

OBSERVATIONS ON SOME FACTORS WHICH MAY INFLUENCE
SUSCEPTIBILITY OR RESISTANCE OF CHICKENS AND TURKEYS TO
HISTOMONIASIS

By

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INTRODUCTION

Histomoniasis (synonyms; blackhead, enterohepatitis) was first described in turkeys by Cushman in 1893. Since that time the literature concerning this disease has increased voluminously. The majority of the investigations have dealt with the etiological agent, mode of transmission, pathology, treatment and prevention of histomoniasis in turkeys. Since the disease spreads rapidly and is highly fatal to turkeys, most of the work has been done utilizing this host. Turkeys of all ages are susceptible, although some workers consider the most dangerous period to be 4 to 25 weeks of age. Mortality is quite variable, sometimes reaching 100 percent.

The literature pertaining to fatal histomoniasis in chickens has been scant. The importance of this disease in chickens has been minimized to the role they play in maintaining and disseminating the etiological agent, and the economic loss in this host is not often realized. Although fatal histomoniasis in chickens does occur, the benign form is seen more often and is followed by recovery, even though the lesions produced are quite similar to the lesions in turkeys dying of the disease. The ability of stress to trigger fatal histomoniasis in chickens has been suggested,

although never adequately substantiated with controlled experiments. The mechanisms and circumstances responsible for resistance of chickens and turkeys to histomoniasis and the factors affecting this resistance have not been investigated. The purpose of this investigation was to determine whether various stress conditions (starvation, exposure to temperature extremes, anemia, coccidia infection, vaccination, cortisone) would alter the disease or mortality in chickens and turkeys due to Histomonas. In addition, an answer was sought for the question of whether bacterial flora in the ceca influenced growth of the histomonad or was in some way responsible for the course of the disease. The question of immunity was also raised. It had been shown by Sautter et al. (1950) that whole blood obtained from turkeys, recovering from histomoniasis, would not protect susceptible turkeys from contracting the disease. In the present investigation an attempt was made to determine if serum, obtained from recovered turkeys, influenced the course of histomoniasis in chickens, or if immune or non-immune chicken sera protected turkeys from contracting blackhead. An attempt was also made to determine what effect age, previous exposure, and splenectomy (in chickens only) would have upon histomoniasis in chickens and turkeys.

REVIEW OF LITERATURE

Histomoniasis was first observed in turkeys by Cushman (1893), and later the etiological agent was described by Smith (1895) as Amoeba meleagridis, a protozoan which he observed could produce conditions in turkeys comparable to amoebic dysentery in man. Tyzzer (1920) noted the mastigophoric and pleomorphic nature of this protozoan in the ceca of turkeys and classified it as Histomonas meleagridis (order Rhizomastigina). Delaplane (1932) isolated flagellated cecal protozoa, which produced fatal histomoniasis, when used to infect other turkeys, thus supporting Tyzzer's studies. Besides the flagellated stage, which possessed from 1 to 4 flagella and occurred in the lumen of the turkey ceca, Tyzzer (1919) observed aflagellated tissue forms which he divided into three stages - invasive, vegetative, and resistant. The invasive stage was considered to be that stage of Histomonas found in early lesions. The cytoplasm, containing inclusions, was basophilic and consisted of clear ectoplasm and finely granular endoplasm. The vegetative stage was larger than the invasive stage and appeared in slightly older lesions. The cytoplasm, without inclusions, was still basophilic and transparent. The resistant stage, found in the oldest lesions of the disease, was

smaller than the two previous stages and possessed an acidophilic cytoplasm filled with small granules or globules.

Other protozoa have been incriminated from time to time as the etiological agent of enterohepatitis. Cole and Hadley (1908) reported the coccidium, Eimeria avium, as the causative agent, and they maintained that the amoeba which Smith observed was really a schizont stage of this parasite. Similarities and dissimilarities between coccidiosis and enterohepatitis were indicated by Schofield (1926). Trichomoniasis, another protozoan disease, has been confused with histomoniasis. Symptoms and pathology are somewhat similar for both diseases. Hadley and Amison (1911) and Jowett (1911) believed that blackhead was caused by trichomonads, but their work was repudiated by the works of Tyzzer (1919, 1920). More recently Allen (1936) has advocated two types of enterohepatitis, one type produced by Histomonas meleagridis and the other by Pentatrichomonas gallinarum. Lesions of both types may be differentiated macroscopically (Santos 1944, Allen 1941). Other etiological agents of histomoniasis besides protozoa have been advocated. Yeast-like fungi have been incriminated several times in this capacity. Enigk (1935) and Menzani (1939) reported a blastomycete as the cause of the disease. Kuprowski (1955) regarded the hepatic lesions, characteristic of the disease, as anemic infarcts. He isolated Candida albicans from the livers of two out of eleven turkeys dying from

blackhead, and considered this fungus to be responsible for the disease.

The growth of Histomonas in vitro was first accomplished by Boeck (Tyzzer 1934). Using Locke-egg-serum medium he isolated Histomonas, together with bacterial flora, from cecal discharges of a chicken. Drbohlav (1924), also using Locke-egg-serum medium, noted the importance of hydrogen ion concentration, as did Tyzzer (1934) with his medium. Bishop (1938) isolated and maintained Histomonas in Laidlaw's medium. She observed, as did Tyzzer (1934), that Histomonas may be rendered non-pathogenic by propagation in vitro over a period of time. Delappe (1953a), using Laidlaw's medium, demonstrated the use of antibiotics in facilitating in vitro isolation of Histomonas. He also observed the effects of pH changes, age of original inoculum, anaerobic environments, and oxidation-reduction potentials upon growth of the histomonad in culture (1953b, 1953c, 1953d). So far no one has been successful in growing Histomonas free of bacteria, nor has culturing been very successful for diagnosis.

Histomoniasis is a disease of fowl not autochthonous to any one part of the world, as affirmed by reports from such widespread areas as Australia (Hungerford 1937); Brazil (Santos 1944); Dutch East Indies (Picard 1929); Germany (Enigk and Wetzel 1938); Italy (Menzani 1939); Japan (Niimi 1930); Poland (Kuprowski 1950) and Russia (Vorob'ev and Kolotilov 1954).

Most of the literature on histomoniasis has been concerned with turkeys, mainly due to its economic importance in this host. However, it is also of primary importance in chickens. It has been observed by many workers that chickens experience a mild form of the disease, recover, and remain carriers and disseminators of the disease. Some investigators noted that histomoniasis results in retarded development of the chicken (Baker 1933) and sometimes mortality. Kaupp (Eveleth 1943) observed the death of 42 out of 43 five-week-old Silver Spangled chickens. Eveleth (1943) reported an outbreak in chickens in which there was a loss of 250 out of a flock of 500 with more showing symptoms. He also noted retarded growth in infected chickens. Hungerford (1937) reported an epidemic in New South Wales which killed 200 out of a flock of 640 chickens; in another flock of 400, twenty-five percent died. Eriksen (1925) stated that losses may vary considerably in different flocks of chickens. Two flocks were observed to suffer losses of more than 50 percent while other flocks, complicated with coccidiosis or brooder pneumonia, also showed high mortalities. It is the opinion of Lund (1956) that histomoniasis in chickens is serious when their resistance is lowered by other diseases, by vaccination, or by undue exposure to adverse conditions.

Other hosts than turkeys and chickens have been reported to be infected with histomoniasis. Wenrich (1943)

described the morphology of Histomonas from pheasants, and Lucas et al. (1956) reported the partridge as a host for this organism. In Russia geese were observed to be infected (Vorob'ev and Kolotilov (1954). Other hosts are grouse, quail, guinea hens, pea fowl, ducks and pigeons.

Transmission of histomoniasis may occur in several ways, when considering both natural and artificial means. Prior to the discovery by Graybill and Smith (1920) of the role of the common cecal worm, Heterakis gallinae, in the transmission of this disease, it was assumed that infection by the consumption of freshly passed histomonads was the common mode of transmission. However, Graybill and Smith (op. cit.) produced histomoniasis in healthy turkeys by feeding them feces (containing embryonated Heterakis eggs) of infected birds. Feeding bird feces, not containing Heterakis eggs, failed to produce the disease in turkeys. These workers, likewise, produced blackhead in turkeys by feeding embryonated Heterakis eggs only, but they did not determine whether the histomonad was carried along on the outer surface of the egg shell or within the egg. Tyzzer and Fabyan (1920) suspended embryonated Heterakis eggs in 1.5 percent nitric acid for 3 days to render them bacteriologically sterile and, at the same time, destroy any histomonads that might have been adhering to the outside of the egg shells. Exposure of the unprotected histomonad in feces to 1.5 percent nitric acid was observed to be lethal

to the organism. Upon feeding these "sterilized" embryonated eggs to turkeys the disease was produced, therefore indicating the location of the histomonad. The ingestion of male Heterakis or non-embryonated Heterakis eggs did not produce histomoniasis (Tyzzer 1934), nor did a ground up suspension of embryonated eggs or larvae (Swales 1948). This indicated that hatchable embryonated eggs were necessary for producing the disease. The histomonad itself has never been demonstrated satisfactorily in the Heterakis egg. Niimi (1930) claims to have seen the causative organism, but his work has never been substantiated. Many methods for demonstration have been tried by different workers, but to no avail. Histomonas has been observed in the Heterakis larvae by Tyzzer (1934), who pictured the histomonad in the intestinal epithelium of an eleven-day-old larva. Swales (1948) artificially hatched embryonated eggs, sterilized the larvae in 50 percent hydrogen peroxide, and introduced them directly into the ceca of a turkey by means of a laparotomy to produce histomoniasis. He concluded that the living larvae must be present to initiate the disease process, but upon repeated examination of larvae was unable to demonstrate the histomonad at any time. Out of 100 worms from a turkey dying of blackhead, Desowitz (1950) observed sections from one showing 2 enlarged intestinal epithelial cells filled with amoebulae which he believed to be Histomonas. Connell (1950) observed in second stage Heterakis larvae abnormalities,

such as cuticular swellings and diffuse thickenings, and suggested that they might be caused by a stage of Histomonas.

Although no stage of Histomonas has ever been satisfactorily demonstrated in the Heterakis egg, it has been observed by many workers that the organism, in this protected niche, is quite resistant to environmental conditions. Graybill (1921) stated that "infectious soil that had remained unoccupied for a period of five months, beginning in the depths of a severe winter, still harbored viable ova of Heterakis and proved highly dangerous to young poults." Four healthy turkeys which he placed in the yard in question contracted histomoniasis. Tyzzer (1934), Van Es and Olney (1934), and Niimi (1937) also observed this overwintering of the histomonad within the still viable Heterakis eggs. Farr (1956) seeded outdoor plots of earth to a depth of two inches with fresh droppings of chickens and turkeys containing Histomonas and the eggs of several nematodes, one of which was Heterakis. After a period of 66 weeks samples from these plots, when fed to healthy turkeys, produced histomoniasis. During this period of time temperatures ranged from 5° F to 100° F.

Transmission of histomoniasis by the ingestion of free histomonads in droppings probably occurs only rarely. The causative agent is unable to survive longer than a few hours outside the avian host. Tyzzer and Collier (1925) stated that "the protozoan is discharged in a form that is

incapable of surviving long outside the body, but which may produce infection if immediately ingested." Tyzzer (1932) and others reported that very irregular results follow attempts to transmit the infection by feeding droppings of infected birds. Graybill and Smith (1920), Niimi (1937), and others failed to produce blackhead when they fed droppings containing free histomonads. Oral inoculation of emulsified diseased organs or of cultures have also produced variable results. Lund (1955) obtained very low mortality (1 or 2 percent) in turkeys when he infected them per os with unprotected histomonads. He stated that "ingestion of histomonads along with solids or materials requiring digestion apparently reduced the infectivity of the organisms, and the ability of the organisms to provoke symptoms was reduced correspondingly." No deaths resulted from these infections. Horton-Smith and Long (1956) showed that a low pH (2.9-3.3) in the gizzards of chickens is lethal to the unprotected histomonad. They observed that starved chickens maintained a higher pH (6.3-7.0) in the gizzard, thereby enabling them to infect chickens orally with histomonad suspensions. However, the highest incidence of infections was obtained by administering histomonad suspensions to starved chickens that had received one gram of alkali mixture (40 percent calcium carbonate, 17 percent magnesium trisilicate, 43 percent colloidal kaolin) beforehand. These investigators also observed the effect of storing histomonad

suspensions at different temperatures for varying lengths of time. The highest proportion of infections occurred when the material was administered immediately after its preparation. At 4.4° C for 2 hours no infections were produced. Tyzzer et al. (1921) reported that freezing killed the etiological agent of histomoniasis.

Other methods of transmission of histomoniasis have been used experimentally. Tyzzer (1934) found that rectal inoculation was more reliable than oral inoculation in producing infection. Farmer and Stephenson (1949) reported that rectal inoculation of emulsified ceca produced higher mortality in turkeys than other methods of infection. Lund (1955) attempted to administer quantitative rectal inoculations of histomonad suspensions, and noted that the incidence of infection, rate of mortality and time of appearance of symptoms varied with the size of infective dose. One-hundred thousand histomonads when introduced rectally into turkeys resulted in shorter prepatent periods, 100 percent incidence, and 100 percent mortality.

McGuire and Morehouse (1958) experimentally produced histomoniasis by transfusing blood from the cecal or mesenteric veins of diseased donor birds into the wing vein of susceptible turkeys. He observed lesions in many of the internal organs, but cecal involvement did not occur. No infection was produced when he transfused blood from the heart or wing vein of infected donor birds to susceptible

turkeys. Tyzzer and Fabyan (1920) produced histomoniasis in turkeys by the injection of histomonad suspensions subcutaneously and intramuscularly. Lesions appeared at the site of inoculation, the histomonad sometimes metastasizing to the lungs and liver. Infection of chickens by these methods proved unsuccessful. Mechanical transmission of blackhead experimentally by arthropods has been reported by Frank (1953). Grasshoppers retained viable Heterakis eggs for at least 96 hours as indicated by infection of turkeys. Flies also were capable of carrying Heterakis eggs mechanically, but are probably of little importance in natural transmission of the disease.

The lesions and corresponding symptoms of histomoniasis have been described many times. The symptoms which appear are not specific for this disease. Somnolence, droopy wings and ruffled plumage, passage of sulfur-colored droppings, anorexia, emaciation and discoloration of the head are some of the symptoms that have been observed. The term "black-head" is actually misleading since the blue-black discoloration of the head seldom occurs in infections with Histomonas. However, due to the common usage of this term, it has been retained in the literature. The prepatent period for black-head varies according to the method of infection. Following the ingestion of embryonated Heterakis eggs symptoms appear in 11 to 17 days, and mortality occurs in 14 to 26 days. Rectal inoculation shortens the prepatent period

and time for mortality to occur by approximately one week. Many workers have described the gross pathologic conditions, mainly in reference to the liver and ceca. Characteristic liver lesions were described by Lund (1955) as being circumscribed (sometimes coalesced), grayish-white in color, and depressed or raised in the center. He described cecal lesions as consisting of inflammation and ulceration, while the ceca often contained thick greenish-white material or large caseous cores. Peritonitis occurred often. That the ceca are the primary site of infection was shown by Durant (1930), Delaplane and Stuart (1933) and Schlotthauer et al. (1933) when they prevented infection with blackhead by ablating the ceca. Lesions have been produced in other organs than the liver and ceca. Farmer et al. (1951) reported lesions in the spleen, and microscopic examination of stained tissue sections revealed the presence of Histomonas. Upon subcutaneous inoculation of turkeys secondary lesions of the lungs, liver, and rarely of the kidneys were observed by Tyzzer and Fabyan (1920). Levine (1947) also reported blackhead lesions in the kidney, and upon microscopic examination he was able to observe the histomonad. Blood induced infections may produce lesions in the lungs, kidneys, spleen, pericardium, proventriculus, and pancreas (McGuire and Morehouse 1958). Malewitz and Calhoun (1958) demonstrated Histomonas, microscopically, in lesions of the kidney and spleen. They also observed lesions in the lungs,

pancreas, and heart, but were unable to demonstrate the presence of the organism. Their method of infection utilized embryonated Heterakis eggs. Using this same method of inoculation the author has observed lesions in the liver, ceca, proventriculus, spleen, mesentery, heart (see Appendix, plate I), and kidney and intestinal wall of turkeys and the ceca, liver, proventriculus (see Appendix, plate V), and spleen of chickens.

Microscopic examination of diseased tissues by means of tissue sectioning and staining has been done by Tyzzer and Fabyan (1920), Farmer et al. (1951), McGuire and Morehouse (1958), and Malewitz and Calhoun (1958). In general lesions are characterized by degeneration, necrosis, hemorrhage, congestion, serous exudate, lymphocytic infiltration, and giant cells.

Pathological manifestations in the blood have been observed by several workers. Johnson and Lange (1939) reported alterations in the differential count and the development of an anemia in turkeys exposed to Histomonas. This work was confirmed by McGuire and Cavett (1952) who, in addition, observed changes in blood glucose (severe hypoglycemia occurred in turkeys just before death), erythrocyte counts and values obtained from the hematocrit.

Factors relating to resistance to infection, immunity, and recovery have not been investigated sufficiently. Curtice (1907) stated that turkeys may contract histomoniasis at

any age and that immunity is not acquired by previous infection. Using experiments Tyzzer (1934) described an apparent development of a resistance to reinfection, following several rectal inoculations of an attenuated strain of Histomonas. He suggested that it was necessary to inoculate birds when young and that there was a need for constant reinfection in order to maintain a state of premunition. He reported that inoculation of turkeys was less uniformly successful than the inoculation of chickens, and a loss of infection included a loss of immunity. Swales and Frank (1943) exposed three-, ten-, and seventeen-day-old turkeys to litter containing embryonated Heterakis eggs. In most cases fatal infection was not initiated until the birds were 3 weeks old, and during this time the turkeys did not acquire a low grade infection capable of immunizing them against fatal histomoniasis later in life. Sautter et al. (1950), DeVolt et al. (1954), and McGregor (1951) reported very little immunity resulting in turkeys when medication, having a suppressive effect upon the parasite, was removed. Sautter et al. (op. cit.) also noted that whole blood obtained from immune turkeys (recovered from blackhead) failed to protect susceptible turkeys. Waletsky (1950) considered that there was no certainty that recovered birds either with or without the help of drugs became immune to reinfection. Lund (1957) reported that rectal inoculation of a non-pathogenic strain of Histomonas failed to protect turkeys

3 to 6 weeks later when they were challenged with large doses of pathogenic histomonads. Small challenge doses provided some protection. He observed that the introduction of non-pathogenic histomonads per os (via embryonated Heterakis eggs) afforded no protection against challenge doses of the pathogenic strain administered by either route. Desowitz (1951), using rectal inoculations, showed age to be a factor influencing fatal infections of histomoniasis in chickens. Sixty to 71 percent mortality occurred in six to twenty-one-day-old chickens as compared to 30 percent mortality in thirty-four-day-old chickens. On the other hand, Kendall (1957), using either embryonated Heterakis eggs or rectal inoculations of histomonad suspensions, failed to observe this age influence in turkeys ranging from 7 days to 20 months of age.

Serological tests have been conducted by Rettger and Kirkpatrick (1927) and Weldin and Kay (Delaplane and Stuart 1935), but the results were variable and unsatisfactory for diagnosis of either acute or chronic cases of histomoniasis.

MATERIALS AND METHODS

A. Experimental materials

1. Chickens and turkeys

All chickens and turkeys used in this investigation were straight run, and obtained the same day as they hatched or one day later. Most of the chickens were White Leghorns, although some Vantress Cross White Plymouth Rocks were used. The majority of the chickens were obtained via parcel post from Hess's St. Louis Hatchery, St. Louis, Michigan. Some were supplied by the Poultry Husbandry Department of Michigan State University.

Two breeds of turkeys were used. Broad Breasted Bronze turkeys and some Beltsville Small White turkeys were supplied by Janssen Farms Hatcheries, Zeeland, Michigan. Beltsville Small White turkeys were also supplied by Wyse Brothers Hatchery, Archbold, Ohio and the Poultry Husbandry Department of Michigan State University.

2. Feed

Non-medicated turkey starter crumbles (28 percent protein, antibiotic supplement) and non-medicated chick starter mash (20 percent protein, antibiotic

supplement), which were used throughout the investigation, were formulated by the A. E. Staley Manufacturing Co., Decatur, Illinois, and obtained through a local distributor.

3. Infective materials

Infective materials consisted of embryonated Heterakis gallinae eggs or cecal washings from turkeys suffering from histomoniasis. Some of the Heterakis eggs were supplied in 0.5 percent formalin by Dr. Gerald Brody, Moorman Manufacturing Co., Quincy, Illinois. However, the majority of the infective material was obtained and processed as follows. Ceca from chickens, generally over a year old, were collected several times from poultry slaughter houses in Linden, Michigan and Middleton, Michigan. These ceca were then brought into the laboratory, slit open, and their contents flushed into a metal seive suitable for retaining the mature Heterakis. Washing was continued until the worms were clean, after which they were flushed into a large transparent glass bowl. Gravid females were isolated and ground up using a mortar and pestle. This material, which contained the unembryonated eggs, was suspended in tap water, centrifuged, and the supernatant discarded. The sediment was resuspended in tap water and poured

into petri dishes. Approximately 300 units of Mycostatin per ml. of water were added to prevent the growth of mold. An embryonation period of 17 days at room temperature was allowed, after which the suspension was again centrifuged and the supernatant discarded. The sediment was diluted in a flask and the number of embryonated eggs per ml. determined. Immediately after shaking the container, so as to obtain a uniform distribution of eggs, 0.02 ml. of the suspension was transferred to a glass slide, a cover slip added, and the number of embryonated eggs counted. Six of these counts were made and the mean, multiplied by 50, designated the number of embryonated eggs in one ml. The embryonated egg suspension was stored at 5° C.

In several instances the infective material consisted of cecal washings (physiological saline) obtained from clinical cases of blackhead in turkeys which had been infected with 620 embryonated Heterakis eggs. This material was then used the same day as a rectal inoculum.

4. Other materials

Fowl pox vaccine (chick-embryo origin) was obtained from Pitman-Moore Co., Division of Allied Laboratories, Inc., Indianapolis 6, Indiana. The

Apothecary Shop, Lansing, Michigan, supplied the cortisone acetate for intramuscular injection.

B. Care, management, and distribution of chickens and turkeys prior to the beginning of experiments

Upon receiving the chickens and turkeys they were placed in electric brooders which had been operating one day prior to their arrival. Here they remained for 3 to 4 weeks with feed and water supplied ad libitum. After 3 to 4 weeks the birds were transferred from the brooder to wire bottom poultry batteries in separate rooms (microbiology building and animal disease barn) according to the grouping in the particular experiment. Infected and uninfected birds were always retained in different rooms.

C. Infection of chickens and turkeys

1. Per os inoculation

Chickens and turkeys which were to be infected with Histomonas via embryonated Heterakis eggs were starved for 16 hours. The appropriate number of eggs was introduced directly into the crops of the birds by means of a one ml. syringe and a rigid five-inch piece of plastic tube cemented to the base of a hypodermic needle. An appropriate number of eggs was considered to be that number of embryonated eggs which when inoculated per os in turkeys

produced 50 percent mortality or greater. This number ranged from 300 to 700 embryonated eggs.

2. Rectal inoculation

A fasting period of 16 hours also preceded rectal inoculations. Birds were suspended by their legs and inoculated rectally with 5 ml. of the histomonad suspension prepared from cecal washings of infected turkeys. A 5 ml. syringe and a five-inch piece of number 8 French catheter tubing was used in administering the rectal inoculation. Five ml. of air was injected immediately following the injection of the histomonad suspension. These birds remained in an inverted position for 30 minutes to insure a "take" in the infection.

Chickens and turkeys were generally infected by either method between 3 to 6 weeks of age.

D. Exposure to various stresses

1. Starvation

Chickens and turkeys were starved every other day for 19 days. Starvation periods, during which time feed only was withheld, ranged from 24 to 34 hours with an average of 27.9 hours. No weights were recorded, but it was obvious that all birds were somewhat emaciated at the end of the 19 days.

2. Simultaneous Eimeria tenella infections in chickens

Approximately 20,000 sporulated oocysts of Eimeria tenella were introduced directly into the crops of chickens 6 days after they had received embryonated Heterakis eggs via the same route. This number of sporulated oocysts was selected by infecting chickens beforehand and observing bloody droppings but no mortality. Feed was removed several hours before infection with the coccidia.

3. Temperature variation

Chickens and turkeys which were to be exposed to temperatures of 4° C. or 38° C were first transferred to a twenty-two by fourteen by ten-inch wire cage (5 birds per cage). They were then placed in the walk-in incubator, maintained at 38° C, or the walk-in refrigerator, maintained at 4° C. Exposure to these temperatures occurred every other day for 20 days. For chickens the exposure periods ranged from 15 to 19 hours with an average of 16.6 hours. Turkeys were exposed from 15 1/2 to 18 1/2 hours or for an average of 16.3 hours. Aside from leaving several open containers filled with water in the 38° C incubator no attempt was made to regulate humidity, but it was measured by placing a hygrograph on the shelf below the cages containing the birds. The hygrograph was first

standardized by placing it in a sealed glass container with open dishes of concentrated sulfuric acid for 11 days and by adjusting the recording pen to zero on the hygrograph chart. The humidity during the exposure of chickens varied from 38 to 65 with an average range of 47.1 to 58.7. During the exposure of turkeys the humidity ranged from 33 to 59.5 with an average range of 37.4 to 54.6.

All birds exposed to either 4° C or 38° C were without feed and water during the exposure for the sake of convenience.

4. Anemia

Chickens and turkeys were rendered anemic by periodic withdrawals of blood from either the heart or the medial wing veins. Hemoglobin determinations were made utilizing the acid hematin technique and the Bausch and Lomb Spectronic 20 colorimeter.

5. Fowl pox vaccination

Chickens and turkeys were vaccinated with fowl pox vaccine (chick-embryo origin) by the wing web (stick) method. For all vaccinations both wing webs were pierced.

6. Cortisone acetate injections

Daily intramuscular injections of cortisone acetate were administered to chickens at a rate of 1.7

to 3.0 mgm. per pound of body weight for 21 days.

Turkeys received daily intramuscular injections

at a rate of 1.7 to 2.5 mgm. per pound for 21 days.

E. Surgical transplant of cecal material

Cecal contents from 5 protozoan free turkeys, 40 days old, were collected and suspended in a small quantity of saline. This material was then used for surgical implantation of chicken ceca. Likewise, cecal material from 5 protozoan free chickens, 39 days old, was collected, suspended in a small quantity of saline, and used for implantation of turkey ceca.

Feed and water were removed from chickens and turkeys for 24 hours preceding the operation in order to evacuate the ceca. Approximately 0.02 ml. of Halatal was administered intravenously via the medial wing vein. The anesthetized bird was then secured to a wooden platform, containing several holes, by means of cloth bands. The area to be incised was first plucked free of feathers and swabbed with 70 percent alcohol. A one-inch incision was made between the last two left ribs (the same operation used for caponization). A surgical spreader held the wound open while a hooked probe was used to lift the ceca and intestine, at their point of union, to the surface. One ml. of the previously prepared saline suspension of cecal material was injected into each cecum with a syringe and number 20 gauge hypodermic needle.

A small quantity of neomycin sulfate was sprinkled into the abdominal cavity, and two stitches (cotton thread) around the rib on either side of the incision were sufficient to close the wound. As soon as the birds recovered from the effects of the Halatal they were returned to their cages and supplied with feed and water. For several days after the operation air pockets occurred under the skin of the turkeys, especially. Consequently, the skin was punctured periodically to reduce the accumulation of air.

All instruments used in the operations were cleaned in 70 percent alcohol, dried, and flamed before use.

F. Serum processing and injection

Whole blood from the appropriate group of birds (usually 10 to 15 in number) was collected (heart puncture) and pooled in a sterile flask containing a known amount of sodium citrate (to give 2 percent sodium citrate by volume). The receiving flask was prepared by adding to it a definite quantity of 10 percent sodium citrate and placing it in the hot air oven for 2 hours at 150° C. At the end of this period all water had evaporated leaving a white residue of sodium citrate adhering to the walls of the flask. When the desired volume of whole blood had been obtained, it was immediately centrifuged for 5 to 10 minutes at 1200 RPM. The supernatant plasma was drawn off with bulb pipettes fitted with rubber

suction tubes and transferred into suitable screwtop bottles. The plasma was then heated in a constant temperature bath at 56° C for 30 minutes, after which it was cooled and frozen for 2 days. On the third day the plasma was thawed and centrifuged in order to remove the fibrinogen precipitate. The supernatant serum was poured off into suitable bottles and used for intravenous injection (medial wing veins) the same day.

All materials used in the collection and processing of blood were sterilized in the hot air sterilizer before use.

G. Post mortem examination of chickens and turkeys

All infected and control birds were necropsied as soon as possible after death. Carcasses of birds which were not immediately necropsied were maintained at 4° C until the autopsy could be performed. Control birds and infected birds which did not die from histomoniasis 30 to 35 days after infection were sacrificed by disengaging the cervical vertebrae. Specimens were observed for gross lesions from which smears were often made in order to observe the histomonads. Liver lesions were graded as follows: mild liver involvement or less than 10 lesions, moderate involvement or 10 to 20 lesions and severe liver involvement or greater than 20 lesions.

Presence of Histomonas in the ceca was determined by microscopic examination of cecal smears.

H. Care of tissues for sectioning, and staining methods

Tissues selected for sectioning were cut 5 millimeters or less in thickness and fixed in 10 percent formalin for 24 hours. After the removal of the formalin from the tissues they were stored in 70 percent alcohol until they could be dehydrated and embedded in paraffin. Sections were cut at 6 to 8 μ and stained with Harris' hematoxylin and counterstained with eosin. Cecal smears and smears obtained from the various lesions were stained with May-Greenwald and Giemsa stains.

RESULTS

A. Surgical implantation of cecal material

Cecal contents were collected from chickens or turkeys, 39 to 40 days old, and prepared for implantation as described in the materials and methods. Birds were infected with embryonated Heterakis eggs, and the results recorded in table 1 which also contains data from splenectomized birds. Chickens, which received cecal material from turkeys 3 days prior to infection with Histomonas, via Heterakis eggs, showed a significantly lower incidence of cecal lesions and liver lesions of the lowest grade (less than 10), as compared to infected chickens, which did not receive cecal implantation. However, the infected, treated birds showed approximately the same total incidence of liver lesions (sum of three grades) as infected, untreated birds, and the incidence of cecal histomonads in both groups was not significantly different. One infected chicken (without cecal implantation) possessed lesions in the spleen. Both groups of infected turkeys (with or without implantation of chicken cecal contents) showed nearly the same results (see table 1). No lesions or cecal histomonads were observed in non-infected chickens and turkeys.

TABLE 1

HISTOMONIASIS IN SPLENECTOMIZED CHICKENS AND IN CHICKENS AND TURKEYS RECEIVING
SURGICAL IMPLANTATIONS OF CECAL CONTENTS BEFORE INFECTION

Groups	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions					No. of Birds Containing <u>Histomonas</u>
			Ceca	Liver			Spleen	
				<10	10-20	>20		
<u>Chickens</u>								
Controls	15	0	0	0	0	0	0	0
Infected	15	0	8	4	1	0	5	11
Infected and Implanted	20	0	3*	1*	0	1	2	13
Implanted	10	0	0	0	0	0	0	0
Splenectomy and infected	5	0	2	2	0	0	2	4
<u>Turkeys</u>								
Controls	15	0	0	0	0	0	0	0
Infected	15	10	11	1	0	10	11	12
Infected and implanted	15	10	10	1	0	10	11	11
Implanted	10	0	0	0	0	0	0	0

*Differs significantly (5 percent confidence level) from infected group.

B. Effect of stress upon histomoniasis in chickens and turkeys

Starvation of chickens infected with Histomonas (via embryonated Heterakis eggs) apparently had no influence upon the lesions produced, as indicated by data compiled in table 2. However, fewer infected and starved chickens showed Histomonas in cecal smears than did unstarved, infected chickens. Turkeys which were infected and starved showed a higher incidence of liver lesions (total of three grades), but differences in occurrence of the three grades of liver lesions were not significant. Although no weights were recorded, all starved birds were noticeably emaciated at the end of the starvation periods. Necropsy of non-infected birds showed no lesions, and cecal smears were negative for Histomonas.

Concurrent infections of histomoniasis and coccidiosis (Eimeria tenella inoculated per os 6 days after the inoculation of embryonated Heterakis eggs) in chickens did not significantly alter characteristics of the histomonad infection alone, as indicated in table 3. No deaths resulted from E. tenella infection alone, but clinical symptoms (bloody discharges, ruffled plumage etc.) were observed. Although 2 of 100 chickens suffering from both coccidial and histomonad infections died, it was not enough to be of statistical significance. The data in table 3 was accumulated from two experiments.

TABLE 2
HISTOMONIASIS IN STARVED CHICKENS AND TURKEYS

Groups	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions					No. of Birds Containing <u>Histomonas</u>
			Ceca	Liver				
				<10	10-20	>20	Total	
<u>Chickens</u>								
Controls	15	0	0	0	0	0	0	0
Infected	20	0	3	6	0	0	6	18
Infected and starved	20	0	3	8	0	1	9	12*
Starved	20	0	0	0	0	0	0	0
<u>Turkeys</u>								
Controls	15	0	0	0	0	0	0	0
Infected	20	10	14	2	1	10	13	19
Infected and starved	20	11	15	5	3	11	19*	20
Starved	20	1**	0	0	0	0	0	0

*Values differ significantly (5 percent confidence level) from unstarved, infected birds.

**Undetermined cause of death.

TABLE 3

CONCURRENT INFECTIONS OF HISTOMONIASIS AND COCCIDIOSIS IN CHICKENS

Groups	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions				No. of Birds Containing <u>Histomonas</u>	
			Ceca	Liver				
				<10	10-20	>20		Total
Controls	30	0	0	0	0	0	0	
<u>Histomonas</u>	44	0	8	13	0	0	13	36
<u>Eimeria</u>	42	0	0	0	0	0	0	0
<u>Histomonas</u> and <u>Eimeria</u>	100	2	14	33	2	2	37	79

No lesions, cecal histomonads or coccidia were observed in non-infected birds.

In another experiment, both chickens and turkeys infected with embryonated Heterakis eggs were bled periodically in an attempt to render them anemic. Initial (day of infection), final (day on which survivors were necropsied), and lowest average hemoglobins and ranges are recorded in table 4. Hemoglobin determinations on unbled, non-infected birds were also recorded. In infected, anemic chickens the lowest average hemoglobin (6.9) occurred on the ninth day after infection. The lowest average hemoglobin of infected, anemic turkeys fell to 9.7 on the eleventh day after infection. Corresponding hemoglobins for non-infected, anemic birds were slightly higher. Data on mortality, tissue damage, and incidence of Histomonas in cecal smears are given in table 5. No significant variation between data from infected, anemic and infected, unbled chickens and turkeys was noted. Non-infected birds were negative for lesions and cecal histomonads.

When chickens and turkeys, infected with histomoniasis, were exposed to 4° C or 37° C every other day for 20 days after infection, variations in mortality, incidence of lesions, and incidence of Histomonas in cecal smears were negligible (see table 6). Thirteen of 20 infected chickens died 16 to 17 days after infection, during

TABLE 4

HEMOGLOBINS OF CHICKENS AND TURKEYS INFECTED WITH HISTOMONAS MELEAGRIDIS

Groups	No. of Birds per Group	Total ml. Blood re- moved per Bird	Hemoglobin Values (grams per 100 ml.)					
			Initial Ave.	Initial Range	Lowest Ave.	Lowest Range	Final Ave. Final Range	
<u>Chickens</u>								
Controls	15	0	11.5	11.0-12.3	11.2	10.6-12.5	11.2	10.6-12.5
Anemic	15	20	10.8	10.3-12.2	7.7	6.7-9.0	10.8	10.3-12.0
Infected and anemic	20	20	11.0	9.8-12.0	6.9*	4.8-9.0	10.6	9.4-12.3
<u>Turkeys</u>								
Controls	10	0	14.2	13.2-15.8	14.2	13.2-15.8	14.4	13.5-16.0
Anemic	5	84	14.3	13.4-15.7	10.1	9.7-10.5	14.1	13.0-14.9
Infected and anemic	10	84	14.5	13.0-16.0	9.7**	6.9-10.5	10.9	7.8-12.7

*Ninth day after infection.

**Eleventh day after infection.

TABLE 5

HISTOMONIASIS IN ANEMIC CHICKENS AND TURKEYS

Groups	No. of Birds per Group	No. of Deaths	Ceca	No. of Birds with Lesions				No. of Birds Containing <u>Histomonas</u>	
				Liver			Kidney		
				<10	10-20	>20			Total
<u>Chickens</u>									
Controls	15	0	0	0	0	0	0	0	0
Infected	20	0	5	1	0	0	1	0	12
Anemic	15	0	0	0	0	0	0	0	0
Infected and anemic	20	0	2	3	0	0	3	0	10
<u>Turkeys</u>									
Controls	10	0	0	0	0	0	0	0	0
Infected	10	5	6	0	0	6	6	0	7
Anemic	10	0	0	0	0	0	0	0	0
Infected and anemic	10	5	6	1	0	5	6	1	8

TABLE 6

HISTOMONIASIS IN CHICKENS AND TURKEYS EXPOSED TO
40° C or 37° C TEMPERATURES

Groups	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions					No. of Birds Containing <u>Histomonas</u>	
			Ceca	Liver			Spleen		
				<10	10-20	>20		Total	
<u>Chickens</u>									
Controls	20	0	0	0	0	0	0	0	0
Infected	20	0	5	2	0	0	2	0	15
40° C	10	0	0	0	0	0	0	0	0
37° C	10	0	0	0	0	0	0	0	0
Infected and 40° C	20	0	3	5	0	0	5	0	14
Infected and 37° C	20	13*	4	4	0	0	4	0	15
<u>Turkeys</u>									
Controls	10	0	0	0	0	0	0	0	0
Infected**	10	5	6	1	0	5	6	0	7
40° C	10	0	0	0	0	0	0	0	0
37° C	10	0	0	0	0	0	0	0	0
Infected and 40° C	10	2	3	3	0	2	5	0	7
Infected and 37° C	10	4	5	1	0	4	5	1	8

*Deaths probably due to over-exposure.

**Same group of turkeys in previous experiment (table 5).

which time they had been exposed to 37° C for 19 hours (average exposure period was 16.6 hours). It is probable that these deaths were a result of over exposure, although no mortality occurred in non-infected chickens exposed for the same length of time at this temperature. The pathological conditions for both groups were very similar. Infected turkeys (not subjected to temperature exposures) were the same group of turkeys used in conjunction with the previous experiment. Necropsy of non-infected birds showed no lesions, and cecal smears were negative for Histomonas.

Table 7 summarizes the data obtained from fowl pox-vaccinated or cortisone-treated chickens and turkeys infected orally with embryonated Heterakis eggs. No significant difference was observed between chickens and turkeys, which received histomonad-containing Heterakis eggs, and chickens and turkeys, which received both Heterakis eggs and fowl pox vaccination or cortisone treatment. A different collection of Heterakis eggs was used for this experiment, and an increased incidence of proventriculus and kidney lesions was noted. In one turkey, lesions were observed in the proventriculus, spleen, mesentery, and heart (see Plate I). Microscopic examination revealed the presence of histomonads in the proventriculus, mesentery (see Appendix, plates II and III) and spleen, but none were observed in the

TABLE 7

HISTOMONIASIS IN FOWL POX-VACCINATED OR CORTISONE-TREATED CHICKENS AND TURKEYS

Groups	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions					No. of Birds Containing <u>Histomonas</u>	
			Ceca	Liver			Prov. Kidney Spleen		
				<10	10-20	>20			Total
<u>Chickens</u>									
Infected	29	0	5	8	0	0	8	0	26
Infected and vaccinated	21	0	5	5	1	1	7	0	18
Vaccinated	21	0	0	0	0	0	0	0	0
Controls	15	0	0	0	0	0	0	0	0
Infected and Cortisone	10	0	1	1	0	0	1	0	10
Cortisone	5	0	0	0	0	0	0	0	0
<u>Turkeys</u>									
Infected	25	20	23	1	0	21	22	6	25
Infected and vaccinated	25	22	25	1	0	23	24	9	25
Vaccinated	20	0	0	0	0	0	0	0	0
Controls	15	0	0	0	0	0	0	0	0
Infected and Cortisone	10	6	6	1	2	5	8	2	10
Cortisone	5	0	0	0	0	0	0	0	0

*This turkey also possessed lesions in the heart and mesentery.

heart lesion which was characterized by a loose fibrous thickening of the epicardium (see Appendix, plate IV). One vaccinated chicken possessed lesions in the ceca, liver, and proventriculus (see Appendix, plate V). Non-infected chickens and turkeys were negative for lesions and cecal histomonads.

C. Immunity and resistance

Chickens, which had been splenectomized and infected with Histomonas (via embryonated Heterakis eggs), survived and showed no significant difference in tissue destruction or incidence of Histomonas in cecal smears, when compared with non-splenectomized chickens suffering from blackhead (see table 1).

In order to determine if host age influences resistance mortality, incidence of lesions and incidence of Histomonas in cecal smears were observed in chickens and turkeys of different ages (see table 8). No mortality was observed in 162 chickens ranging in ages from 18 to 131 days. The incidence of cecal and liver lesions and cecal histomonads was consistent. Turkeys of all age groups, ranging from 10 to 62 days, suffered from 40 to 100 percent mortality losses (see table 8). Out of a total of 142 turkeys, representing all age groups, 97 or 68.3 percent died. No age group of turkeys demonstrated a resistance to histomoniasis, as indicated by the mortality and tissue damage. The incidence of

TABLE 8

HISTOMONIASIS IN CHICKENS* AND TURKEYS** OF DIFFERENT AGES

Age in Days	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions					No. of Birds Containing <u>Histomonas</u>
			Ceca	Liver	Spleen	Kid- ney	Prov.	
<u>Chickens</u>								
18	6	0	0	1	0	0	0	5
21	20	0	5	2	0	0	0	15
22	20	0	5	1	0	0	0	12
25	8	0	1	1	0	0	0	6
27	29	0	5	8	0	0	0	26
28	20	0	3	6	0	0	0	18
32	5	0	2	3	0	0	0	3
40	29	0	10	14	1	0	0	27
42	15	0	8	5	1	0	0	11
56	5***	0	5	5	0	0	0	5
131	5***	0	5	5	0	0	0	5
<u>Turkeys</u>								
10	5	2	2	2	0	0	0	2
17	4	2	2	2	0	0	0	3
21	22	13	18	15	0	0	3	21
25	4	4	4	4	0	0	0	4
29	25	20	23	22	0	2	6	25
31	20	10	14	13	0	0	0	19
35	10	5	6	6	0	0	0	7
36	5	2	2	2	0	0	0	4
42	7	5	6	6	0	0	0	7
43	15	10	11	11	0	0	0	12
62	25	24	24	25	0	1	5	25
Totals								
Chic-								
kens	162	0	49	51	2	0	0	133
Turkeys	142	97	102	108	0	3	14	129

*Represents a majority of White Leghorns and some Vantress Cross White Plymouth Rocks.

**Represents a majority of Beltsville Small Whites and some Broad Breasted Bronze.

***Sacrificed 17 days after infection.

cecal histomonads was high in both chickens and turkeys (82 and 91 percent, respectively), thus proving a high degree of experimental exposure to the histomonad. The data accumulated in table 8 represents infections in Vantress Cross White Plymouth Rock and White Leghorn chickens and Beltsville Small White and Broad Breasted Bronze turkeys.

One or 5 inoculations of embryonated Heterakis eggs were administered orally to two groups of chickens in order to observe the effects of challenging doses upon the course of the disease. Surviving birds which received only one oral inoculation were sacrificed 30 days later, whereas surviving birds which received 5 oral inoculations (two-week intervals) were sacrificed 30 days after the fifth inoculation (60 days after the first inoculation). Data for this experiment are contained in table 9. No mortality occurred in either group (1 or 5 inoculations), and pathological conditions were consistent except for the incidence of liver lesions. Nine of 24 chickens, receiving a single inoculation, possessed less than 10 liver lesions, and 3 chickens had 10 to 20 lesions in the liver. Only 4 of 24 chickens, receiving multiple inoculations had less than 10 liver lesions, and more severe liver lesions were not observed in this group. Although these variations in grades of liver lesions were not very large, the difference between

TABLE 9

HISTOMONIASIS IN CHICKENS AND TURKEYS RECEIVING MULTIPLE INFECTIONS OF HISTOMONAS
OR SERUM FROM RECOVERED BIRDS

Groups	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions					Intes- tine	Spleen	No. of Birds Containing <u>Histomonas</u>
			Ceca	Liver			Kid- ney			
			40	10-20	20	Total	Prov.			
<u>Chickens</u>										
Controls	14	0	0	0	0	0	0	0	0	0
Infected	24	0	8	9	3	12	0	0	1	23
Infected and serum	22	0	6	9	0	9	0	0	1	22
Serum	12	0	0	0	0	0	0	0	0	0
Multiply infected	24	0	9	4	0	4*	0	0	0	22
<u>Turkeys</u>										
Controls	25	0	0	0	0	0	0	0	0	0
Infected	25	24	24	1	0	25	5	1	0	25
Infected and serum	20	8(3)**	10*	4	0	8*	12*	0*	0	17
Serum	5	0	0	0	0	0	0	0	0	0
Multiply infected	25	18*	21	3	3	18*	24	3	1	23

*Values are significantly different (5 percent confidence level) from those of the infected group.

**Only 3 deaths had occurred 22 days after infection. Five more occurred 35 to 37 days after infection.

the total number of birds possessing any grade of liver lesions (total incidence) was statistically significant. One chicken was observed with lesions in the spleen. Turkeys were also inoculated orally with embryonated Heterakis eggs and challenged at two-week intervals. However, in this case the first two inocula administered to turkeys which were to receive multiple doses, contained an insufficient number of histomonads to produce mortality. Succeeding inocula, obtained from a new collection of Heterakis were sufficient to produce mortality, as indicated when 24 of 25 turkeys, which received one inoculation, died. Eighteen of 25 turkeys, which received 5 inoculations (last 3 inoculations sufficient to produce mortality) died of histomoniasis. As can be seen from the results in table 9, the incidence of severe liver lesions (greater than 20) was less for turkeys receiving multiple inoculations. The occurrence of any grade of liver lesions (total), however, was nearly the same for both groups, since 6 of the birds receiving multiple inoculations had less than 20 liver lesions.

Table 9 also contains data obtained from infected turkeys (one inoculation sufficient to produce mortality), each of which received an intravenous injection of serum collected from chickens that had recovered from histomoniasis. In this group of turkeys mortality, incidence

of cecal lesions and severe liver lesions, total of three grades of liver lesions, and incidence of proventriculus lesions were significantly lower than those values obtained from infected turkeys not receiving chicken serum. Only 3 out of the 20 infected, serum treated turkeys had died after 22 days. Thirty-two days after infection, at which time survivors were generally autopsied, blackhead symptoms were again observed, and 3 to 5 days later 5 more turkeys died. Since turkeys were injected with serum 3 days after infection, a period of 32 days had elapsed between the serum injection and the second occurrence of fatal blackhead. Forty-two days after infection no further symptoms were observed and the 12 surviving birds were necropsied. In like manner, infected chickens were injected intravenously with serum obtained from turkeys that had recovered from blackhead. The occurrence of cecal, liver, and spleen lesions and cecal histomonads was approximately the same for both infected groups, either with or without serum injection. No blackhead lesions were observed in non-infected birds.

The next experiment was, in part, a repetition of the previous one (infected turkeys received immune chicken serum). Non-immune serum was collected from chickens which had been reared in a separate room and which had no contact with histomoniasis. Three groups of 22 turkeys were infected orally with Histomonas (via

embryonated Heterakis eggs). One group received immune chicken serum four days after infection, another group received non-immune chicken serum four days after infection, and the third group received no serum at all. Three groups of 8 turkeys were treated in the same manner, only they had been infected rectally with a suspension of histomonads. The injection of immune chicken serum into orally infected turkeys decreased significantly the severity of histomoniasis (see table 10). Lower mortality and incidence of cecal lesions and severe liver lesions were observed in serum-treated turkeys than in untreated turkeys; however, the total incidence of the mild, moderate, and severe liver lesions in each group was not significantly different since most of the liver lesions in serum-treated turkeys were mild or moderate, whereas most of the liver lesions in untreated turkeys were severe. On the other hand, orally infected turkeys treated with non-immune chicken serum showed approximately the same mortality and incidence of blackhead lesions and cecal histomonads as did orally infected turkeys not injected with serum. One-hundred percent mortality occurred in rectally infected turkeys of all three groups, although it did not occur at the same time. Rectally infected turkeys, given immune or non-immune serum or no serum at all, suffered 100 percent losses in 16, 14, and 12 days, respectively. No lesions were observed in non-infected turkeys.

TABLE 10

HISTOMONIASIS IN TURKEYS RECEIVING IMMUNE OR NON-IMMUNE CHICKEN SERUM

Groups	No. of Birds per Group	No. of Deaths	No. of Birds with Lesions					No. of Birds Containing <u>Histomonas</u>
			Ceca	Liver			Provent- riculus	
				<10	10-20	>20		
Controls	18	0	0	0	0	0	0	0
Oral infection	22	13	18	2	0	13	15	21
Oral infection and immune serum	22	5*	13*	1	4*	5*	10	20
Oral infection and non-immune serum	22	13	19	2	1	13	16	21
Rectal infection	8	8	8	0	0	8	8	8
Rectal infection and immune serum	8	8	8	0	0	8	8	8
Rectal infection and non-immune serum	8	8	8	0	0	8	8	8
Immune serum	10	0	0	0	0	0	0	0
Non-immune serum	10	0	0	0	0	0	0	0

*Values differ significantly (5 percent confidence level) from those obtained by oral infection and no serum injection.

DISCUSSION

Diseases in poultry may range from little host specificity to species specificity. Certain diseases (often of viral etiology) terminate fatally in both chickens and turkeys), whereas others may affect chickens more severely than turkeys or vice versa. Still other diseases are host specific (coccidiosis). Poultry diseases of protozoan etiology generally fall under the last two categories. Histomoniasis, as indicated in the review of literature, affects turkeys more severely than chickens. From this aspect the chicken might be considered a more normal host. Since fatal blackhead does occur in chickens under field conditions, an attempt was made in this investigation to determine what factors are responsible for this fatal termination, and also what factors provide the chicken with a greater resistance to the disease.

At the beginning of this investigation it was often observed that cecal histomonads in stained preparations from chickens and turkeys showed the presence of bacteria within their cytoplasm. A review of the literature indicated that Histomonas has not been cultured in vitro in the absence of bacteria. The disease, itself, is initiated in the ceca where there is an abundance of bacteria. In view

of these facts surgical implantation of cecal contents from turkeys to chickens, and vice versa, was performed in order to determine whether bacterial flora might affect the disease. From results in table 1 it appears as though implantation of turkey cecal contents into ceca of chickens effected a decrease in the incidence of cecal lesions and mild liver lesions (less than 10) as compared with the incidence of these lesions in infected, untreated chickens. Therefore, the incorporation of turkey cecal contents into ceca of chickens definitely did not result in conditions favorable for progressive histomoniasis. Implantation of chicken cecal contents into ceca of turkeys was without effect upon histomoniasis in this host.

It is the opinion of some workers that fatal histomoniasis occurs in chickens when their resistance has been lowered by vaccination, other diseases, or some adverse condition. In the present investigation several stresses were used in an attempt to break down the resistance of chickens or alter the susceptibility of turkeys to black-head. Periodic starvation of birds to the point of emaciation did not suffice to lower the resistance of chickens sufficiently to produce mortality due to histomoniasis, although it did decrease the incidence of cecal histomonads (see table 2). Although there was no significant change in mortality, the disease in starved turkeys resulted in a higher incidence of cecal lesions. Therefore, the indications

are that starvation may have had some influence upon the disease in turkeys (increased liver lesions), but no influence upon the disease in chickens.

An induced, anemic condition was not sufficient to alter histomoniasis in either chickens or turkeys, as indicated in table 5. A slight anemia resulted from histomoniasis in chickens and turkeys, as indicated by the comparison of hemoglobins of infected and non-infected birds which were bled periodically (see table 4). That a slight anemia develops in infected turkeys has been demonstrated by Johnson and Lange (1939) and McGuire and Cavett (1952). Likewise, the repeated exposure of infected chickens and turkeys to 4° C or 37° C did not change the mortality or pathological conditions characteristic of the disease (see table 6).

Under field conditions when two diseases such as coccidiosis and histomoniasis occur simultaneously it is difficult to determine whether one influences the other. However, under experimental conditions, more accurate information can be obtained. Chickens were infected with just enough sporulated oocysts of Eimeria tenella to produce clinical symptoms, but not mortality. The results in table 3 indicate that coccidiosis did not significantly increase mortality or the incidence of lesions due to histomoniasis. These results do not support the belief of Jowett (1911) that a disruption of the intact epithelium

by coccidia aids the invasion of Histomonas.

Lund (1956) has stated that vaccination may lower the resistance of chickens thereby allowing them to contract fatal histomoniasis. Results obtained in the present investigation, using fowl pox vaccination, disagree with Lund's belief. Infected and vaccinated chickens and turkeys failed to show any significant change in mortality or incidence of lesions and cecal histomonads when compared with infected, unvaccinated chickens and turkeys (see table 7).

Since none of the afore-mentioned stresses were capable of inducing fatal histomoniasis in chickens or influencing mortality in turkeys, the investigation was continued along the lines of natural or acquired resistance. Kendall (1957) infected turkeys of different ages (7 days to 20 months) orally with embryonated Heterakis eggs or rectally with histomonad suspensions, and observed uniform susceptibility to histomoniasis. On the other hand, Desowitz (1951) infected chickens of different ages (6 to 34 days) rectally with suspensions of histomonads, and he observed less mortality (30 percent) in thirty-four-day-old chickens than in six to twenty-one-day-old chickens (60 to 71 percent mortality). However, this method of infection is unnatural, and the number of histomonads introduced into the ceca was probably much greater than the number which would be introduced orally by means of embryonated Heterakis eggs.

In the present work data were accumulated from many experiments in which all infections were produced by the oral inoculation of embryonated Heterakis eggs. One hundred and sixty-two chickens ranging in ages from 18 to 131 days showed uniform resistance to histomoniasis (see table 8). Pathological conditions occurred with uniform frequency, and chickens in all age groups recovered. The greater proportion of these chickens (133 of 162) retained histomonads within their ceca for at least 30 to 42 days after infection, at which time they were necropsied. Turkeys, on the other hand, showed uniform susceptibility to histomoniasis (see table 8). Out of 142 turkeys 97 or 68.3 percent died of histomoniasis. Mortality for the different age groups (ranging from 10 to 62 days) varied from 40 to 100 percent. These results are in agreement with Kendall's (op. cit.), namely, that turkeys of all ages are susceptible to histomoniasis.

It was apparent that under experimental conditions a percentage of turkeys acquired an infection which was relatively non-pathogenic since the incidence of lesions and cecal histomonads was higher than the occurrence of mortality (see table 8). Resistance to succeeding pathogenic challenge doses may indicate a state of premunition in turkeys, as reported by Tyzzer (1934). Other workers have also noted the existence of a temporary resistance to reinfection. Results of the present investigation, listed in

table 9, agree with results obtained by other workers. Turkeys were infected with embryonated Heterakis eggs five times (two week intervals). The first two inoculations were sub-lethal, although symptoms did appear. Succeeding challenge doses were lethal, as indicated when 24 of 25 turkeys, that had not received an inoculation previously, died as a result of one inoculation. Turkeys which received multiple inoculations suffered mortality losses after each of the last three inoculations. However, these turkeys showed less mortality and a lower incidence of severe liver lesions when compared with turkeys receiving a single inoculation (see table 9). Statistical analysis showed the differences to be significant at the 5 percent confidence level. In reference to these results it can be said that turkeys may possess some resistance to reinfection, but this resistance is by no means complete, since mortality did occur after repeated inoculations. Chickens, which received a total of five pathogenic inoculations at intervals of 2 weeks, showed a lower total incidence (significant at the 5 percent confidence level) of liver lesions when compared with chickens that had received one inoculation. Here again the indications are that a resistance to reinfection exists, only in this case the initial resistance is greater at the first oral inoculation since no mortality occurred. Subsequent infections, though, did not increase the pathological conditions characteristic of the disease.

That turkeys, which recover from histomoniasis, possess an incomplete and temporary immunity has been pointed out previously in the review of the literature. It has been demonstrated by Sautter and co-workers (1950) that whole blood, obtained from turkeys which had recovered from blackhead, failed to protect susceptible turkeys from the fatal disease. To the author's knowledge this has not been tried in chickens suffering from histomoniasis, or for that matter, blood from infected, recovered chickens has not been injected into infected turkeys. In the present investigation serum was obtained from chickens and turkeys that had recovered from histomoniasis. The turkey serum was injected into chickens 3 days after infection with Heterakis eggs, and the results in table 9 indicate that the serum had no effect upon the disease in chickens. For this reason serum from control turkeys (no contact with blackhead) was not injected into infected chickens. On the other hand, when serum from recovered chickens was injected into turkeys 3 days after infection with Heterakis eggs, an impressive change in the course of the disease was noted (see table 9). Mortality was decreased considerably and lesions were less severe and occurred less frequently. By 32 days after the oral inoculation of turkeys with embryonated Heterakis eggs (29 days after serum injection) only 3 of 20 turkeys had died, and they showed typical histomonad lesions. In previous experiments surviving birds were necropsied at this

length of time after infection, but symptoms of histomoniasis were again observed (32 days after infection), and necropsy was delayed for 10 days, during which time 5 more deaths occurred and symptoms of blackhead disappeared. The occurrence of the last 5 deaths was not attributed to reinfection by the ingestion of food or water that had been contaminated with Heterakis eggs passed by gravid worms in the turkeys, since there was insufficient time for the worms to mature, the eggs to embryonate, and turkeys to become infected by ingesting the embryonated eggs. Nor were these deaths attributed to infection by the ingestion of free histomonads in the feces since all turkeys were retained in batteries with wire bottoms. Therefore, it appears that the chicken serum afforded the last 5 turkeys that died protection from histomoniasis for approximately 3 weeks. By 32 days after the infection of a similar group of turkeys which had received no serum injection, 24 of 25 had died. All of these turkeys, except the lone survivor, which possessed less than 10 liver lesions and no cecal lesions, showed very severe liver and cecal lesions. Five of the 24 birds which had died also showed grayish necrotic lesions on the wall of the proventriculus (see Appendix, plate I). The lesions always occurred on that portion of the proventriculus which remained in contact with the highly necrotic liver, and they gradually spread from that area. It is the author's opinion that such lesions were not initiated by histomonads

from the blood stream, but that a direct transfer of the histomonads from the liver (through the disrupted liver capsule) to the wall of the proventriculus initiated the lesions. Previously, McGuire and Morehouse (1958) reported proventriculus lesions in one turkey that had been infected intravenously (blood inoculum derived from cecal or mesenteric veins of infected donor), but they did not state whether or not the lesions resulted from histomonads in the blood stream. That the proventriculus lesions resulted from a direct transfer of histomonads from the liver is supported by the observation in one turkey of a similar lesion on the wall of the small intestine where it lay in contact with the necrotic liver. Microscopic examination of proventriculus sections revealed coagulation necrosis and inflammation, and the histomonads were observed in abundance (see Appendix, Plate III). Fresh saline smears also revealed the presence of the organisms. None of the turkeys which had received serum injections showed lesions in the proventriculus.

At this stage of the investigation it remained to be determined whether the serum obtained from recovered chickens contained immune bodies or whether the protection afforded turkeys was derived from some inherent property of chicken serum. Therefore, serum was collected from chickens which had no contact with histomoniasis, and from other chickens which had been infected for 21 days. The

ability of serum, obtained from infected, recovered chickens, to protect turkeys from the progressive form of the disease was confirmed, as indicated by data in table 10. Turkeys, which were infected with Histomonas and injected with non-immune chicken sera, experienced no protection against the disease. Mortality and incidence of lesions were not significantly different from the results obtained from infected turkeys which received no serum injection. This would seem to indicate the existence of humoral antibody in recovered chickens. Three groups of 8 turkeys were infected rectally with histomonad suspensions, the results of which are shown in table 10. One group received immune chicken serum, another group received non-immune chicken serum, and the third group received no serum. All three groups of turkeys died, but 100 percent mortality of turkeys receiving immune serum occurred later than it did in the other two groups, thereby suggesting the benefit of some protection derived from the serum.

Since the information on the ability of immune chicken serum to afford some protection to turkeys from histomoniasis came late in this investigation, time did not permit sufficient supportive experimentation on antibody production. Five chickens were splenectomized and inoculated per os with embryonated Heterakis eggs. This operation was not sufficient to induce fatal histomoniasis; nor did the incidence of lesions and cecal histomonads differ significantly

from results obtained from non-splenectomized, infected chickens (see table 1).

Bjorneboe and co-workers (1951) had shown that cortisone may affect the concentration of humoral antibody, so chickens and turkeys were inoculated orally with embryonated Heterakis eggs and treated with daily intramuscular injections of this hormone for 21 days after infection. Pullin (1955) injected turkeys intramuscularly with 1.5 mgm. of cortisone per pound, since this was the suggested dosage for rheumatoid arthritis in man. He gave daily injections for 19 days and observed no toxic effects in turkeys resulting from the cortisone. Since there was no toxic effects at this dosage, an increased dosage was used in the present investigation. Chickens received 1.7 mgm. per pound of body weight for the first 5 days, and the dosage was gradually increased to 3.00 mgm. per pound by the tenth to thirteenth day, after which it was gradually decreased on successive days to 2.33 mgm. per pound. Turkeys also received 1.7 mgm. per pound for the first 5 days, after which the dosage was gradually increased daily to 2.43 mgm. per pound on the nineteenth to twenty-first day. Pullin (op. cit.) observed no effect of cortisone upon histomoniasis in turkeys, but he had not used chickens in his experiments. In the present investigation Pullin's results were confirmed, and daily cortisone injections in chickens also failed to alter the disease significantly (see table 7). Since

cortisone has been shown to decrease antibody concentration and inflammatory reactions, one might expect the severity of histomoniasis in chickens and turkeys to increase. However, this was not observed, possibly due to insufficient dosage.

An insight to the exact cause of death of turkeys suffering from histomoniasis might shed some light upon fatal histomoniasis in chickens. McGuire and Cavett (1952) observed the development of a severe hypoglycemia in turkeys just before they died of blackhead, and they stated that this factor might be the cause of death. From time to time during the present investigation when turkeys were observed a few hours away from death intravenous injections of 5 percent glucose (5 to 15 ml.) were administered in an attempt to prolong the life of the bird, but to no avail. On another occasion 6 turkeys were inoculated rectally with histomonad suspensions, and on each day thereafter 4 ml. of a 5 percent glucose solution was injected intravenously as long as the turkeys lived. From the fifth day after infection (symptoms appeared) until death the turkeys were also injected intramuscularly with 2 ml. of a 5 percent glucose solution. The injections of glucose did not prolong the lives of turkeys infected with histomoniasis. These results indicate that the hypoglycemia which develops may not be severe enough to cause death of turkeys.

SUMMARY

Surgical implantation of cecal contents from non-infected control chickens into ceca of turkeys, followed by infection with embryonated Heterakis eggs, failed to alter the course of the disease significantly in turkeys. The surgical implantation of cecal contents from non-infected control turkeys into ceca of chickens, followed by Histomonas infection, failed to produce conditions favorable for progressive histomoniasis. Infected chickens receiving implants showed a lower incidence of cecal lesions and of mild liver lesions, possibly indicating the creation of an unfavorable cecal environment.

Starvation of chickens suffering from histomoniasis did not increase the severity of the disease. A significantly lower incidence of cecal histomonads was observed. Starvation of turkeys suffering from histomoniasis increased significantly the incidence of liver lesions, but did not affect mortality.

Concurrent infections of histomoniasis and coccidiosis in chickens did not increase significantly mortality and incidence of lesions due to histomoniasis.

Rendering chickens and turkeys anemic did not change the pathogenesis of histomoniasis as it generally occurs

in these hosts. Slight anemias in chickens and turkeys were developed as a result of the disease alone.

Exposure of chickens and turkeys to 4° C or 37° C temperatures failed to influence any aspect of the disease in these hosts. Likewise, the vaccination of chickens and turkeys with fowl pox virus failed to change significantly mortality, incidence of cecal histomonads, or incidence of lesions.

Turkeys of all age groups (10 to 62 days) were found to be equally susceptible to histomoniasis when inoculated per os with embryonated Heterakis eggs. Chickens of all age groups (18 to 131 days), on the other hand, were found to be equally resistant to the disease when inoculated with embryonated Heterakis eggs. Pathological conditions occurred with uniform frequency. Chickens in all age groups recovered, while turkeys suffered mortality losses from 40 to 100 percent.

Turkeys, which received 2 sub-lethal oral inoculations of embryonated Heterakis eggs, followed by 3 lethal inoculations (two-week intervals), showed less mortality and a lower incidence of severe liver lesions (greater than 20 lesions), thereby indicating some resistance to reinfection. Mortality occurred, however, after each lethal dose. Chickens, which received 5 lethal oral inoculations of embryonated Heterakis eggs (lethal for turkeys), showed a decrease in the incidence of liver lesions (any grade), again indicating

some resistance to reinfection. No mortality was observed in these chickens.

Serum, collected and pooled from turkeys (20 to 21 days after oral inoculation with embryonated Heterakis eggs), and injected into chickens (3 to 4 days after oral inoculation of embryonated Heterakis eggs), had no effect upon histomoniasis in chickens. In like manner, serum, collected and pooled from uninfected control chickens, when injected into turkeys had no significant effect upon the pathogenesis of histomoniasis. However, when serum was collected and pooled from chickens (20 to 21 days after per os inoculation with embryonated Heterakis eggs), and injected into turkeys (3 to 4 days after infection), mortality decreased significantly. Corresponding significant decreases occurred in the incidence of cecal, liver, and proventriculus lesions. Immune chicken serum afforded less protection (delayed 100 percent mortality) to turkeys when they were infected rectally with histomonaad suspensions.

Splenectomy, followed by oral inoculation of embryonated Heterakis eggs, had no effect upon hitomoniasis in chickens.

Chickens and turkeys, which received daily intramuscular injections of cortisone for 21 days after oral inoculation of Histomonas, via Heterakis eggs, showed no significant change in mortality and incidence of lesions.

Injection of a 5 percent glucose solution into turkeys just before death or daily injections after the

time of infection until death did not prolong the lives of turkeys suffering from histomoniasis.

An increased incidence of lesions on the wall of the proventriculus next to the liver occurred when ground up Heterakis females from the last two collections (from poultry abattoirs) were used as the source of inoculum. It appears that these lesions are not initiated by histomonads from the blood stream, but by a direct contingent migration from the liver, through the disrupted liver capsule, to the wall of the proventriculus. In one turkey an identical lesion occurred on the wall of the small intestine where it lay in contact with the necrotic liver. Microscopic examination of tissue sections revealed coagulation necrosis and inflammation of the muscular layers and the presence of many histomonads.

Lesions resulting from histomoniasis, alone or in conjunction with a particular treatment, have been observed in chickens (478 infected) in the following organs (the number in parentheses indicates the number of observations): ceca (94), liver (130), spleen (3), and proventriculus (1). Histomonads have been observed in these lesions. In like manner, the following lesions have been observed in turkeys (355 infected) suffering from histomoniasis: liver (256), ceca (253), proventriculus (36), kidney (8), spleen (3), intestinal wall (1), mesentery (1), and heart (1). Histomonads have been observed in these lesions with the

exception of the heart.

The incidence of Histomonas in cecal smears from experimental chickens and turkeys infected with 300 to 700 embryonated Heterakis eggs has been high throughout the investigation, indicating a high degree of exposure of birds to the etiological agent.

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APPENDIX

PLATE I. Lesions due to histomoniasis in a turkey. Note lesions in the liver, ceca, proventriculus, spleen, mesentery, and one lesion on the heart.



PLATE II. Heart of a turkey infected with histomoniasis.
Notice the thick fibrous layer on the epicardium.

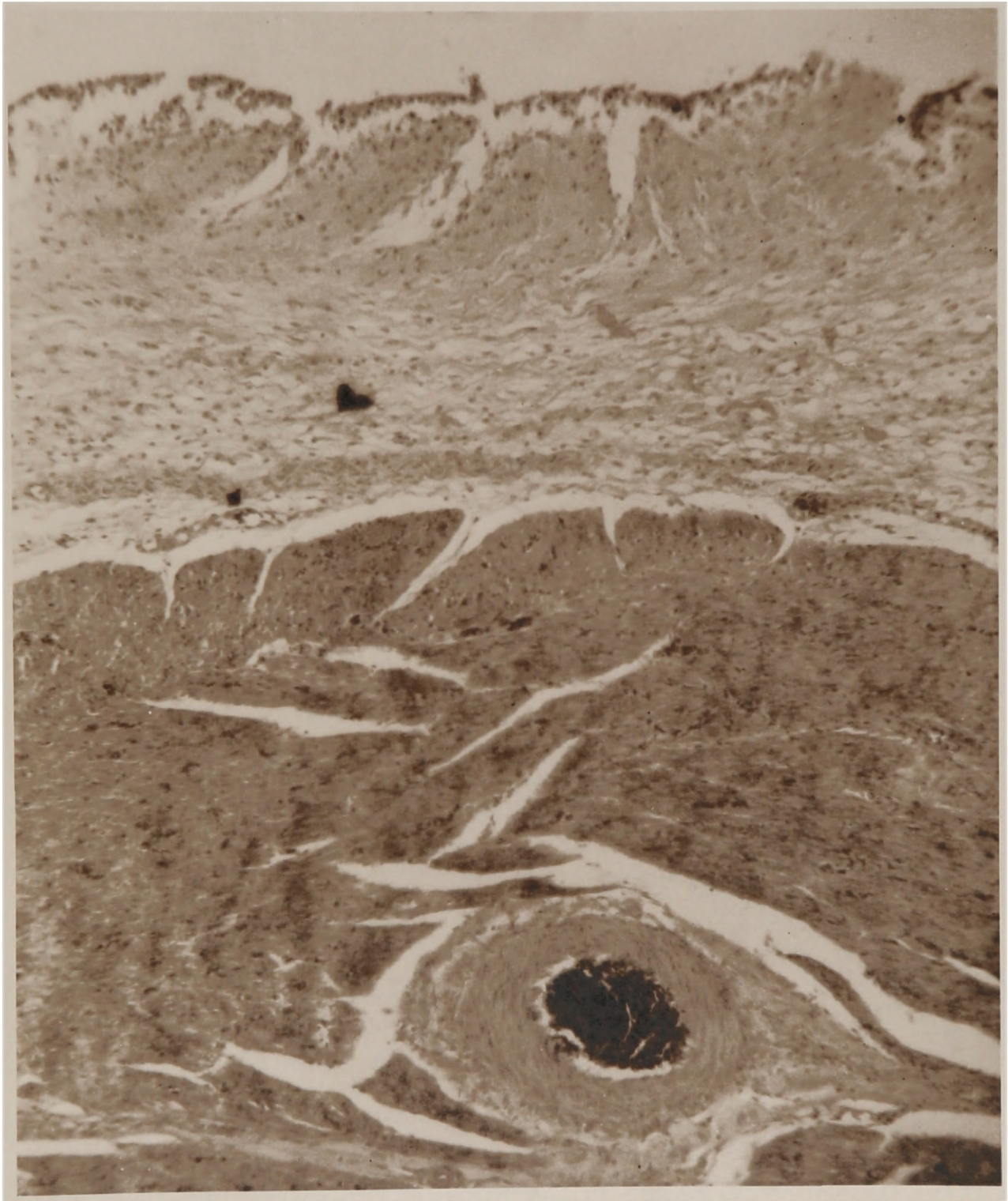


PLATE III. Proventriculus of a turkey infected with histomoniasis.

PLATE III.



PLATE IV. Mesentery of a turkey infected with histomoniasis.
The thickened area of the mesentery shows many
small, rounded histomonads.

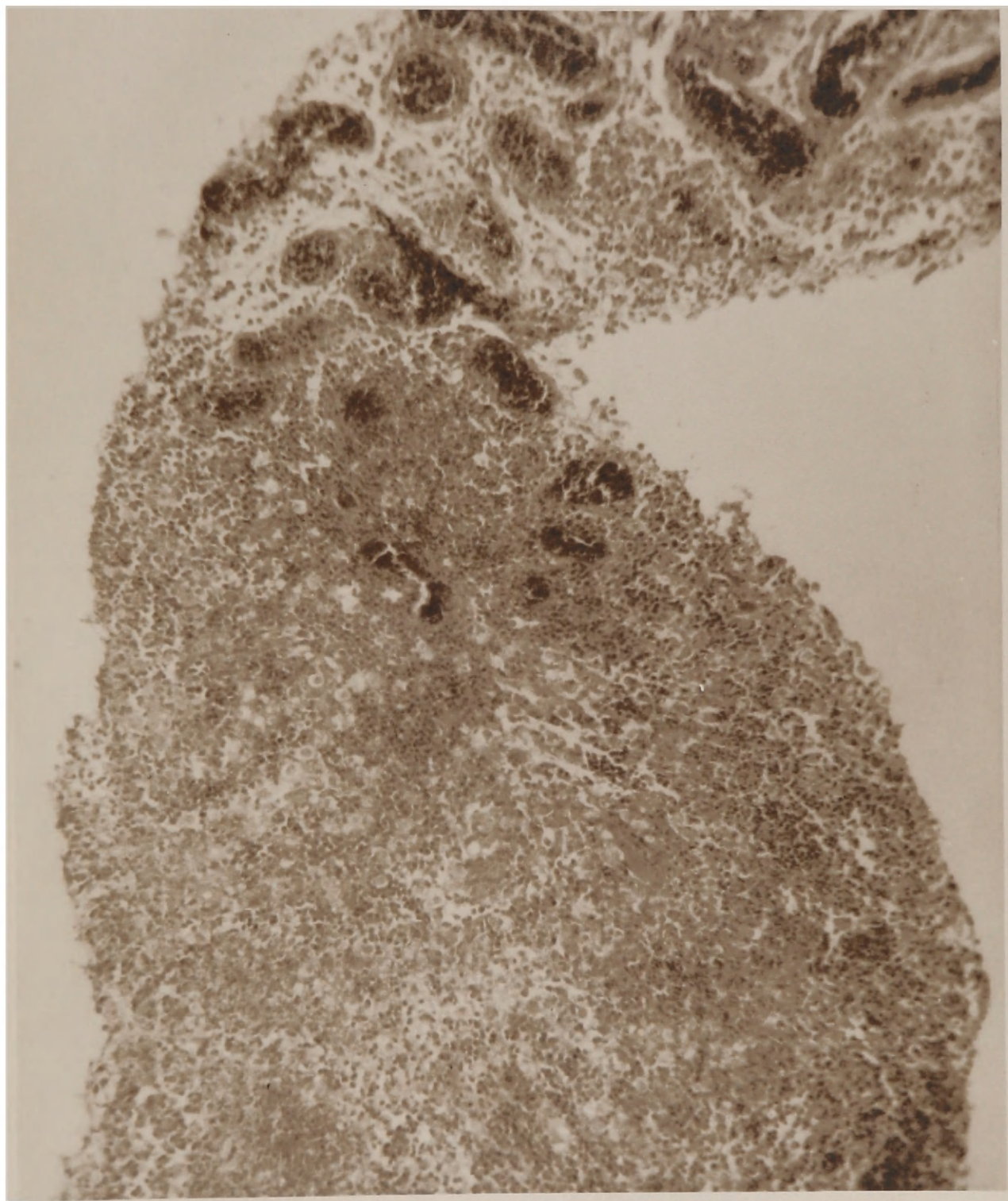


PLATE V. Lesions due to histomoniasis in a chicken. Note lesions in the liver, ceca, and proventriculus.



OBSERVATIONS ON SOME FACTORS WHICH MAY INFLUENCE
SUSCEPTIBILITY OR RESISTANCE OF CHICKENS AND TURKEYS TO
HISTOMONIASIS

By

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AN ABSTRACT

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ABSTRACT

A study was made concerning factors which may influence susceptibility or resistance of chickens and turkeys to histomoniasis. These factors included surgical implantation of cecal contents from chickens to turkeys and vice versa, exposure to various stresses (starvation, Eimeria tenella infections in chickens, anemia, temperature variation, cortisone injection, fowl pox vaccination), and the determination of the effect of age, multiple inoculations, and immune or non-immune serum injections.

Most of the birds were infected orally with 300 to 700 embryonated Heterakis eggs (sufficient to produce 50 percent or greater mortality in turkeys). Some were infected rectally with histomonad suspensions. Surviving birds were necropsied 30 to 35 days after infection.

Surgical implantation of cecal material failed to alter histomoniasis in chickens and turkeys with the exception of a decreased incidence of cecal and mild liver lesions in chickens.

Starvation of infected chickens and turkeys did not affect mortality, but turkeys showed a significant increase in the incidence of liver lesions.

Concurrent infections of histomoniasis and coccidiosis in chickens did not significantly change mortality and incidence of lesions due to histomoniasis.

Rendering chickens and turkeys anemic or exposing them to 4° C or 37° C temperatures or vaccinating them had no significant effect upon histomoniasis in these hosts.

Turkeys of all age groups (10 to 62 days) were found to be equally susceptible to histomoniasis, and chickens of all age groups (18 to 131 days) were equally resistant to the disease. All chickens recovered, while turkeys suffered from 40 to 100 percent mortality losses.

Two sub-lethal oral inoculations of Histomonas (via embryonated Heterakis eggs), followed by three lethal oral doses (at two-week intervals), had the effect of decreasing mortality and incidence of liver lesions in turkeys. Necropsy of chickens after five lethal inoculations (lethal for turkeys) revealed a lower incidence of liver lesions.

Turkey serum (collected 20 to 21 days after oral inoculation with embryonated Heterakis eggs) when injected into chickens had no effect upon histomoniasis. In like manner, serum, collected from non-infected chickens, when injected into turkeys did not alter the disease. However, when serum was collected from chickens (20 to 21 days after per os inoculation with embryonated Heterakis eggs) and injected into turkeys, mortality due to histomoniasis decreased considerably. In two separate experiments corresponding significant (5 percent confidence level) decreases occurred in the incidence of cecal and liver lesions. In one experiment a significant decrease in occurrence of

proventriculus lesions was observed. Immune chicken serum from this same collection afforded less protection (delayed 100 percent mortality) to turkeys when they were infected rectally with histomonad suspensions.

Splenectomy had no effect upon histomoniasis in chickens. Likewise, infected chickens and turkeys, which received daily intramuscular injections of cortisone for 21 days, showed no significant change in mortality and incidence of lesions.

Injections (just before death or daily until death) of 5 percent glucose solution into turkeys did not prolong the lives of turkeys suffering from histomoniasis.

Lesions resulting from histomoniasis have been observed in the ceca, liver, spleen and proventriculus of chickens and the liver, ceca, proventriculus, kidney, spleen, intestinal wall, mesentery and heart of turkeys. Histomonads were observed in these lesions with the exception of the heart.