified as hemocytoblasts. Electron microscopic examination of lymphoid follicles in the *M. meleagridis* infected air sacs revealed a predominance of hemocytoblasts, indicating that the follicles were part of the bursal dependent portion of the lymphoid system and involved in the development of circulating antibody.

The focal encapsulated accumulations of lymphocytes, lymphoid follicles, found in the *M. meleagridis* infected tissues differ, by virtue of their focal arrangement and
circumscribed appearance, from the focal nonencapsulated and diffuse types (65). The characteristic circumscribed appearance has been attributed to lymphocytic proliferation within the confining influences of blood vessels (65) and connective tissue (65,66). Electron microscopy in the present study substantiated light microscopic observations (65,66) that connective tissue surrounded the lymphocytes. However, since lymphocytes generally migrate through either connective tissue or blood vessels, the factors responsible for the characteristic morphology of lymphoid follicles are still indefinite.

The formation of germinal centers, structures similar to lymphoid follicles, in avian spleens has been suggested as the result of an agglutination reaction between reticulo-endothelial cells bearing antigen at their surfaces and lymphoid cells bearing complementary antibody at their surfaces. Enlargement of these centers occurs by growth and multiplication of the involved cells (67). The characteristic morphology of lymphoid follicles may be due to this mechanism rather than to confinement by connective tissue.
SUMMARY

Experimentally induced *Mycoplasma meleagridis* infections of turkey hen reproductive tracts resulted in a high incidence of egg transmission of these organisms. Infections persisted in most hens the entire 12-week course of the study and were widely distributed within the tracts, involving even the surfaces of the ovaries of several hens. Gross lesions did not occur, and microscopic lesions were limited to accumulations of lymphocytes. Lymphoid follicles were the most evident type of lymphocytic accumulations. Infections occurred in peritoneal cavities but not in adjacent air sacs of several hens, indicating resistance to contiguous extension of infection from reproductive to respiratory tracts. Specific agglutinins were demonstrated in serum samples from all exposed hens. Maximum average antibody titers occurred 4 weeks after exposure.

Phallic exposure of 12 mature male turkeys with *M. meleagridis* resulted in infections which persisted in phallic areas of the cloaca for the 12-week duration of the study. Microbiologic examination at 4-week intervals indicated that these infections did not ascend the vasa deferens. Circulating agglutinins could be demonstrated in only 2 toms, indicating that most were serologically-negative carriers of *M. meleagridis*. No gross or microscopic lesions which could be attributed to *M. meleagridis* were found in the male reproductive tracts.
Turkey embryos from naturally and experimentally infected eggs were examined for distribution of infection and lesion development using immunofluorescent and light microscopy. The time of occurrence and distribution of infection suggested that the embryos became infected following ingestion or inhalation of infected amniotic fluid. Specific fluorescence was observed along the epithelial surfaces of respiratory and anterior digestive tracts of embryos after 3 weeks or more incubation. The only inflammatory lesions were exudative airsacculitis and pneumonia which were found at a late stage of embryonic development. A hepatic perivascular granulocytopoietic response was also evident near the time of hatching. The occurrence of lesions only during late stages of development of the embryos was believed to be related to the time of development of inflammatory cells.

The sequential development of lesions in poult air sacs experimentally infected with *M. meleagris* was studied. Lymphocytic perivascular infiltration occurred early and was accompanied by fibrinocellular exudate on the surface of air sac epithelium. The epithelium became hyperplastic in some areas, and underwent necrosis in others. The hyperplastic areas often formed acinar-like structures which coalesced and eventually resulted in separation of air sac epithelium and exudate. Later in the course of the disease, lymphoid follicles were formed within air sac tissues and the exudate became organized.
Electron microscopy revealed structures resembling *M. meleagris* along the surface of air sac epithelial cells. It also indicated that the lymphoid follicles which resulted from air sac infections were intimately surrounded by collagen bundles, and were composed primarily of hemocytoblasts which were believed to be bursal dependent cells involved in circulating antibody production.


64. Clawson, C. C., Cooper, M. D., and Good, R. A. Lymphocyte fine structure in the bursa of Fabricius, the thymus, and the germinal centers. Laboratory Investigation 16: 407-421. 1967.


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