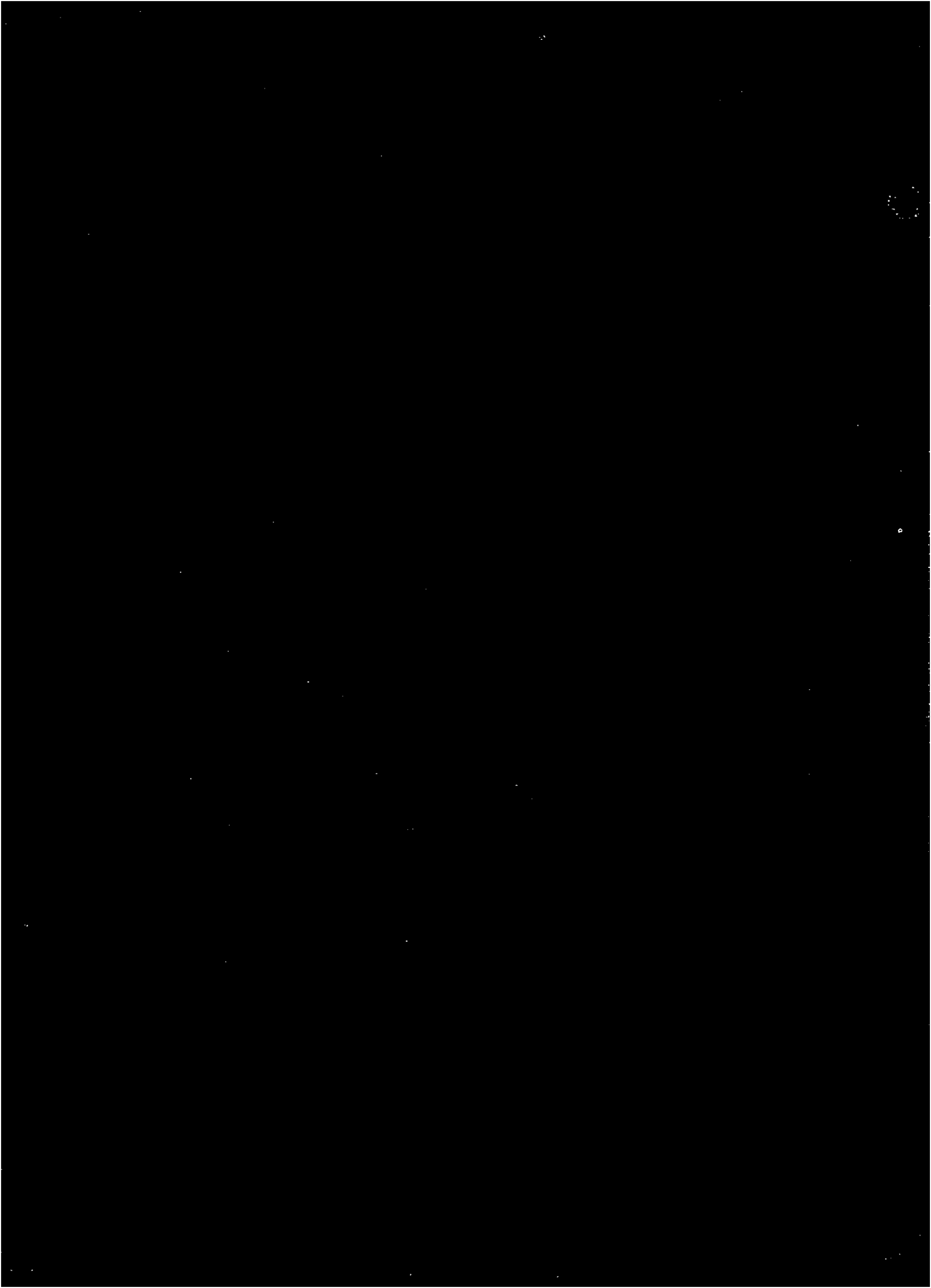

A STUDY ON THE TRANSMISSION OF *HISTOMONAS MELEAGRIDIS* TO POULTS (*MELEAGRIS GALLOPAVO*)

A Thesis
Presented to
the Faculty of the Graduate School
University of Missouri

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
J. S. Ahluwalia
January 1962

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

Histomoniasis (Blackhead or Infectious Enterohepatitis) is a protozoon disease caused by a microscopic single-celled parasite Histomonas meleagridis. It was a devastating disease of turkeys in the East and Midwest of the United States of America a generation or two ago. Blackhead still remains the outstanding disease among turkeys when evaluated in terms of its prevalance and financial loss to poultry growers. The United States Department of Agriculture (1954) has estimated that Histomoniasis causes an annual loss of 3.8 million dollars in turkeys and 149 thousand dollars in chickens due to mortality alone.

In nature birds most commonly become infected by the ingestion of infected Heterakis eggs or they may acquire infection directly from other infected turkeys and chickens.

The infective agent enters the digestive tract of the bird and become localized in the blind pouches--the ceca. An inflammatory ulcerative condition develops in the ceca which permits the parasites to invade other tissues. Probably through the blood stream the parasites reach the liver where they produce numerous characteristic abscesses varying in size from pin-point to 15 m.m. or more in diameter. Because of extensive tissue destructions, usually of

the liver and ceca, death results in many infected birds. The death rate in individual flocks of turkeys may run as high as 50-100 percent. Eriksen (1925) reported losses from Blackhead in Missouri chicken flocks which ranged from one bird in a group of 350 to more than 50 percent in two other flocks. Turkeys of any age may contract Blackhead, but losses are usually greatest among turkeys eight to eighteen weeks of age. They often die two or three days after showing the first signs of the disease.

Outbreaks are most common in the spring and fall and usually are more serious in wet seasons than in dry ones. A study in Minnesota in 1951 revealed that of every 1000 poults started, six died from Blackhead after being kept in the brooder house and eighty-seven more died when the poults were placed on range. The number of cases of Histomoniasis recorded at the Poultry Diagnostic Laboratory, School of Veterinary Medicine, University of Missouri, from 1956 to 1960 are shown in Table I. Histomoniasis is apparently decreasing every year and there may be at least three reasons for this.

1. More has been learned about the spread of the disease. Most growers keep turkeys isolated from any contact with chickens, or older turkeys, or contaminated soil or litter that may harbor the disease.
2. Drugs have been developed which help to control the disease.

3. Turkey growers can usually diagnose the disease by examining the ceca and liver which show characteristic lesions.

In spite of these advances, Histomoniasis remains a costly problem in areas where the soil retains its moisture and where high environmental temperatures are not sustained.

Further investigations must be made regarding the life history; transmission and pathogenecity of Histomoniasis. Several investigators have studied the comparative effectiveness of methods used for producing infection. The studies presented here were made to determine how readily normal Heterakis free poults would become infected with Histomonas from various sources i.e. ceca; liver; cecal contents and whole blood, using various routes of transmission.

PROBLEM

To select a problem in veterinary protozoology it was thought that Histomoniasis (Blackhead or Infectious Enterohepatitis) would be most interesting due to the fact that this disease as yet has not been recorded in India. Due to the modern facilities of transportation diseases from one country are spreading to another. There is every possibility that Histomoniasis may be a disease of the future for India, where the poultry industry is fast

developing.

This disease is also of great economic importance in Missouri where turkey rearing is an important industry.

In the course of investigations into the prevention and treatment of Histomoniasis, one of the major difficulties has always been the lack of an adequate supply of infected turkeys under controlled conditions. For statistical purposes it is essential that diseased birds should be of comparable age and weight in order that the efficiency of the preventive or curative methods can be accurately determined. Furthermore, it is desirable that as far as possible the incidence of Histomoniasis in groups of birds used for experimental purposes should be 100 percent. It is therefore important to have a method of producing Histomoniasis at will. With all of the above mentioned objects in view, the problem of experimental transmission of Histomoniasis by different routes was selected.

these parasites. Hadley and Amison (1911) and Hadley (1916) later became advocates of the theory of the flagellate nature of the infection. They believed that a Trichomonas which ordinarily lives the life of a harmless resident of the intestines of fowls may assume a new role under conditions that lower the resistance of the bird. Hadley also contended that flagellosis (as he called it) of the ceca and liver could not be regarded as an infectious disease, since Trichomonas existed in the intestinal tract as a facultative parasite and its disease producing powers were wholly extrinsic to its own physiological organization.

Jowett (1911) in South Africa likewise held the view that a Trichomonads "Trichomonas eberthi" (Kent) was a normal inhabitant of the ceca of healthy birds and that under certain conditions this flagellate became pathogenic and produce Blackhead. In his description of this parasite Jowett mentions the presence of a well developed undulating membrane and axostyle. Because of this suggested connection with Histomonads, it is interesting to note that this author also encountered spherical, oval or pear shaped organisms with only two flagella and devoid of an undulating membrane and axostyle.

Doflein (1911) expressed the opinion that the position and pathogenic importance of the Blackhead parasite was not clear. He referred to it as "Entamoeba meleagri-

dis" in his textbook on protozoology.

Tyzzer (1919) discredited the Amoeba, Coccidia and Trichomonad hypothesis and published the first of a series of articles which served to counteract the confusion that earlier existed regarding the causative agent of Blackhead. In his earlier work Tyzzer mentions no flagella arising from the extra nuclear body. He did describe the amoeboid movements of the organism. In a later paper (1920) entitled "The Flagellate Character and Reclassification of the Parasite Producing 'Blackhead' in Turkeys. Histomonas (gen. nov.) meleagridis (Smith)," he renamed Smith's organism Histomonas meleagridis. He described the "pulsating movements" of the parasites when observed under the microscope in a warm chamber at 41° C. to 42° C., and gave considerable evidence that the parasite migrates through the tissue by amoeboid movements. A rudimentary flagellum on the surface of the organism was also observed.

In addition he called attention to the existence of various developmental phases in the tissue which he named "invasive phase," the "vegetative phase" and the "resistent phase" of the parasite.

The first form i.e. invasive phase is found in early lesions of the disease and rarely in older lesions. It is amoeboid in nature and measures 8 to 17 microns, although some organisms as long as 30 microns have been noted. It is found between the cells and is never intra-

cellular. The cytoplasm is basophilic and consists of clear cytoplasm and a finely granular endoplasm. There is a small extra nuclear body present although its location is variable. Ingested particles may be found in vacuoles in the cytoplasm, but bacteria are never observed in this first stage.

The second form is the vegetative stage which is found in slightly older lesions and measures 15 to 21 microns in length and about 12 microns in width. They are present in great numbers and are associated with distension and disruption of the tissues. The cytoplasm is still basophilic, clear and transparent but without ingested particles.

The third form is the resistant stage and is found in the oldest lesions of the disease. These forms are small, varying in size from 4 to 11 microns in diameter. The cytoplasm is acidophilic and is filled with small granules or globules which give it a rather course appearance. The organisms are spherical in shape or compressed in masses with their shape modified by contact with other flagellates. The number of organisms in such masses may vary from two to several dozen. After being enclosed in the tissue the parasite becomes surrounded by a transparent thick layer which appears almost cystlike in nature. Although these forms are described as resistant stages, this is a misnomer. Even though a cystlike membrane

surrounds the parasite, it is still very susceptible to environmental conditions and can live only a very short time outside the body.

In addition to the tissue stages, the parasite may develop flagellated stages in the lumen of the cecum or in culture media.

Free flagellated forms were first seen by Tyzzer and Fabyan (1922). These were taken from the ceca of experimentally infected turkeys. They stated that in the ceca of turkeys late in the disease a form of Histomonas meleagridis was found in considerable numbers of which one or two short flagella were demonstrable. In 1924 Tyzzer found flagellate forms in the ceca of chickens. Drbohlav (1924) obtained the flagellated forms in cultures from the ceca of diseased birds. Flagellate type parasites showed a great variety of amoeboid movements and ingested bacteria, cell fragments and starch grains.

Tyzzer (1934) later shed more light on the nature and behavior of Histomonas. In cecal discharges under optimum conditions, it is fairly rounded but with irregular surface extensions. It exhibits active amoeboid movements and rhythmic rotary movements.

In artificial culture the organisms usually attains a larger size than in cecal discharges and exhibits active amoeboid movements, at other times they are more rounded and undergo "rhythmic flagellate motility." The flagel-

lated phases may measure from 4.5 to 25 microns in diameter and flattened amoeboid forms measure considerably more. While normally uniflagellate and aflagellate forms are common, those with two or even four flagella are not infrequently encountered in cultures. Devolt and Davis (1936) largely confirmed Tyzzer's observations on the behaviour and nature of the organism from tissues, in cecal discharges, and in culture.

Wenrich (1943) made comparative studies of Histomonads from the ceca of pheasants and chickens. Measurement of 400 organisms from pheasants gave a range of 9 to 23 microns in diameter with an average of 13.9 microns. In chickens a range from 5 to 18 microns in diameter with an average of 7.86 microns. The cytoplasm is usually composed of a clear, outer ectosarc and a coarsely granular endosarc. It may contain bacteria, starch grains and other food particles, including an occasional red blood cell. The nucleus is often vesicular, with a single dense karyosome, or it may contain as many as eight scattered chromatin granules. Near the nucleus is a basal granule or blepharoplast from which the flagella arises. There is typically a single, short flagellum but as many as four may be present. Movements may be amoeboid, and there may be a pulsating, rhythmic intracytoplasmic movement. The flagella produce a characteristic, jerky, oscillating movement resembling that of Trichomonads but Histomonas can be

parts of the United States of America. Gilbert (1899) found it in Ottawa, Canada. In 1908, F. V. Theobold called attention to its presence in England. Brigham in 1907 reported its prevalence in Maryland. In the state of Missouri the disease was reported sometime in the year 1915 and Eriksen (1925) indicated that the losses from Blackhead in Missouri chicken flocks ranged from one to fifty per-cent.

Transmission experiments from diseased to healthy turkeys were done apparently for the first time by Veranus Moore in 1896 at the B.A.I. Laboratory, Washington, D.C. He obtained specimens from Cushman of the Rhode Island Experiment Station. Turkeys obtained from nearby flocks were inoculated. He succeeded in transmitting the disease to one of two poults by feeding them with diseased liver and ceca. In the case of four other young turkeys that were confined in a pen with two affected ones and fed daily with their excreta, the disease was transmitted to three. In 1900 Chester reported the transmission of the disease to a fowl, but he refers to the causative organism as a "coccidia." No doubt the finding of Graybill and Smith in 1920 showing that the cecal worms (Heterakis gallinae) played an important role, invalidated certain earlier experiments in which cecal worm was not considered. The disease has since been transmitted by many investigators by different routes of inoculation, with different degrees

of success. Histomoniasis can be experimentally produced by oral or by rectal inoculation of the liver and cecal lesions from acute cases, cecal discharges of carriers, culture of Histomonas and embryonated Heterakis eggs or by intravenous, intrahepatic, intracecal, subcutaneous, and intramuscular inoculation of aspectically obtained infected tissues.

In the following pages literature of some of the important routes of transmission will be discussed, with their degree of success on the method and material they utilized for producing experimental Histomoniasis. (Table 2).

I. Transmission by Oral Inoculation

At first, neither Smith nor Tyzzer had been able to find definite indication of the presence of Histomonas meleagridis in the cecal contents or discharges of infected birds. Smith (1915) stated "Although the ceca are the chief seat of the disease, and are evidently the region where the tissues are first invaded by way of digestive tract, a study of the contents has thus far yielded nothing definite." Tyzzer (1919) stated that "The contents of the ceca are remarkably free from parasite. It has thus far been impossible to identify it in the discharges examined during the life of the infected bird." These workers, like most of the others, were of the opinion that the

TABLE II

Examples in the Literature of Experimental Transmission of Histomoniasis (Enterohepatitis, Blackhead) in Turkeys.

Route of Transmission	Material or Method	Author	Apparent Success
Oral inoculation	Infected liver and cecal tissue	Moore (1896)	50%
Oral inoculation	Excreta	Moore (1896)	75%
Oral inoculation	Embryonated eggs of Heterakis gallinae	Graybill and Smith (1920)	High
Oral inoculation	Disinfected embryonated eggs of Heterakis gallinae	Smith and Graybill (1920) Tyzzer (1925)	High
Oral inoculation	Liver and lung lesions	Tyzzer Fabyan & Foot (1921)	0
Oral inoculation	Liver lesions	Tyzzer & Collier (1925)	38%
Oral inoculation	Culture of Histomones meleagridis	Allen (1941)	92%
Oral inoculation	Culture of Histomones meleagridis	Devolt & Holst (1948)	96.4%
Oral inoculation	Emulsified cecal lesions	Farmer & Stephenson (1949)	6.6%
Oral inoculation	Emulsified liver lesions	Farmer & Stephenson (1949)	0

TABLE II (continued)

Route of Transmission	Material or Method	Author	Apparent Success
Subcutaneous inoculation	Liver lesion	Devolt & Davis (1936)	80%
Intramuscular inoculation (pectoral muscle)	Liver lesions to pigeons	Tyzzer & Fabyan (1920)	17%
Intramuscular inoculation (pectoral muscle)	Cecal lesions and Heterakis eggs	Tyzzer Fabyan & Foot (1921)	0
Blood inoculation (wing vein)	Blood from posterior mesentric vein and cecal veins	McGuire & Morehouse (1958)	25%
Blood inoculation (wing vein)	Blood from heart and wing vein	McGuire & Morehouse (1958)	0
Intracecal inoculation	Subcutaneous and lung lesions	Tyzzer, Fabyan & Foot (1921)	0
Intracecal inoculation	Cecal "cores" from infected turkeys	Tyzzer, Fabyan & Foot (1921)	0
Intracecal inoculation	Artificially hatched disinfected larvae of H. gallinae	Swales (1948)	100%
Intra-hepatic inoculation (through body wall)	Bacteria free liver-lesion suspension	Harrison, Hansen, Devolt, Holst & Tromba (1954)	30% to 53%

TABLE II (continued)

Route of Transmission	Material or Method	Author	Apparent Success
Intra-hepatic inoculation (by laparotomy)	Bacteria free liver lesion suspension	Harrison, Hansen, Devolt, Holst & Trombe (1954)	83%
Intravenous inoculation (wing vein)	Liver suspension	Tyzzer et.al. (1921)	40%
	Earthworms from infected soil	Curtice (1907)	17%
Anthropods per os.	Blow flies, grasshoppers, crickets, etc.	Tyzzer et.al. (1920 & 1921)	0
Anthropods per os.	Flies (Musca domestica)	Devolt & Davis (1936)	6%
Direct infection	Running turkeys on infected ground	Graybill (1921)	12%
Direct infection	Running turkeys on infected grounds with yearling turkey hens	Smith (1917)	48%
Direct infection	Running turkeys on infected ground	Devolt & Davis (1936)	53%
Direct infection	Turkeys kept with chicks	Devolt & Davis (1936)	63%
Direct infection	Running turkeys on infected ground	Farmer & Stephenson (1949)	49%
Direct infection	Running turkeys on infected grounds with poultry	Farmer & Stephenson (1949)	87%

natural outlets for the parasite were the cecal discharges and the bile, hence the feces. The parasite was supposed to be acquired by the new host in contaminated ingested material. Tyzzer (1924) encountered Histomonas in large numbers in the ceca of chicks early and late in the infections, but not during the acute attack. This observation confirms the existence of a free stage in the cecal contents.

Tyzzer (1919) and Tyzzer and Fabyan (1920) observed that the parasites (resistant forms) are occasionally found in stained sections in giant cells. It has not yet been proved that the forms found in the giant cells are capable of multiplying in normal turkeys. They may have become damaged by the digestive enzymes of the giant cells.

Tyzzer, Fabyan and Foot (1921) attempted to produce Blackhead by the intestinal route. They were of the opinion that the disease could be produced by means other than feeding infective tissue. Temporary obstruction was experimentally produced by the injection of melted paraffin into the lumen of the cecum. Paraffin alone or in conjunction with Blackhead virus, fails to cause cecal infection. Tyzzer and Fabyan (1922) later produced Blackhead with typical lesions in poults by feeding considerable amounts of active liver lesions.

Tyzzer and Collier (1925) suggested that "the passage of the Blackhead protozoon into the turkey's cecum in

sufficient numbers for it to become established, appears to be the only condition necessary for the invasion of the tissues in young turkeys, so that lowered resistance and cecal injury are without etiologic significance." They infected young turkeys by feeding them fresh liver lesion of infected birds. The ingestion of such material by turkeys at the age of five days, produced infection in seven of a group of eleven. In a later attempt to produce Blackhead by feeding fresh liver lesion to seven poults fifteen days old failed. Thus in a total of 18 turkeys seven became infected.

Allen (1941) cultured the characteristic Histomonads liver lesions on a modified Boeck and Drbohlava medium to which sterile rice starch was added. The culture containing Histomonads were administered per os. Twenty-four of the twenty-six poults inoculated died of acute Histomoniasis.

Devolt and Holst (1948) worked on the preventive action of "vioform" against infectious Enterohepatitis (Blackhead) of turkeys. Turkeys varying in age from one to two months were injected with Blackhead culture into the crop. Approximately 97 percent of the birds died of Histomoniasis.

Farmer and Stephenson (1949) administered emulsified liver lesion orally to 16 turkey poults and found negative results. When emulsified cecal lesion were given orally

to 15 poults, one of them died of Blackhead.

Lund (1956) described the effects of oral administration of unprotected Histomonas to turkey poults 6 to 19 weeks of age. Out of 109 birds inoculated with Histomonads in dilute suspension in saline, 66 remained healthy while 43 became infected. The infection occurred in about 40 percent, morbidity about 20 percent, and mortality less than two percent. Pathological changes more or less characteristic of Histomoniasis occurred in the ceca of 23 of the 43 infected birds. In another experiment a total of 61 turkeys comprised of three groups, received Histomonads in a dense suspension of cecal contents in saline or in egg albumen. Forty-seven failed to become infected, 6 were definitely infected at necropsy and eight were questionable. The organisms in the last named turkeys presented an atypical appearance, if they were actually Histomonads. In his third experiment Lund did not observe any definite results from feeding macerated liver lesions containing Histomonads until doses of almost five million organisms were used. When a greater concentration of parasites were given 20 percent of the birds acquired Histomonads, but none of them died and none were visibly sick. His feeding experiment indicated that orally acquired Histomonads may be of little importance from the standpoint of infectivity compared to those organisms that are expelled in the droppings of infected birds. He also indicated that similar

as in starved chickens not receiving an alkali. He suggested that resistant Heterakis ovum provides protection to the protozoon during its passage through the acid environment of the gizzard. Infections are readily obtained by the administration of embryonated ova of the nematode. The acidity of the gizzard of feeding chickens appears to provide a barrier to the passage of the unprotected protozoon into the intestine.

This hypothesis might be true in the case of turkeys but there is no doubt that some birds become infected only by the ingestion of infected tissue lesions, and that the results might depend on the dose of the infective material containing Histomonads.

II. Transmission by Rectal Inoculation

As first shown by Browne (1922) and later by Hill and Schillinger (1923) that fluid introduced into the cloaca of the chicken is commonly passed into the ceca. Advantage of this physiological response was made by various investigators to produce a type of Blackhead which is similar to that seen in nature. They have shown that rectal inoculation furnishes an easy and rather reliable method of transmitting infection to both turkeys and chickens.

A high percentage of successful results were first demonstrated by Tyzzer (1924) and Tyzzer and Collier (1925).

A suspension of ground liver lesions was employed for producing Blackhead by rectal injection. Out of 20 exposed turkeys of different ages (five to fifty-eight days old) 16 became infected. The death of the infected birds occurred between ten to twenty days after the first injection. In several cases they made rectal injections of the material on successive days. Histomonas meleagridis was found in the cecal contents with involvement of both ceca. Delaplane (1932) confirmed the transmission experiments of Tyzzer and Collier. The infective material consisting of liver, ceca, and both liver and ceca were injected (1 ml.) per rectum to 54 turkeys and 21 chicks. Forty-nine turkeys and 13 chicks developed typical Blackhead in their livers. Both ceca of the birds were involved with one exception. In another experiment, the protozoa was artificially cultivated on Boeck's Locke egg media. Feces of the birds were used to inoculate the media. The rectal injection of this culture (1.5 ml.) resulted in infection in one of five turkeys. The organisms were subsequently recultivated from the cecal contents of this bird. He demonstrated that the incubation period in young artificially infected birds was apparently short. The average being ten days after infection. Typical cecal lesions of Blackhead were observed as early as the fourth day following exposure. Both cecal and liver lesions were observed seven days following exposure. He

further suggested that ground diseased ceca of turkeys or chickens are as capable of infecting turkey poults as ground diseased liver, when the material is injected rectally.

Tyzzer (1933) infected ten hen chicks by this method. In 1934 he infected 15 of 16 poults and 18 of 25 hen chicks by rectal inoculation. He suggested that the inoculum is better retained in birds that have fasted overnight than in those that are fully fed. Furthermore he states that the cloacal injection of cultures causes Blackhead more regularly in young turkeys than in young hen chicks. All the cultures contained cecal bacteria. However cloacal injection of cultures frozen overnight at 15° C. failed to cause Blackhead. Tyzzer stated that Histomonad is alone the cause of the disease. Rectal inoculation with young cultures was again attempted in 1936. Infection was obtained in 161 out of 162 chicks, but those subcultured for lengthy periods became attenuated and did not produce Blackhead. Bishop (1938) infected hen chicks by rectal inoculation with cultures of Histomonas meleagridis and obtained good results.

Farmer and Stephenson (1949) compared various methods used for the experimental infection of turkeys with Blackhead. The effect of inoculating diseased tissue into the turkeys per rectum was regarded by far the most successful method. Young turkeys four weeks old were injected

rectally with a cecal suspension in Ringers solution. Two and five tenths ml. of this suspension was given to each bird after an enema of 5 ml. of Ringers solution. The injection was repeated after an interval of two hours. Experimental birds died 9 to 14 days after exposure. Positive results were obtained in 20 of 24 cases. Sixteen birds were given diseased ceca rectally without an accompanying enema. Thirteen poults developed Blackhead. When a suspension of liver lesions were given rectally without an enema, 7 out of 16 birds were positive. Eight of 16 poults developed Blackhead when exposure was preceded by an enema.

In the next experiment 78 four-week old poults were exposed by the same route as in the previous experiment. They were divided into three groups on the basis of the volume of inoculum used and the time interval between injections. The first group received 2.5 ml. each on two occasions with an interval of five minutes between injections. Twenty-three of 26 poults died of Blackhead. The second group received 2.5 ml. of suspension each on two occasions with an interval of two hours between injections. All poults died from Blackhead within 14 days following injection. All showed typical liver and cecal lesions. The third group received 5 ml. each as a single injection and 24 of 26 poults died from Blackhead. These experiments confirmed the efficacy of rectal exposure. Farmer

and Stephenson recommended it as suitable for infecting turkeys to be used for experimental work in connection with the testing of drugs for prophylactic or therapeutic purposes.

Sautter and Pomeroy (1950) did work on the chemotherapy of experimental Histomoniasis in turkeys. They used the rectal injection method for producing the disease. Young turkeys were given an inoculum consisting of livers from poults recently dead or destroyed at the height of the disease. A new group of poults was started every six to eight days. It was found that when a culture was obtained from a bird of a flock suffering from low mortality, the bird died in 15 to 18 days on the first passage, 12 to 15 days on the second passage and 6 to 10 days on third passage. In contrast when cultures were obtained from a flock suffering from an acute outbreak with accompanying high mortality. Inoculations from such a flock produced deaths of recipients in 6 to 10 days on the first passage. Death occurred much earlier with repeated passages. He suggested that three passages increased the virulence of the organism and that virulence also depends on the birds from which the culture was prepared.

III. Transmission by Combined Oral and Rectal Inoculations

Devolt and Davis (1936) for the first time combined oral and rectal inoculation for transmitting Blackhead with

suspensions of macerated liver and cecal tissue. One ml. of the inoculum was passed through the mouth into the crop of the poult by the aid of a glass pipette and with the same pipette a dose varying from 1/16 to 1/4 ml. was administered per rectum. A total of 27 poults were used for the experiment. They were divided into three groups: A, B, and C. Eight poults were used in group 'A', four received four doses each, two received three doses each, and two received only one inoculation. Six poults developed Blackhead and two died of general weakness. In group 'B' ten poults received three doses each and all developed Blackhead. In group 'C' two received a single dose, four received double doses each and the remaining three received three doses each. All developed Blackhead. Of the 27 poults, Blackhead was produced in 25 of them.

In another experiment 12 poults were inoculated repeatedly with ground liver showing typical lesions. Some of them received as many as twelve inoculations per os. and per vent. All of these poults died of Histomoniasis during the course of two months. Thus the inoculations were equally effective but much slower in action than those of the previous experiments. He suggested a wide variation in the pathogenicity of strains of Histomonas and showed that cultures lose pathogenicity after being carried in vitro for several months. It seems probable on the basis of this information that the difference in results are

attributable to a difference in pathogenicity of the parasites in the suspensions.

Later Devolt and Davis (1936) conducted a similar transmission experiment using Histomonad cultures in Lockes modified egg medium. A suspension was obtained from a 48-hour culture. The oral dose was about 1 ml. and from 1/16 to 1/4 ml. was given per vent. Thirty-three birds from 8 to 23 days old were inoculated three to four times at different intervals in different groups. Typical Blackhead was produced in 29 of 33 birds inoculated. He described it as "although the inoculation of young poults with cultures of Histomonads has regularly reproduced the disease, it must be borne in mind that numerous bacteria of undetermined species (bacteria normally found in the intestinal tract) were present in the cultures."

In another experiment while studying the parasitological and clinical periods of Infectious Enterohepatitis produced from culture, a total of 12 poults, 23 days old, were inoculated three times at intervals of three to four hours with a Locke's egg medium culture containing large number of Histomonas meleagridis. Each poult was given both oral and rectal injections as in other experiments. Results showed the implantation of the organism in the ceca of 10 poults. Nine died from the disease and one developed the disease without showing symptoms of sickness. The inoculations induced an acute form of the disease.

The cecal droppings were positive for Histomonas from 2 to 8 days post inoculation. The high degree of potency ceased with the failure of the ceca to discharge their contents as the ailment progressed to its advanced stages. The findings of occasional Histomonads in the sulphur-colored droppings thereafter prevented a period of sub-potency from being sharply outlined. As a rule no droppings were voided on the day or two days preceding death.

Devolt and Davis (1936) also used infectious cecal droppings as an inoculum for transmitting Histomoniasis. Five poults were inoculated with sterile Locke's solution and positive droppings. The droppings were secured from a donor bird during its potent-period. Each was given 1 ml. suspension per os. into the crop and about 1/8 ml. per vent. Three such doses were given to each poult at regular intervals. The ceca of four of the five were positive for parasite but only two of the four infected turkeys died of Blackhead. Typical lesions were observed in the affected organs and culture was recovered from the ceca. He suggested that it thus appeared that Histomonads are infectious at once after being voided in the droppings.

IV. Transmission by Subcutaneous Inoculation

Blackhead was transmitted by subcutaneous inoculation by Tyzzer and Fabyan (1920); Tyzzer, Fabyan and Foot (1921) and Devolt and Davis (1936).

Tyzzer and Fabyan (1920) suggested that a distinct form of Blackhead may be produced in turkeys by subcutaneous inoculation of liver lesions from acute cases and that this disease may be propagated apparently indefinitely by sub-inoculation into healthy turkeys. Fourteen turkeys were inoculated subcutaneously with bits of fresh liver lesions in the left breast. Most of them either succumbed to the disease or were in a dying condition when killed. In several of the inoculated turkeys the subcutaneous lesion failed to attain great size, indicating that the lesion had no appreciable effect on the growth and health of the turkeys. The progress of the disease depends on the involvement of vital organs. Secondary lesions were found regularly in the lungs, rarely in the liver and kidneys. The incubation period was 11 to 17 days.

Tyzzer et. al. (1921) reported that subcutaneous injection of scrapeings of the cecal mucosa of several old turkeys did not produce disease. Heterakis freshly obtained from cases of Blackhead were washed several times in salt solution and were injected subcutaneously into turkeys. All gave negative results. It was suggested that acute inflammation resulting from the injection of cecal contents may interfere with the development of Histomonas. The results of the inoculation of necrotic portions of subcutaneous lesions, or material from healing lesions indicate that Histomonas does not persist long in dead tissues.

In another experiment, Tyzzer, Fabyan and Foot (1921) produced Blackhead by subcutaneous inoculation in a number of turkeys, which he designated as "Inoculated Blackhead." A high percentage of success was obtained by this method. Liver showing characteristic lesions of acute Blackhead after being subjected to freezing, refrigeration or room temperature was inoculated subcutaneously into turkeys from 3 to 6 weeks old. It was found that Histomonas deteriorates more rapidly at room temperature than at lower temperature. At refrigerator temperature 5° C. it may remain alive for at least four days, but was quickly killed by actual freezing. These results were based on subcutaneous inoculation experiments. The ailment was described as consisting of a subcutaneous lesion at the point of inoculation accompanied by secondary involvement of the lungs and rarely the liver and kidneys.

Devolt and Davis (1936) reported that subcutaneous inoculation resulted in "Inoculated Blackhead" in a high percentage of poults. The inoculum was obtained from turkey livers showing characteristic lesions of Blackhead. In carrying out the experiment, an incision three-fourths to one inch long was made through the skin on the unfeathered area over the shoulder joint. With the help of a clean scalpel handle, the skin and fascia was loosened and a piece of liver tissue was then pushed through the incision down to the end of the loosened tract beneath the

skin. At the same time a small amount of a suspension prepared by crushing up a few pieces of diseased liver tissue in Locke's solution or sterile saline was injected through the skin around the implanted liver piece with the expectation that it would prevent destructive drying of the parasites before they could penetrate the muscles. With this method, Blackhead was produced in 16 of 20 poults between the ages of 27 and 60 days. The disease was fatal in 15 poults. In these sixteen cases death occurred at various times between the 13th and the 28th day following inoculation. In all cases the formation of local lesions at the point of inoculation was complicated by lesions in one or both lungs and in some cases the liver was also abscessed showing characteristic Blackhead lesions.

V. Transmission by Intramuscular Inoculation

Tyzzer and Fabyan (1920) produced "Inoculated Blackhead" in pigeons by intramuscular injection of liver lesions. Out of 18 pigeons inoculated, 3 showed an indurated mass with microscopically active lesions. The remaining pigeons were negative showing a slight local reaction or with an indefinite swelling at the point of injection.

Tyzzer et. al (1921) tested the infectiousness of various materials from Blackhead lesions also including Heterakis eggs. A series of turkeys were inoculated

intramuscularly. The contents of diseased ceca was collected from a case of Blackhead and was injected on 2 separate occasions into the breast muscles of a healthy turkey. Such material failed to produce Blackhead. Ripe Heterakis egg, when fed to turkey produced Blackhead, however when injected into the breast muscles of a normal turkey negative results were obtained. He suggested that Histomonas, even if present in the muscles is incapable of developing.

VI. Transmission by Blood Inoculation

The "blood phase" of the life cycle of Histomonas meleagridis was postulated some time back but it was incompletely proven. No positive proof of the presence of protozoa in the blood stream has been published. Thus far no other hypothesis has been advanced for its migration from the site of infection in the ceca to the liver. McGuire (1955) has proved that the micro-organism travels from the ceca to the liver via the hepatic portal circulation.

McGuire and Morehouse (1958) produced "Blood induced Blackhead" in 7 of 28 inoculated poults in 5 separate experiments. Various amounts of whole blood were withdrawn into sterile citrated syringes from the posterior mesentric veins, small cecal veins, and heart or wing veins. No infection resulted from the transfusion of

heart or wing vein blood in any of the recipients. A fatal case of Blackhead developed by inoculating 0.75 to 3.0 ml. of whole veinous blood from the cecal veins of diseased donor turkey into the wing vein of susceptible turkeys. Lesions of Blackhead were found in the liver; with atypical involvement of lungs, kidneys, heart, spleen, pancreas and proventriculus, but in no instance was the ceca or any portion of the lower intestinal tract involved. He suggested that this protozoa is capable of entrance and survival in the turkey blood stream. Furthermore it can establish itself and cause pathology in nearly any type of tissue to which it can gain access. In contrast Durant (1929) demonstrating that the ceca are the primary site of natural Blackhead, when the ceca were surgically obligated or removed, no infection resulted. Farmer and Stephenson (1949) indicated a definite time relation between the development of the infection in the ceca and appearance of liver lesions. McGuire and Morehouse also gave indirect evidence of the passage of Histomonas from the ceca to the liver through the hepatic portal system. The liver apparently acts as an efficient filter for these organisms, usually preventing pathology in other suitable tissues when the infection proceeds by its natural routes.

VII. Transmission by Various Other Methods

Many investigators have tried other methods of transmitting Blackhead with varying degrees of success. Some of the important methods will be briefly discussed.

There is abundant evidence that Histomonas is not transmitted through the turkey's egg. Curtice (1907) first made this assertion. Smith (1917) carried along 23 incubators hatched and brooder-raised poults for more than 6 weeks without the appearance of Blackhead among them. Since the eggs from which the poults were hatched were from an infected flock, the experiment supplied considerable evidence that the protozoon was not egg borne.

1. Intra Cecal Inoculation

This method was first tried by Tyzzer, Fabyan and Foot (1921). They suggested that Histomonas under normal physiological conditions, might be destroyed in passing through the alimentary tract before reaching the ceca. This was considered as a possible basis for the failure to infect birds by feeding fresh lesions. They performed laprotomies on three turkeys and injected a suspension of ground subcuteneous and lung lesions directly through the wall of the ceca into its lumen. The turkeys did not develop Histomoniasis. They further suggested that some form of Histomonas, not found in acute lesions, might be necessary for the penetration of the cecal mucosa before

causative agent of Blackhead; yet other organisms have been frequently found, associated with the Blackhead liver lesion. (Devolt and Davis 1935; Niimi 1936; Allen 1935; Harrison 1952).

Harrison et. al. (1954) reported intra-hepatic inoculation with a bacterial free liver lesion suspension of Histomonads. One to 2 ml. of this suspension was injected into the liver depending upon the size of the poult. In his first experiment (1951) a total of 17 bacteria-free livers were inoculated into the livers of 40 poults through the body wall. Twelve poults developed lesions. In the second experiment (1952) 8 bacteria-free donor livers were used for the preparation of a suspension. It was injected through the body wall into the livers of 32 poults. Seventeen developed typical lesions. In the third experiment (1952) five bacteria-free donor livers were used for inoculating 24 poults. Incisions were made into the body cavity and the suspension was injected into the middle parietal surface of the left liver lobe. With this method infection developed in 20 poults. In another experiment four poults were inoculated with Seitz (Ek) filtrates as above. None of these poults developed symptoms of sickness and none contained Histomoniasis lesions when killed.

3. Intravenous Inoculation

Tyzzer et. al. (1921) inoculated turkeys intra-

venously to determine whether certain organs or tissue were more favorable for the development of Histomonas. The liver suspension in doses of 0.5 to 2 ml. were injected into the wing vein of ten turkeys. The disease was produced in only a relatively small number of birds. Intervenous inoculation of birds produced lesions mainly in the lungs and smaller secondary lesions were distributed in various organs. In order of frequency they are as follows: lung, liver, kidney, proventriculus, pancreas, small intestine, spleen, cecum and ovary. Although skeletal muscles are readily invaded, no metastatic lesions were found in them.

4. By Arthropods

The possibility of arthropods as intermediate hosts in the transmission of Blackhead was considered by Smith in his original report but later he discarded the possibility. Curtice (1907) mentions that many poults which he had raised free from Blackhead "undoubtedly ate all sorts of insects." Tyzzer and Fabyan (1920) fed young turkeys grasshoppers, crickets and also about 135 Blow flies (Calliphora erythrocephala) which had fed on finely minced lung lesions from a case of inoculated Blackhead. The turkeys remained normal and continued to grow. Tyzzer et. al. (1921) did the same type of experiment with Blue-bottle flies and found negative results. Devolt and Davis (1936) suggested the possibility that flies (Musca

domestica) might carry the disease either as biological host or as mechanical vectors. On the basis of 49 poults exposed to flies during the course of three experiments extending over 3 years, only three cases of Blackhead occurred. Apparently flies act as mechanical vector of the disease on certain occasions when the parasites cling to their legs or other parts of the body.

It is quite clear now that arthropod plays no direct part in the dissemination of the disease, and the absence of the protozoon in the peripheral circulation would indicate that biting insects such as certain lice play no role in transmitting the disease. (Devolt and Davis 1936).

5. Direct Infection

Transmission of Histomoniasis by running turkeys on infected ground, Graybill (1921) produced 12 percent infection in a flock of artificially-reared turkeys. Smith (1917) obtained 48 percent infection in young poults exposed to infected grounds along with yearling turkey hens. Rettger and Kirkpatrick and McAlpine (1929) reported an incidence of 17 to 21 percent for poults kept in stationary pens as against 7 to 8 percent for poults on a rotation system. Devolt and Davis (1936) described the transmission of Histomoniasis by contaminated soil as a potent factor. Fifty-three of 84 poults contracted Blackhead from contaminated soil removed from the chicken and turkey breeder yards. Seven died from other causes and 24

remained uninfected. In these experiments losses occurred from two to three weeks after the young poults were exposed. Later he showed an incidence of 46.2 percent in poults under similar conditions and 15 percent in adult turkeys. When poults were kept with chicks Blackhead was produced in 62.5 percent of the poults.

Farmer and Stephenson (1949) suggested that it has not yet been possible to obtain more than 87 percent infection by running turkeys on infected grounds along with other poultry. Turkeys 6 to 8 weeks old were placed on infected grounds upon which turkeys had developed Blackhead. Sixty-nine turkeys were divided into three equal groups. The first group was run on the ground with 18 fowls, the second group was run on infected ground with no fowls and the third group was run on "clean" ground not previously occupied by infected turkeys or other fowl. After one month the results were as follows: In the first group 18 turkeys died from Blackhead, of the five survivors, two showed evidence of Blackhead and three were negative. In the second group 11 turkeys died from the Blackhead while all 12 survivors were negative. In the third group only one turkey died of Blackhead and the remaining 22 birds were negative.

These experiments indicate that the parasite in the turkey and chicken is the same, and the turkey may acquire both the cecal worm and the protozoon parasite from the

chicken.

6. Other Possible Factors in the Transmission of Blackhead

Smith and Graybill (1920) made some very important observations in mentioning that Blackhead disease and Heterakis appeared among young turkeys when kept in an unused horse paddock, enclosed by a high iron fence and not occupied by poultry for many years. The pasture was plowed early one spring and sowed to oats and grass. The explanation offered for this type of infection is the attraction of wild birds in large numbers to the food supply in the turkey enclosures. These birds were supposed to have deposited infective materials which were taken up by the turkeys.

Cram (1927) suggested that earthworm may act as a mechanical vector for the eggs of Heterakis gallinae: "Earthworm may ingest the eggs and carry them in the intestine, and birds may become infected by eating such earthworm; the earthworm may also pass these eggs in their casts and thus infect otherwise uninfected ground." Curtice (1907) produced 17 percent infection of Blackhead by oral inoculations of earthworms from infected soil.

INCIDENCE OF HISTOMONIASIS IN VARIOUS HOSTS OTHER THAN THE TURKEY

1. The Chicken:--Infections in chickens have been widely reported by Chester and Robin (1900); Curtice (1907); Theobald (1907); Milks (1908); Cole, Hadley and Kirkpatrick (1910); Higgins (1915); Tyzzer (1919 and 1924); Smith and Graybill (1920); Kaupp (1922) and Eriksen (1925). The infection in the common fowl usually runs a much milder course than in the turkey but is otherwise very similar. Smith and Graybill found that in chicks experimentally infected with Heterakis eggs, the initial lesions appeared in the ceca, usually followed by a microscopic focal collection of lymphocytes or yellowish necrotic foci in the liver. The inflammation and thickening of the cecal wall and subsequent formation of a "core" resemble somewhat the condition in the turkey but liver lesions bear no comparison in the two birds. Smith and Graybill state that all their chicks would probably have survived had they not been killed, for the processes of repair had been initiated, whereas experimentally infected turkeys usually die.

Milks, Kaupp and Eriksen indicate, that at times the disease in chickens may run a much more severe course. Kaupp observed the death of 42 out of the 43 silver spangled Hamburg chicks about five weeks of age. Eriksen necropsied a total of 25 birds from 17 Missouri flocks.

The losses ranged from one bird in a flock of 350 to more than 50 percent in two other flocks. During necropsy cecal and liver lesions were observed. Occasionally the liver was enlarged to several times its normal size and was studded with grey and greyish yellow areas 3 to 8 mm. in diameter which penetrated deeply into the liver tissue. Histomonas was observed in sections of both the ceca and in the liver. The age of the experimental chicks was from 7 to 10 weeks. Thus infection in chickens may assume a serious nature at times, although under ordinary circumstances it appears to be well tolerated by the host.

Tyzzer and Fabyan (1922) found that subcutaneous exposure of infectious material to chicks had a local and transient effect on the birds.

2. The Pigeon:--The ceca of the pigeon is a small lateral diverticulae and is not known to herbor Histomonas. Tyzzer and Fabyan (1920) produced transient but well defined localized lesion in the subcutaneous tissue and pectoral muscle. The inoculum was liver tissue organisms from active cases of Blackhead in turkeys.

3. The Ruffed Grouse:--According to Tyzzer and Fabyan (1920), and Graybill, (1925) this bird commonly succumbs to the disease in captivity, but natural infection is unknown.

4. The Common Quail:--According to Tyzzer and

in this bird also. Tyzzer stated that pheasants show great numbers of Heterakis, but he has never been able to produce Blackhead in turkeys by feeding the Heterakis eggs from the pheasant. Neither has he been able to produce carriers in half-grown pheasants by rectal inoculation of infected tissue. Cecal material collected from pheasants and inoculated per rectum into young turkeys has always failed to infect the latter. Pheasants are somewhat resistant to Blackhead infection.

9. The Peafowl:--Graybill (1925) states that the disease occurs occasionally in this bird. Dickinson (1930) necropsied two peafowl which had presumably become infected from running with a flock of turkeys. Both showed cecal and liver lesions typical of fatal Blackhead.

10. Mammals:--According to Tyzzer and Fabyan (1920) rabbits, guinea pigs, mice and Japanese waltzing mice are not susceptible to inoculation with the organism. It is likely that all mammals are resistant to Histomoniasis.

SURVIVAL OF HISTOMONAS MELEAGRIDIS

Histomonas meleagridis is unable to survive more than a few hours outside the Avian host. This was first demonstrated by Tyzzer and Collier (1925). They stated that "the protozoon is discharged in a form that is incapable of surviving long outside the body but which may

produce the infection if immediately ingested."

Tyzzer et. al. (1921) reported that Histomonas meleagridis as found in turkey liver lesions, survive for at least 4 days at 5° C., deteriorates more rapidly at 22° C., and is immediately destroyed by freezing. Tyzzer and Collier (1925) reported the infectivity of Histomonas was from one to five days at room temperature, "Liver lesions kept for 24 hours at room temperature were administered to 2 turkeys by mouth and by rectum. Samples of the same material were given after an interval of 3 to 5 days at room temperature to 2 other turkeys. None of the 6 turkeys became infected. The infectivity of this strain of Histomones, when fresh was not determined, but material was obtained from acute cases of Blackhead indicating that strain was potent when fresh." Tyzzer (1932) and others later reported that very irregular results follow attempts to transmit the infection by feeding droppings from infected birds. Graybill and Smith (1920), Niimi (1937) and others failed to produce Blackhead by feeding droppings containing free Histomonads. This indicates that susceptible birds contract Blackhead only rarely by ingesting droppings containing the parasites. These investigations indicate that the cecal worm probably plays an important role in the spread and perpetuation of Histomoniasis under field conditions and that the ingestion of Histomonads unprotected by cecal worm eggs may be of little etiological

importance.

Graybill (1921) stated that "infectious soil that had remained unoccupied for a period of five months beginning in the depth 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 contracted Blackhead within 28 days. Tyzzer (1934) reported that Histomonas in Heterakis eggs survive New England winters in the soil. According to Van Es and Olney (1934) birds placed in Nebraska poultry yards that had not been used from November to June acquired Blackhead, showing that the parasites had remained viable over the winter months. Niimi (1937) stated that Histomonas may survive in the worm eggs longer than one year.

Farr (1956) reported that Histomonas meleagridis and eggs of Heterakis gallinarum (H. gallinae) contained in the feces on the soil in four outdoor experimental plots at Beltsville, Maryland, were still infective after 66 weeks. Farr (1961) further determined the length of time of Histomonas meleagridis and the eggs of other nematodes which can remain infective outdoors on soil. Histomonas meleagridis was recovered from experimental poults fed material that had been on the soil for as long as 150.8 and 148.8 weeks and that Heterakis gallinarum eggs produced infection through 229.8 and 210.5 weeks.

The results of these investigations indicated that

(1948) pointed out that the poults were not immediately affected by exposure to Histomonas at 3, 10 and 17 days of age. In the majority of cases the fatal infection was not initiated until the birds were about 3 weeks old. Desowitz (1951) inoculated chickens of various ages per rectum with a suspension of liver and cecal material from a turkey affected with Histomoniasis. It was found that susceptibility to infection was dependent upon the age of the recipients. The highest mortality (71 percent) occurred in a group of birds 21 days old. The lowest (30 percent) in a group 34 days old. This resistance might take the form either of an actual barrier to invasion by the organism or an increased resistance to the clinical disease. Kendall (1957) indicated that there is no significant difference in susceptibility referable to age whether challenged experimentally by rectal inoculation of a suspension of Histomonads or the ingestion of the embryonated eggs of Heterakis gallinae.

It is generally recognized that turkeys surviving Blackhead are resistant to further attacks although Hinshaw (1943) mentions the possibility of relapses during the breeding season. Swales and Frank (1948) kept small flocks of recovered birds on contaminated soil. They remained healthy although the uninfected birds from the same lots that were isolated on wire, retained high susceptibility for at least ten months. They further pointed out that in

spite of contact with infective material, the poults exposed at a non-susceptible age did not acquire a low grade infection capable of immunizing them against fatal Blackhead in later life, suggesting that the host-parasite balance was not established. Kendall (1957) also confirmed that resistance to reinfection can arise by previously infected birds and that resistant birds may harbor a latent infection.

Tyzzer (1934) was able to alternate the virulence of a strain of Histomonas. This was used successfully under experimental conditions to protect turkeys against virulent strains. Thus it appears that immunity can be developed as the result of a non-fatal form of the disease or of the invasion of a virulent strain of Histomonas meleagridis. Tyzzer's work suggested that it was essential to inoculate birds when young and that there was a need for constant reinfection in order to maintain the state of premunition which was apparently the criterion of resistence.

The development of an acquired resistance was accepted by some later workers. Sautter, Pomeroy and Roepke (1950) tested the effect of blood from immunized turkeys in preventing loss from Histomoniasis. By contrast Waletzky (1950) considered that there was no certainty that recovered birds, either with or without the help of drugs become immune to infection. Swales (1950) suggested that birds treated in the later stages of the disease or those

treated in such a way that disease processes in the ceca were not completely suppressed, were resistant when challenged.

Lund (1956) reported that the ingestion of unprotected Histomonads established infections in about half of the poults so exposed. Up to 20 percent of the birds so infected showed pathological changes characteristic of Histomoniasis. A bird having Heterakis infection if given unprotected Histomonads is more liable to come down with Blackhead than the unparasitized bird. Ingestion of Histomonads along with solids or materials requiring digestion apparently reduced the infectivity of the organisms. Dilute suspensions of Histomonads in saline probably are retained in the gizzard for a considerably shorter time than are heavy suspensions, or other substances susceptible to digestion.

Horton-Smith and Long (1955) presented evidence which indicates that the low pH of the gizzard of chickens is detrimental to unprotected Histomonads. The same is probably true for turkeys. Histomonads contained in extremely fluid cecal discharges, probably could remain infective for a time, especially if voided in puddles of water or in waterers.

Welter (1960) determined whether various stress conditions (starvation, exposure to temperature extremes, anemia, coccidia infection, fowl pox vaccination, cortisone

injection, splenectomy) would alter the disease or mortality in chickens and turkeys, infected with Histomonas meleagridis. He found that starvation of chickens suffering with Histomoniasis did not increase the severity of the disease. Starved turkeys showed a higher incidence of liver lesions than did unstarved turkeys; however both groups of turkeys suffered similar mortality losses. Anemia, exposure to temperature extremes, coccidia infection, fowl pox vaccination, cortisone injection and splenectomy has no significant effect upon Histomoniasis in turkeys and chickens.

LIFE CYCLE IN RELATION TO NATURAL TRANSMISSION

Reproduction of Histomonas meleagridis is by binary fission. There is no evidence of a sexual cycle. Wenrich (1943) considered the large four-flagellate forms in the ceca to be adult. There are no cysts and the naked trophozoites are delicate. They do not survive more than a few hours when passed in the feces.

Turkey can be infected by ingesting trophozoites in infective material but the most important mode of transmission is in the eggs of the cecal worms--Heterakis gallinae. Its discovery by Smith and Graybill (1920) was a milestone in the history of Parasitology. In his first experiment two turkeys were fed feces of adult turkeys and cultures of embryonated Heterakis ova from chickens. The

Heterakis ova were kept in petri dishes in normal saline solution for 17 days. Both infected turkeys became sick fifteen days after dosing with the ova and both died within a week. In another experiment an overdose of Heterakis eggs which were placed in 0.5 percent solution of bichloride of mercury for 30 seconds, also produced Blackhead infection.

This method of transmission was later confirmed by many workers. Tyzzer and Fabyan (1922) proved that the ovum of Heterakis may actually harbor Histomonas. Heterakis material treated for three days with 1.5 percent nitric acid which rendered the medium bacteriologically sterile, was proved capable of transmitting Blackhead when fed to incubator hatched turkeys. The discharges of Blackhead carriers free from embryonated eggs will not transmit the disease after treatment with 1.5 percent nitric acid (Tyzzer 1926).

Generally the nematodes are not present in the ceca during the acute stage of Blackhead. Failure to find the worms in enormous number in the ceca after the ova were fed, does not necessarily indicate that the worm had no role in transmission. Tyzzer and Fabyan (1922) found evidence that the worms are destroyed in the diseased ceca.

Lund and Burtner (1957) found that less than 0.5 percent of embryonated eggs from experimentally infected chickens contained the protozoa. The Heterakis eggs must

hatch and liberate larvae in order to transmit the protozoa. Feeding unembryonated eggs and male Heterakis will not produce the infection. The disease follows the feeding of the embryonated eggs more often when they are pooled from a number of different birds.

The weak link in the chain of circumstances incriminating Heterakis eggs is the failure to demonstrate the protozoa inside their membrane. The proof of their existence in a stage of a parasite, would establish the nematode as a true invertebrate intermediate host.

Tyzzer (1925) found the protozoa in a half grown Heterakis from birds with Histomonas and again in 1934 in the cells of the intestinal wall of ten, twelve and twenty-one days old worm from experimentally infected birds. Swales (1948) demonstrated the transmission of Histomonas in Heterakis larvae mechanically separated from Heterakis eggs and showed that the etiological micro-organism is present within infective larvae. Connell (1950) observed refractile swellings in the cuticle of hatched second stage larvae of Heterakis gallinae, which he attributed to the presence of Histomonas, perhaps in the process of becoming capable of initiating Blackhead in a suitable host. Desowitz (1950) examined female Heterakis gallinae recovered from the turkeys dying of Histomoniasis. In one, of 100 examined, he found small intracellular parasites in the epithelium of a gut, but he did not consider himself

justified in identifying these parasites as development forms of Histomonas meleagridis.

Kendall (1959) found Histomonas in a four-day old H. gallinae larvae. The parasite was smaller than Histomonas meleagridis seen in the tissue, but in other respects were identical. He further suggested that difficulty experienced in finding the protozoon in its vector may possibly be explained in the assumption that the rate of infection of the eggs of Heterakis (and hence of the young larvae) is very low. Perhaps infected eggs do not greatly exceed the ratio of 1 in 1,000 (Kendall 1957) as 1,000 eggs were normally necessary in order to set up infection with Histomonas in turkey poults.

Another factor in the transmission of Blackhead in nature is the length of time the Heterakis eggs harbor the viable protozoon. Farr (1956 and 1961) recovered both Heterakis larvae and Histomonas from experimental turkeys and chickens fed material that had remained on soil outdoors (in Maryland) for 149 to 151 weeks i.e. approximately three years.

So it can be said that Blackhead of turkeys is the first disease found to have a helminth intermediate host. When the embryonated ova hatch in the alimentary tract of the turkeys, the young worm larvae penetrate the epithelium of the cecal glands carrying their Histomonads with them where they are deposited in a site favorable for their

establishment. The worm thus not only serves as the intermediate host for Histomonas meleagridis but also provides a portal of entry by demaging tissue.

HISTOLOGY

Ceca

Zeitchmann (1911) and Looper and Looper (1929) made the most complete studies of the turkey ceca. The ceca appear as a pair of long blind-ended tubes which open into the large intestine immediately behind the annular thickening which separates it from the small intestine. The ceca arise well forward near the last dorsal vertebra and following the small intestine curve ventrally backward and finally bend forward to end blindly. The length varies from 17 to 25 cm. in a 5 months old turkey. The ceca of the turkey are much thicker walled and more highly developed than those of common fowl and have an important physiological function. It is assumed that the filling as well as the discharge is periodical and is accomplished by the reversal of peristaltic movement. Fermentation evidently occurs normally in these organs since considerable gas is usually present.

Bittner (1924) and Otte (1928) divided the ceca into three parts, a neck with many villi, a middle portion with few villi, and the vesicular blind end which is thin walled and free from villi.

The ceca contain four layers namely serous; muscular; submucous; and mucous. The mucous membrane is lined with columnar epithelium, containing globlet cells, and villi of varying lengths depending on the region. The submucous layer contains white fibrous and yellow elastic tissue containing nerves, blood vessels, and lymph plexuses. The lamina muscularis varies in thickness and arrangement. The serous layer is rich in nerve elements.

In the proximal portion there are prominent villi. The muscularis mucosa and submucosa are in thin layers and are crowded close to the base of the villi. The lamina muscularis is marked by a thin inner circular layer and a thin outer longitudinal layer. No lymphoid tissue was observed in the section of a 36 hour chick by Calhoun (1954) but after 5 months she found a mass of lymphoid tissue with several nodules.

In the mid-portion the villi are shorter and broader. In the constricted part of the mid-portion, the villi are longer, the muscles thicker, and the whole circumference smaller than in the dialated portion of a corresponding level. Plicae circulares are present at this level. Lymphoid tissue became more prominent with advanced age. Near the blind end of the ceca of the 36 hour chick, the inner circular and outer longitudinal muscular layers are nearly the same width. True villi and lymphoid tissue are not present in this area. Goblet cells are present in the

epithelium.

In the distal portion of the ceca of a 20 day chick, the inner circular muscle increased to about three times the width of the longitudinal muscle. The surface of the mucous membrane approaches a villi-like arrangement between the plicae circulares. Goblet cells and lymphoid tissue are also present. In older birds so called tonsils composed chiefly of lymphoid tissue are present in each cecum near its junction with the intestine.

Liver:

The microscopic anatomy of the liver of turkey has been discussed by Calhoun (1954). The liver is a two lobed organ lying posterior to the rudimentary diaphram. Its posterior borders are quite noticeably notched. The gall bladder is located on its visceral surface, and from it a ductus cysticus carries the bile to the duodenum, while a second duct, the ductus hepaticus, comes directly from the left liver lobe and empties into the duodenum in close proximity to the first.

The livers of birds differ a little from that of mammals. The interlobular septa are probably less apparent than those of the domesticated animals. The portal canal contains the portal vein, lymph vessel, hepatic artery and bile ducts. The interlobular veins are not prominent.

The central veins are distinguished by the prominent sinusoids which entered them and they are lined with

a thin endothelial membrane. The sinusoids are also lined with endothelial cells. Kupffer cells are distinct.

The liver epithelium is arranged in a tubule of four to seven cells about an intralobular bile capillary. This tubular arrangement is well marked in a cross section and appeared plate-like in longitudinal section. The liver cell with its distinct nucleus is a pyramidal cell with its apex bordering the lumen of the tubule.

Elastic tissue is confined to the walls of the blood vessels, to the connective tissue septa surrounding them, and to the capsule of Glisson surrounding the liver. White fibrous tissue is distributed similarly.

The liver cells are supported by a mesh work of reticular tissue. The fat cells decrease until the twenty-first day. The fat globules are confined to a small area about the central veins and after the twenty-fifth day, occasional fat droplets are found scattered throughout the liver.

Spleen

The spleen is a somewhat elongated spherical organ in turkeys. Its histology has been recently described by Malewitz and Calhoun (1958). A thin capsule of connective tissue, smooth muscle and occasional elastic fibers surrounds the spleen. Trabeculae were not prominent. The capsule was covered by a thin serosa.

The parenchyma of the spleen consists of a reticular network with numerous small circumscribed white pulp areas surrounded by areas of red pulp. Several (2-4) central arteries are present within the white pulp which consists chiefly of lymphocytes. The surrounding red pulp is composed of numerous eosinophilic cells, macrophages, and intermingled lymphocytes. Sinusoid also contains red blood cells in the red pulp.

Kidney

Malewitz and Calhoun (1956) described the histology of the kidney and they stated that the lobed kidney is covered by an extremely thin connective tissue capsule, and covered by serosa on the peritoneal side. The lobes, in turn, are semilobulated. Each kidney lobule consists of an outer cortical and inner medullary area. Par radiata and par convoluta are not apparent.

The cortex contained typical malphigian or renal corpuscles and the proximal and distal convoluted tubules. Maculae densae and intertubular cells are also present. Collecting tubules (straight tubules) are present in the medulla and converged to form the ducts of Belline near the ureter. The proximal convoluted tubules are characterized by eosinophilic cuboidal cells, a brush border, a central nucleus and a small lumen. The distal convoluted tubule has a wide lumen lined by pale staining cuboidal cells without a brush border. The collecting tubules are

lined with cuboidal cells. Tall columnar epithelium line the ducts of Bellini. The kidney has a rich blood supply.

CHAPTER III

MATERIALS AND METHODS

Equipment and Chemicals

1. New battery brooder - 5 deck
2. Balance for weighing
3. Syringes, all glass, 1-5 ml. capacity
4. Pestels and mortars
5. Beakers
6. Incubator 37° C.
7. Microtome, American Optical
8. Pipettes, transfer
9. Scissors and scalpels
10. Staining dishes
11. Microscope
12. Bottles for tissue
13. Microscopic slides 1 x 3 inches
14. Cover slips 22 x 22 mm.
15. Thermometer
16. Cheesecloth
17. Chemicals for staining, washing, etc.
18. Culture medias

Management of Turkeys

All turkey poults used in this study were purchased from one source. All birds were of the same age (hatched

CHAPTER III

MATERIALS AND METHODS

Equipment and Chemicals

1. New battery brooder - 5 deck
2. Balance for weighing
3. Syringes, all glass, 1-5 ml. capacity
4. Pestels and morters
5. Beakers
6. Incubator 37° C.
7. Microtome, American Optical
8. Pipettes, transfer
9. Scissors and scalpels
10. Staining dishes
11. Microscope
12. Bottles for tissue
13. Microscopic slides 1 x 3 inches
14. Cover slips 22 x 22 mm.
15. Thermometer
16. Cheesecloth
17. Chemicals for staining, washing, etc.
18. Culture medias

Management of Turkeys

All turkey poults used in this study were purchased from one source. All birds were of the same age (hatched

on June 5th, 1961, P.M.).

These birds were kept in a new battery brooder having five decks with wire floors. When the turkey poults were six to eight weeks of age they were placed in wire cages, which were steam cleaned.

Commercial poult feed was fed at all times. In the daily care of such birds the feeders and waterers were filled twice a day and the pans were cleaned with hot water daily. Particular care was taken to avoid taking anything from one pen to another. The same feeders and waterers were used for a particular group of experimental poults. The dropping pans were covered with newspapers and these newspapers having droppings on them, were removed two times a day (morning and evening).

All equipment taken into this room were thoroughly cleaned and disinfected. Hands were washed before taking care of these birds and a separate laboratory coat was kept in this room which was worn while performing the necessary duties. The temperature of the automatic battery brooder was kept constant.

The Selection of Material and Its Preparation For Inoculations

Chicks five weeks of age showing typical symptoms of Histomoniasis were selected as a suitable donor for these experiments. These birds were killed. The liver showed only a few early lesions of Histomoniasis. The

ceca were greatly enlarged. Necrosis and caseous cores were present.

The livers, ceca, and their contents were removed asoptically from the chicks. Specific amounts were weighed on a balance. The liver, ceca and cecal contents were placed seperately in sterile morters and was cut up into small pieces with the aid of scissors. The material was then macerated with washed sterile white sand, and a suspension was prepared with normal saline solution. The suspensions were then pressed through several layers of gauze into beakers. By this means large fragments of tissue and debris were eliminated. The infective materials were examined for Histomonads before inoculating into the experimental poults. The chick (donors) livers were also cultured on nutrient agar for the presence or absence of bacteria. These later proved negative.

For whole blood inoculation experiments, the donor birds were selected on the same basis as above. Laprotomies were performed on anesthetized donor birds. Their livers showed early lesions of Histomoniasis. Various amounts of whole blood were withdrawn from the posterior mesentric, cecal, and hepatic veins into sterile syringes containing heparin. Blood smears were made before these blood samples were inoculated into the experimental poults and all appeared negative.

Methods of Inoculation

Various experiments that were conducted concerning the transmission of Histomoniasis were mainly divided into six groups for the purpose of discussion. The effected internal organs were used in five groups and in the last group whole blood was utilized for the transmission of Histomoniasis. Exposures were accomplished using the following routes:

Group A: Oral Inoculation

1. The inoculum by way of mouth into the oesophagus.
   a. Ceca with their contents - two grams.
   b. Liver - two grams.
   c. Liver and ceca with their contents - two grams.
2. The inoculum by way of mouth into the gizzard.
   a. Ceca with their contents - three grams.
   b. Liver - three grams.

Group B: Rectal Inoculation

1. The inoculum by way of rectum into the ceca.
   a. Ceca with their contents - two grams.
   b. Liver - two grams.
   c. Liver and ceca with their contents - two grams.

Group C: Combined oral and rectal inoculation.

1. Liver and ceca with their contents - one gram to each area.

Group D: Subcutaneous Inoculation

1. Liver and ceca with their contents - two grams.

Group E: Intramuscular Inoculation

1. Ceca - one gram.
2. Liver - one gram.
3. Liver and ceca - one gram.

Group F: Whole Blood Inoculation

1. Blood injected into the liver through body wall - 0.2 ml.
2. Blood injected into wing vein - 0.2 ml.

In oral inoculations, the inoculum was administered once only, using an all glass syringe with an attached long cannula. A long firm tube of para rubber was used for inoculating into the gizzard. The same type of glass syringe with a long cannula was also utilized for rectal and combined oral and rectal inoculation. In all types of rectal inoculation, the bird was suspended by the feet with the head down, to permit the inoculum to enter the ceca. The lips of the vent of each bird was pressed together following each such inoculation. Most of the poults retained all of the inoculum.

In intramuscular inoculations, the suspension was injected into the pectoral muscles of the bird, using an all glass syringe and a hypodermic needle. In subcutaneous inoculation, the suspension of the diseased organs were injected through the loose unfeathered skin of the

abdominal area. Care was exercised to avoid blood vessels and muscles that are just beneath the skin.

In whole blood inoculation, the blood was injected into the wing vein with antiseptic precautions. The intrahepatic inoculation of blood was done through the body wall.

All inoculations were done immediately after the preparations of the suspensions.

Methods of Preparing Tissue for Sectioning and Stains

All exposed birds, including controls were necropsied and the suspected tissue was fixed in 10 percent formalin solution. After fixation, the tissue was washed in running water. It was dehydrated in assending grade of alcohol and was cleared in chloroform xylene, embedded in paraffin and sections of the tissue were cut at a thickness of 6 to 10 microns. Mounted sections were all stained with Hematoxylineosin, and duplicate sections were stained using Azure-eosin.

A. Hematoxyline and Eosin Method

1.	Xylene to remove paraffin	3-5 minutes
2.	Xylene (blot excess)	3-5 minutes
3.	Alcohol 95%	3-5 minutes
4.	Alcohol 95%	3-5 minutes
5.	Alcohol 70% (blot excess)	3-5 minutes
6.	Running tap water	3-5 minutes
7.	Hematoxylin stain	3-5 minutes
8.	Running tap water	3-5 minutes
9.	Acid Alcohol Decolorizer	Dip
10.	Running tap water	3-5 minutes
11.	Eosin stains	8-10 minutes
12.	Running tap water	Dip briefly

13.	Alcohol 80%	Dip briefly
14.	Alcohol 95%	Dip briefly
15.	Alcohol 95%	1-3 minutes
16.	Alcohol absolute	Dip briefly
17.	Alcohol absolute	3-5 minutes
18.	Xylene	3-5 minutes
19.	Xylene	10-30 minutes
20.	Mounted in technicon	

Formula of Hematoxylin and Eosin Stain:

1. Hematoxylin: Distilled water 300 ml.
   Hematoxylin 1 gm.
   Ammonium alum in 100 ml. of water - 14 gm.
   Thymol crystals
   Ripen for 30-45 days

2. Eosin: Glacial acetic acid 2% 7.4 cc.
   Eosin Y in 1000 cc. of 95% alcohol - 10 gms.

B. Azure-Eosin Method:

1. Paraffin sections to water level is same as in H & E stain.
2. Azure-Eosin stain - one hour.
3. Dehydrated in acetone (2-3 changes).
4. Clear in a 50:50 acetone xylene mixture and two changes of xylene.
5. Mounted in technicon.

Azure-Eosin Stain:

Azure A 0.1%	4 cc.
Eosin B 0.1%	4 cc.
Citric Acid M/10	1.1 cc.
Disodium phosphate M/5	0.81 cc.
Acetone C. P.	5 cc.
Distilled water	25 cc.

CHAPTER IV

EXPERIMENTAL PROCEDURE AND RESULTS

I. ORAL INOCULATIONS

A total of 17 poults 16 to 40 days of age were used in this series of exposures. The inoculum was introduced by way of mouth into the oesophagus using an all glass syringe to which was attached a 15 g. cannula 3 inches in length. Six poults 16 days old were dosed; one half with 2 grams of diseased liver each, the remaining three in a like manner with ceca and their contents. One bird of each group was killed at 7, 14, and 21 days. Gross and microscopic examination failed to reveal symptoms or lesions of the disease. Five poults 25 days of age were exposed by dosing. Two grams each of liver, ceca and their contents were administered to each bird. As before one poult was killed 7 days post inoculation and another at 14 days. The remaining 3 poults died before the 18th day post inoculation. All 5 birds were typical of Histomoniasis. The incubation period was 7 to 14 days. (Table III).

Six poults 40 days old were dosed by way of mouth into the gizzard (ventriculus) by means of a long para rubber cannula attached to a glass syringe. Three poults received 3 grams each of diseased liver. The remaining 3 a like amount of ceca and their contents. One poult

TABLE III

The effect of dosing poults (into the oesophagus) with two grams of tissues containing Histomonads.

Poult No.	Age when exposed Days	Inoculum	Post-Exposure Days	Black-head Lesions	Remarks
638	16	Ceca & Contents	7	None	Killed
603	16	Ceca & Contents	14	None	Killed
601	16	Ceca & Contents	21	None	Killed
615	16	Liver	7	None	Killed
608	16	Liver	14	None	Killed
606	16	Liver	21	None	Killed
627	25	Liver, Ceca & Contents	7	Ceca	Killed
648	25	Liver, Ceca & Contents	14	Ceca & Liver	Killed
637	25	Liver, Ceca & Contents	15	Ceca & Liver	Died
629	25	Liver, Ceca & Contents	16	Ceca & Liver	Died
634	25	Liver, Ceca & Contents	17	Ceca & Liver	Died

receiving liver died of Histomoniasis on the 12th day. The remaining two were killed on the 7th and 21st days post inoculation. Both were normal. Two of three poults that received ceca and their contents developed Histomoniasis. One died on the 12th day and one was killed on the 7th day. The remaining poult killed on the 21st day was normal. The incubation period in this group was 7-13 days. (Table IV).

Six uninoculated poults served as controls. They were killed at 7, 14, and 21 days. Histomoniasis either gross or microscopic was not present.

The microscopic and macroscopic findings were as follows:

Bird No. 627 (Killed 7 days post inoculation).

This bird was droopy. It appeared weak, somewhat drowsy and stood with lowered head and ruffled feathers. Its appetite was poor. An increased desire for water was observed. The droppings were watery. (Fig. 1 and 1a).

Ceca: Both ceca were enlarged and small pin point abscesses were present. Microscopic examination revealed extensive hyperaemia, swelling and increased thickness of the mucous and submucous layer of the tissue. Detachment of the mucous membrane at the villus tip was well marked. The mucous membrane was also loosened from the underlying tissue and this detachment appeared to be associated with a pink homogeneous transudate. There was polymorphonuclear

infiltration and in some areas an increase in the lymphoid cells. Circular muscle fibers were streaked with reactive cells between their bundles. The capillaries in the lamina propria were engorged. Associated with the inflammatory reaction in the submucous tissue were aggregation of protozoa which appeared to be Histomonads.

Liver: No lesion was present on gross examination. Microscopically cloudy swelling and capillary congestion was present. There were a few foci of small round cell and polymorphonuclear infiltration close to the blood vessels. A few cells were desquamated.

Kidney: Normal.

Spleen: Normal.

Bird No. 648 (Killed 14 days post inoculation).

The bird was emaciated. Its eyes were closed. The wings and tail drooped. A sulphur-colored diarrhoea was in evidence.

Ceca: Both were enlarged. Their walls were thickened. The contents were semi-caseous. Histologically it was evident that the process of inflammation involved all the tissue layers of the ceca. Protozoa were common having acidophilic cytoplasm and with well defined eccentric nuclei. Some of them were lying together in so called "nests." The normal musculature was almost lost. The

villi were desquamated. There was capillary congestion throughout with haemorrhage in some areas. Polymorphonuclear leucocytes and lymphocytes were common near the serous layer.

Liver: The surface of the liver was covered with somewhat spherical depressed areas of necrosis up to a centimeter in diameter. Histologically there were numerous areas of round cell infiltration, more common near the portal vein, capillaries, and bile ducts. Widespread necrosis of the hepatic parenchyma with ill-defined margins was predominant. There was an irregular distribution of polymorphonuclear leucocytes with slight haemorrhage. The protozoa was plentiful and in a few places "nests" lay in clear areas.

Kidney: No gross lesion was present. Apparently normal on microscopic examination.

Spleen: No noticeable change.

Bird No. 637 (Died 15 days post inoculation).

Bird No. 629 (Died 16 days post inoculation).

Bird No. 634 (Died 17 days post inoculation).

All three birds died of Histomoniasis. Previously they showed typical symptoms of the disease. The histopathological changes were more or less the same in all

three poults. (Fig. 2).

Ceca: Both ceca were greatly enlarged 2-3 times normal. The cecal contents were caseous. There was an extending peritonitis which involved the mesentery. Histologically section showed no new feature other than previously described except that "nests" of protozoa were more common. (Fig. 3). The mucous membrane was ruptured releasing dead tissue and inflammatory cells into the lumen.

Liver: Numerous large abscesses yellowish to yellowish-green in color were present which closely resembled those seen in the natural disease. Microscopic examination revealed extensive areas of necrosis where "nests" of protozoa were common. (Fig. 4 and 5). Protozoa like bodies were also present in the hepatic veins. (Fig. 6).

Kidney: No gross lesion was observed. Histologically a few kidney tubules were distorted by necrosis. There was haemorrhage with a few reactive cells present. Protozoa like bodies were observed in the blood vessels. (Fig. 7).

Spleen: No gross lesion was observed. Microscopic examination revealed certain spherical bodies having many of the characteristics of Histomonas. However they were not numerous. (Fig. 8). The macrophoges were diffusely distributed throughout the spleen. Protozoa like bodies were also present in the blood vessels. (Fig. 9 and 10). A

few cells were desquamated and the inflammatory reaction was purely granulomatous.

Bird No. 650 (Killed 7 days post inoculation).

The bird was showing droopiness, watery droppings and appeared weak. Temperature 106.9° F.

Ceca: Upon gross examination both ceca showed hyperaemia and marked distension with a slight thickening of the mucosa. Microscopically the mucous and submucous layers of the tissue were distorted with reactive cells especially eosinophiles. There was also an increase in the lymphoid cells. The muscular layers showed local aggregation of these cells. In some places the mucous membrane stained poorly and was detached from the underlying tissue due to oedematous fluid especially near the tips of villi. (Fig. 11). The protozoa were present mostly in the mucous and submucous layer. The serous layer was normal.

Liver: No lesion was present on gross examination. Microscopically early changes of necrosis were present. A few small areas of round cell infiltration were seen close to the blood vessels.

Kidney: Normal.

Spleen: Normal.

Bird No. 607 (Died 12 days post inoculation).

This bird previously showed typical signs and characteristic sulphur colored droppings.

Ceca: Both ceca were affected and were enlarged four times normal. The foul swelling contents were caseous. A study of tissue sections showed thickened walls with a laminated structure in their lumens which contained protozoa. (Fig. 12). There was well marked congestion. Widespread infiltration of all tissue layers by polymorphonuclear leucocytes and lymphocytes were present. Protozoa were common in all the tissue layers. The protozoa contained well defined eccentric nuclei with an acidophilic cytoplasm. (Fig. 13).

Liver: Large necrotic yellowish-green abscesses were present on the surface of the liver such as is seen in the natural disease. Microscopically the architecture of the liver was lost with irregular distribution of polymorphonuclear leucocytes and lymphocytes. The necrotic areas were rich in protozoa and "nests" of the parasite were not uncommon. Giant cells also contained protozoa like bodies. Some haemorrhage was present.

Kidney: No gross lesion was present. Microscopically the tissue was reasonably normal.

Spleen: Normal.

Bird No. 617 (Killed 21 days post inoculation).

The bird appeared to be healthy. Gross lesions were not visible in the internal organs. Microscopic examination of the ceca revealed slight haemorrhage and local aggregation of lymphoid tissue in the mucous layer.

Bird No. 645 (Killed 7 days post inoculation).

The bird was healthy. No lesions were present.

Bird No. 624 (Died 12 days post inoculation).

The bird was showing characteristic symptoms of the disease at the time of death. There was emaciation with sulphur colored droppings.

Ceca: The ceca were enlarged and their contents were caseous. On microscopic examination all the tissue layers were affected and the lumen was packed with caseous material containing some apparently dead protozoa. An inflammatory change was seen in the muscular and serous layer of the ceca. The mucous and submucous layers were greatly necrosed with a heavy concentration of protozoa. The epithelial lining was totally lost and no villi were present. The protozoa contained granular cytoplasm with eccentric nuclei.

Liver: Numerous large lesions were observed on macroscopic examination. Microscopically there were numerous areas of necrosis with the presence of large protozoa with eccentric

nucleus. Some protozoa were engulfed by giant cells. The necrosis was irregular occurring near the hepatic triad. Polymorphonuclear leucocytes were few in number.

Kidney: There was an increase in the size of these organs. The microscopic study showed congestion and haemorrhage. The tissue was showing necrosis with few reactive cells. The glomeruli were packed with pinkish material. Protozoa like bodies were present near the necrosed tissue. (Fig. 14). Necrosis was irregular in nature and was more common near the blood vessels.

Spleen: Normal.

Bird No. 616 (Killed 21 days post inoculation).

The bird was healthy. On necropsy no lesion was observed.

II. RECTAL INOCULATION

A total of 16 poults 16-25 days of age were utilised for this method. Three types of suspension, that is, ceca with their contents, liver, and combined liver and ceca with their contents were used as inoculums. It was proposed to kill one exposed poult of each group and a control poult at weekly intervals. All control birds remained healthy throughout the three week period. The infected poults were observed each day for abnormal droppings or other characteristic signs.

TABLE V

The effect of rectal inoculations of two grams of tissue containing Histomonads to turkey poults.

Poult No.	Age when exposed Days	Inoculum	Post-exposed Days	Blackhead Lesions	Remarks
611	16	Ceca & Contents	7	Ceca	Killed
612	16	Ceca & Contents	14	Ceca & Liver	Killed
622	16	Ceca & Contents	17	Ceca & Liver	Died
632	16	Ceca & Contents	17	Ceca & Liver	Died
625	16	Ceca & Contents	21	Ceca & Liver	Died
620	16	Liver	7	None	Killed
604	16	Liver	13	Ceca & Liver	Died
613	16	Liver	13	Ceca & Liver	Died
610	16	Liver	17	Ceca & Liver	Died
619	16	Liver	17	Ceca & Liver	Died
609	16	Liver	16	Ceca & Liver	Died
630	25	Liver, Ceca & Contents	7	Ceca & Liver	Killed
633	25	Liver, Ceca & Contents	9	Ceca & Liver	Died
636	25	Liver, Ceca & Contents	9	Ceca & Liver	Died
639	25	Liver, Ceca & Contents	10	Ceca & Liver	Died
643	25	Liver, Ceca & Contents	10	Ceca & Liver	Died

Spleen: Normal.

Bird No. 612 (Killed 14 days post inoculation).

This bird was presenting drooping wings and tail, lowered head, ruffled feathers, and a sulphur colored diarrhoea. (Fig. 17).

Ceca: Both the ceca were enlarged. The lesions consisted of marked inflammatory changes with ulceration involving almost all of the organ. The lumens were packed with semi-caseous material. Microscopically all of the tissue layers were involved with the parasite. Normal structures of the villi and muscular layers were completely lost. Polymorphonuclear leucocytes were numerous throughout the section. "Nests" of protozoa were also present. (Fig. 18).

Liver: The affected liver presented a characteristic appearance with many areas of necrotic and degenerated tissue. These were more or less circular having a yellowish to yellowish-green appearance. On microscopic examination widespread congestion was present and at least half of the normal tissue was damaged showing different degrees of necrosis, most common in the hepatic triads. (Fig. 19). The size of the protozoa was larger than in the ceca. Round cell foci and reactive cells were present near the central canal. Occasional giant cells containing protozoa were present.

Kidney: No gross lesion was present. Microscopic examination revealed localized infiltration of polymorphonuclear leucocytes and congestion. Examination of kidney tubules showed degenerative changes.

Spleen: Normal.

Bird No. 622 (Died 17 days post inoculation).

Bird No. 632 (Died 17 days post inoculation).

Bird No. 625 (Died 21 days post inoculation).

All the three poults showed more or less the same changes of Histomoniasis. The poults were emaciated. A sulphur colored diarrhoea tinted the feathers below their vents. (Fig. 20).

Ceca: Apart from the gross changes previously described, the ceca were greatly enlarged containing a characteristic foul smelling, yellowish-green, caseous exudate and a firm cheesy core. Perforating ulcers produced a peritonitis which involved the mesentry. Microscopically the sections showed no new features except that the granular and non granular leucocytes were few in number. "Nests" of protozoa were more common. The cecal core was made up of cells, debris, and protozoa.

Liver: Gross changes were more or less the same as previously described. Microscopically there were extensive

areas of necrosis with irregular distribution of reactive cells. Protozoa were common. In some areas "nests" of such protozoa were present in the clearer areas of lysed tissue.

Kidney: No gross lesion was observed. Microscopically protozoa like bodies were present near the necrosed kidney tubules.

Spleen: Reasonably normal.

In the second group two grams of infected liver suspension were given to six 16-day old turkey poults. One poult exposed for 7 days showed no lesions. The remaining five poults died of Histomoniasis after 13 to 17 days exposure. They showed characteristic symptoms and lesions. (Table V).

The incubation period in this group was between 7 to 13 days. The following changes were observed in each poult.

Bird No. 620 (Killed 7 days post inoculation).

The bird was apparently healthy. Lesions were not present.

Bird No. 604 (Died 13 days post exposure).

Bird No. 613 (Died 13 days post exposure).

Bird No. 609 (Died 16 days post inoculation).

Bird No. 610 (Died 17 days post inoculation).

Bird No. 619 (Died 17 days post inoculation).

All the 5 poults of this group had previously showed characteristic symptoms and sulphur colored diarrhoea. Drooping wings and emaciation were present in all poults.

Ceca: The ceca showed advanced lesions although there was some variation in the degree of changes. The lumens were packed with foul smelling osseous material and there was evidence of peritonitis extending to the adjacent mesentry. Microscopically the wall of the cecum was thickened and all the tissue layers were effected showing protozoa in "nests" of varying sizes. The largest parasites were present near the muscular layer. Intestinal villi were distorted and normal muscular tissue was very scanty. Polymorphonuclear leucocytes and lymphocytes were numerous. The lumen of the ceca were showing fibrinous exudate, dead tissue, inflammatory cells and few protozoa. (Fig. 21). The blood vessels were engorged.

Liver: Numerous large characteristic lesions were observed on the surface and throughout the organ. There was a variation in the extent of the lesions. Their size ranged from pin-head foci to those well defined. Histological examination of the section showed extensive areas of necrosis with few normal hepatic cells. The necrotic area was rich

in protozoa with well defined cytoplasm. (Fig. 22). "Nests" of protozoa of varying size were common in the clearer areas of lysed liver tissue. (Fig. 23, 24, and 25). The inflammatory process with reactive cells was well established, mostly near the hepatic triads. Blood vessels were engorged. Mild haemorrhage was observed.

Kidney: Lesions were not observed. Microscopic examination revealed cloudy swelling, slight haemorrhage with a distortion of some few kidney tubules.

Spleen: Reasonably normal.

In the third group two grams suspension of combined infected liver and ceca with their contents were given to five 25-day old poults. One bird was killed after 7 days of exposure. It showed characteristic lesions of Histomoniasis. The remaining four poults died of the disease 9 to 10 days after being exposed. They showed typical symptoms and lesions. (Table V).

The incubation period of the disease in this method was 7 to 9 days, a little shorter than the previous methods. The following changes were observed in these poults:

Bird No. 630 (Killed 7 days post inoculation).

The poult was off feed, dull, with its head down and with ruffled feathers. Droppings were watery and yellowish-white in color.

Ceca: Both ceca were affected and were enlarged with semi-caseous material. (Fig. 26). Histological examination of tissue sections presented a thickened mucous and submucous layer with hyperaemia and detached villi. The muscular layer was partly affected but the serous layer was normal. Capillary congestion was present in the muscular layer. Eosinophiles were most common in the mucous and submucous layer. An exudate was present with numerous protozoa having well defined eccentric nuclei.

Liver: The liver showed very few lesions, some of them were slightly raised above the surface of the organ. (Fig. 26). Histologically areas of round cell infiltration were common with necrosis of the hepatic parenchyma. Well stained protozoa resembling the "vegetative phase" of Histomonas meleagridis were present. Necrosis and polymorphonuclear leucocytes were irregularly distributed.

Kidney: No change was observed.

Spleen: Appeared normal.

Bird No. 636 (Died 9 days post inoculation).

Bird No. 633 (Died 9 days post inoculation).

Bird No. 643 (Died 10 days post inoculation).

Bird No. 639 (Died 10 days post inoculation).

All these four poults which died of Histomoniasis previously showed a characteristic sulphur-colored diarrhoea. The pathological changes were more or less the same.

Ceca: All of the ceca showed advanced lesions with extending peritonitis which involved the mesentry. Histologically all the layers of the cecal tissue were showing inflammatory changes. The protozoa could be seen in all parts of the sections and many of them were laying together in "nests." The size of the protozoa were variable. In most cases the nucleus was not visible. A few "divided phase" of the protozoa were present. There was widespread necrosis with reactive cells. Blood vessels were engorged.

Liver: Numerous widespread lesions up to 1.5 cm. were present as viewed through Glisson's capsule. Microscopically necrosis was ill defined and cell substances were lysed leaving only the cell outline. Reactive cells were common in the hepatic triad and around the central canal. "Nests" of protozoa of variable size were common.

Kidney: Reasonably normal.

Spleen: Reasonably normal.

III. COMBINED ORAL AND RECTAL INOCULATION

Three turkey poults 39 days old were given two grams each of a suspension of liver and ceca with their contents. One gram of suspension of affected tissue was passed through the mouth into the crop of the poult and another gram of suspension was administered per rectum in the same way as described for "Rectal inoculation." At weekly intervals one poult was to be killed. One bird exposed to the infection for 7 days was killed. It showed gross lesions in the ceca and liver. The two remaining poults were healthy at 14 and 21 days. (Table VI). Three controls were killed at weekly intervals. All were healthy.

The incubation period in this method was not estimated as typical infection was not present. The following changes were observed in these poults:

Bird No. 698 (Killed 7 days post inoculation).

The poult showed drooped wings, poor appetite, head down and its eyes were closed. The droppings were watery and yellowish in color. Temperature 105.5° F.

Ceca: Both ceca were affected. They were enlarged with semi-caseous material in their lumen. Microscopically all layers of the ceca were showing an acute inflammatory process. The mucous and submucous layers were thickened. Fibrinous exudate was present with massive necrosis. The

villi were distorted. Protozoa were present throughout the tissue but "nests" of parasites were rare. There was a marked polymorphonuclear infiltration. Capillary congestion with limited haemorrhage was seen.

TABLE VI

The effect of combined oral and rectal inoculations using two grams of tissue containing Histomonads to turkey poults.

Poult No.	Age when exposed Days	Inoculum	Post-exposure Days	Black-head Lesions	Remarks
698	39	Liver, Ceca & Contents	7	Ceca & Liver	Killed
689	39	Liver, Ceca & Contents	14	None	Killed
682	39	Liver, Ceca & Contents	21	None	Killed

Bird No. 689 (Killed 14 days post inoculation).

Bird No. 682 (Killed 21 days post inoculation).

Symptoms of the disease were not observed in these two poults. Lesions were not observed upon necropsy. Microscopic examination of ceca revealed hyperplasia of lymphoid area only in the mucous layer. (Fig. 27). Tissue sections of liver, kidney and spleen were normal.

villi were distorted. Protozoa were present throughout the tissue but "nests" of parasites were rare. There was a marked polymorphonuclear infiltration. Capillary congestion with limited haemorrhage was seen.

Liver: This organ showed a few pin-point lesions. A study of tissue sections revealed few areas of well defined round cell infiltration mostly near the capillaries and small bile ducts. Reactive cells were present with slight haemorrhage. The necrosis was irregular with ill-defined margins showing the presence of few protozoa.

Kidney: Reasonably normal.

Spleen: Normal.

Bird No. 689 (Killed 14 days post inoculation).

Bird No. 682 (Killed 21 days post inoculation).

Symptoms of the disease were not observed in these two poults. Lesions were not observed upon necropsy. Microscopic examination of ceca revealed hyperplasia of lymphoid area only in the mucous layer. (Fig. 27). Tissue sections of liver, kidney and spleen were normal.

IV. SUBCUTANEOUS INOCULATION

Three poults 39 days of age were each inoculated subcutaneously with two grams suspension of liver and ceca, with their contents. The exposed poults and controls were killed at 7, 14 and 21 days post exposure. None of the exposed birds developed typical Histomoniasis but a local reaction at the site of injections were well marked. (Table VII). The control birds remained healthy.

The following changes were observed in these poults:

Bird No. 649 (Killed 7 days post inoculation).

The first local reaction consisted of a slight swelling in the subcutaneous tissue with early necrosis. The poult was dull, off feed and continued to fall on the affected side of the body. Temperature was 106° F.

Ceca: Appeared normal.

Liver: Normal.

Kidney: The organ was swollen. Section revealed slight haemorrhage, also degenerative changes of a few kidney tubules and glomeruli.

Spleen: Appeared reasonably normal.

Local reaction: The skin showed hyperkeratosis with

TABLE VII

The effect of subcutaneous injection of two grams of tissue containing Histomonads to turkey poults.

Poult No.	Age when exposed Days	Inoculum	Post-exposure Days	Lesions	Remarks
649	39	Liver, Ceca & Contents	7	Local*	Killed
631	39	Liver, Ceca & Contents	14	Local*	Killed
641	39	Liver, Ceca & Contents	21	Local*	Killed

*Local reaction at the site of inoculation.

thickening of the dermis. There was a mass of necrosis containing reactive cells, mononucleated cells and exudate. They were showing an inflammatory reaction with haemorrhage.

Bird No. 631 (Killed 14 days post inoculation).

Bird No. 641 (Killed 21 days post inoculation).

Both poults were dull with a loss of appetite and both were showing the same types of changes. There was a widespread swelling around the inoculated areas. The skin was white to dark in color as a result of necrosis, haemorrhage, and exudate. The necrotic area was depressed. Its edges were indurated and raised imparting a ringlike shape to the outer affected part. Gravitation produced lameness.

Ceca: Appeared normal. A study of sectioned tissue revealed a slight thickening of the submucous layer from odematous fluid.

Liver: Reasonably normal. Microscopically a few round cell foci near the hepatic triad and apparent hyperplasia of blood vessels were the only changes.

Kidney: Same type of changes were present as in the previous bird.

Spleen: Normal.

Local reaction: Apart from the local changes previously described, the necrotic mass contained caseous material. Degenerative changes were also present on the related pectoral muscles.

V. INTRAMUSCULAR INOCULATION

Thirteen poults 16-39 days of age were inoculated intramuscularly with three different types of suspension: that is, ceca, liver, and combined cece and liver. It was proposed to kill one exposed bird in the three different groups at weekly interval. Also a control bird was killed at the same interval. All the control birds remained healthy. The exposed birds were closely observed each day for abnormal droppings or other changes.

In the first group one gram of cecal suspension from an infected bird was injected intramuscularly to each of five 16-day old poults. One poult died two days post exposure from a non-specific cause. Another poult died after 11 days post exposure. It showed a large local reaction at the site of inoculation. Degenerative lesions of the liver were present without Histomonas. The three remaining poults remained healthy other than one which showed a local reaction in the pectoral muscle. (Table VIII). The following changes were observed in these poults:

TABLE VIII

The effect of intramuscular inoculations of one gram of tissue containing Histomonads to turkey poults.

Poult No.	Age when exposed Days	Inoculum	Post-exposed Days	Blackhead Lesions	Remarks
647	16	Ceca	2	Pectoral Muscle	Died
644	16	Ceca	11	Pectoral Muscle & Liver	Died
635	16	Ceca	21	Local	Killed
642	16	Ceca	28	None	Killed
628	16	Ceca	35	None	Killed
623	16	Liver	7	None	Killed
602	16	Liver	12	Pectoral Muscle & Liver	Died
621	16	Liver	13	Pectoral Muscle & Liver	Died
605	16	Liver	13	Pectoral Muscle & Liver	Died
618	16	Liver	13	Pectoral Muscle & Liver	Died
691	39	Ceca & Liver	7	Pectoral Muscle	Killed
680	39	Ceca & Liver	14	Pectoral Muscle	Killed
694	39	Ceca & Liver	17	Pectoral Muscle	Died

Bird No. 647 (Died 2 days post inoculation).

A local swelling at the site of inoculation was present. The pectoral muscle was showing slight necrosis with a fibrinous exudate and reactive cells.

Bird No. 644 (Died 11 days post inoculation).

The poult was emaciated. A local swelling at the site of inoculation was present. The droppings were normal.

Ceca: Normal.

Liver: This organ was showing degenerative changes on both gross and microscopic examination.

Kidney: No gross lesion was present. Sections showed slight inflammatory process in the kidney tubules.

Spleen: The tissue was abscessed.

Pectoral muscle: Microscopically at the point of injection the muscle cells were distorted, necrosed with infiltration of polymorphonuclear leucocytes especially near the blood vessels. Congestion of vessels was also present.

Bird No. 635 (Killed 21 days post inoculation).

Bird No. 642 (Killed 28 days post inoculation).

Bird No. 628 (Killed 35 days post inoculation).

All three poults appeared healthy. One bird showed a slight local reaction at the site of inoculation. Temperature 107° F.

Ceca: Normal. An increase in lymphoid cells in the mucous layer was seen microscopically.

Liver: Appeared normal.

Kidney: Normal. Microscopic examination revealed haemorrhage with necrosis of a few kidney tubules with hyaline casts.

Spleen: Normal.

Pectoral muscle: There was necrosis with reactive cells and congestion seen upon microscopic examination.

In the second group five 16-day old poults were each injected with one gram of suspension of infected liver intramuscularly. One poult killed at 7 days post exposure showed no signs of the disease. The remaining four poults died on the 12th and 13th days post exposure. They showed lesions in the pectoral muscles and in the liver. (Table VIII). Two other poults were inoculated with normal liver as inoculated controls. Lesions did not develop following the inoculations.

The following changes were observed in five exposed poults:

Bird No. 623 (Killed 7 days post inoculation).

The poult was healthy. All organs were normal.

Bird No. 602 (Died 12 days post inoculation).

Bird No. 618 (Died 13 days post inoculation).

Bird No. 605 (Died 13 days post inoculation).

Bird No. 621 (Died 13 days post inoculation).

All the four poults were showing more or less the same types of changes. They were weak, emaciated, dull, and had previously shown loose white droppings. Local swelling was present at the site of inoculation.

Ceca: No gross lesion was present. Microscopically the mucous membrane was lost. The villi were showing necrosis with the presence of reactive cells. Protozoa like bodies were present. Blood vessels were engorged.

Liver: A few areas of necrosis were present on the surface of the liver. Tissue section study revealed necrosis with irregular margins and protozoa like bodies resembling Histomonas meleagridis. (Fig. 28 and 29). Oedematous fluid was present with few mononuclear cells. Polymorphonuclear leucocytes were present especially around the margins of the necrosed areas. The necrosis was not uniform. Few cells were distorted. (Fig. 30).

Kidney: The kidneys were swollen. Microscopically

degenerative changes of the tubules were present.

Spleen: Normal.

Pectoral muscle: The muscle was swollen and necrosed on the effected side. Microscopic examination revealed a mass of necrosis with proteinacious fluid. Protozoa of various sizes were present most having an eccentric nucleus with granular cytoplasm. There were mononuclear cells, infiltrated around the necrosed margins. The normal structure of the muscle tissue was lost. Haemorrhage and fibrinous exudate were present. (Fig. 31).

The third group was comprised of three 39 day old poults. One gram of a suspension of infected ceca, and liver were injected intramuscularly. Two of the three poults were killed on the 7th and the 14th days post exposure. Both showed a local reaction in the pectoral muscles. The third poult died 17 days post exposure. (Table VIII).

The changes in each poult were as follows:

Bird No. 691 (Killed 7 days post inoculation).

Bird No. 680 (Killed 14 days post inoculation).

Both poults were apparently healthy with slight local swelling at the point of injection. Lesions were not present in the ceca, liver, kidney and spleen. The pectoral muscles showed widespread necrosis with large numbers

of reactive cells and oedematous exudate. (Fig. 32). Protozoa were present in the necrosed tissue. (Fig. 33).

Bird No. 694 (Died 17 days post inoculation).

The poult was emaciated. Upon necropsy the ceca, liver, kidney and spleen were reasonably normal. The pectoral muscle was swollen at the point of injection. Histologically apart from the changes already described the muscle tissue showed some haemorrhage and its normal structure was lost.

VI. BLOOD INOCULATION

A total of seven poults 35-42 days of age were inoculated with 0.2 ml. of infected whole blood withdrawn from the posterior mesentric, cecal and hepatic veins. Two different routes of transmission were used. One poult of each group of exposed birds including the controls were killed at weekly interval. All control birds remained healthy. The exposed poults were observed each day for abnormal droppings or other signs of the disease.

In the first group five poults 35 days old were given 0.2 ml. of infected whole blood intra-hepatically through the body wall. One poult killed after 14 days of exposure had developed the disease and showed atypical lesions in the liver and lungs. Another poult died of Histomoniasis 20 days following exposure. The remaining three birds were killed on the 7th, 21st and 27th days of

exposure. They showed no sign of the disease at necropsy. (Table IX).

The incubation period of the disease in this group was between 14-20 days. The following changes were observed in these poults:

Bird No. 690 (Killed 7 days post inoculation).

The poult was normal. There was no local reaction and no lesion was observed.

Bird No. 686 (Killed 14 days post inoculation).

The poult was slightly weak. Droppings were normal. Temperature 105° F. No clinical symptom of Histomoniasis was present.

Ceca: Gross lesions were not present. A study of tissue sections revealed increased lymphoid cells in the mucous layer.

Liver: Upon gross examination the organ showed a single massive necrotic area 3 x 2½ cm. in diameter at the site of inoculation. Microscopic examination revealed massive necrosis. (Fig. 34). Protozoa with eccentric nucleus and granular cytoplasm were common. (Fig. 35 and 36). "Nests" of protozoa were also present. The tissue showed inflammatory process with many reactive cells, haemorrhage and exudate, dead tissue, and hyaline casts. (Fig. 37).

Kidney: Normal.

TABLE IX

The effect of 0.2 ml. of whole blood from infected donors to poults.

Poult No.	Age when exposed Days	Site of Inoculation	Post-exposed Days	Blackhead Lesions	Remarks
690	35	Liver*	7	None	Killed
686	35	Liver*	14	Liver & Lung	Killed
695	35	Liver*	20	Liver	Died
681	35	Liver*	21	None	Killed
697	35	Liver*	27	None	Killed
6851	42	Wing Vein	7	None	Killed
646	42	Wing Vein	14	None	Killed

*The inoculum was given through the body wall into the liver.

Spleen: No gross lesion was present. Microscopic examination showed distortion of few cells with protozoa like bodies 12 to 20 microns in size in the blood vessel.

Lung: All of this organ was congested. It consisted of firm masses of yellowish caseous material of varying sizes. Smaller nodules of caseous material were separated at their boundaries by a thick red oedematous degenerating tissue. Microscopic examination of sectioned tissue revealed generalized areas of coagulation necrosis and the alveoli were filled with oedematous exudate. (Fig. 38). Numerous polymorphonuclear and mononuclear leucocytes were present especially eosinophiles and myelocytes. (Fig. 39). There was haemorrhage and degeneration of blood vessels. Protozoa like bodies resembling Histomonas meleagridis were present near the blood vessels. (Fig. 40).

Bird No. 695 (Died 20 days post inoculation).

The poult had previously shown typical symptoms of the disease with the exception of sulphur colored droppings. Dullness, emaciation, and drooped wings were typical of Blackhead symptoms.

Ceca: Normal.

Liver: The liver surface was covered with spherical necrotic foci varying from pin-point up to a centimeter or more in diameter. The lesions were typical as those seen in a

natural outbreak of Histomoniasis. A study of tissue sections revealed massive necrosis with sero-cellular exudate. (Fig. 41). Lymphocytes, multi nucleated giant cells, heterophiles, and other inflammatory cells were present near the necrotic areas. The tissue was rich in protozoa and in a few giant cells protozoa like bodies were present. (Fig. 42). The blood vessels were engorged, contained cellular debris, erythrocytes, other cells but few protozoa.

Kidney: Gross lesion were not observed. Microscopically slight necrosis with some haemorrhage, distortion of few cells and infiltration of mononuclear cells were the only changes present.

Spleen: No gross lesions. Section study showed increased connective tissue to be present and end arteries were very distinct. (Fig. 43). Engulfed protozoa were present. (Fig. 44).

Lung: Histopathological changes were the same as previously described.

Bird No. 681 (Killed 21 days post inoculation).

Bird No. 697 (Killed 27 days post inoculation).

Both of the poults were apparently healthy at the time of necropsy. No lesion was observed. Microscopic examination revealed normal structure of the tissues.

In the second group two 42-day old turkey poults were given 0.2 ml. of infected whole blood by intravenous injection into the wing vein. The poults were killed on the 7th and 14th days post exposure. None of the poults developed typical Histomoniasis. (Table IX). The following changes were seen in these two birds:

Bird No. 6851 (Killed 7 days post inoculation).

Bird No. 646 (Killed 14 days post inoculation).

The general condition of both poults was good. Temperature 107° F.

Ceca: No gross lesion was present. A study of the tissue sections revealed a slight thickening of the submucous and mucous layer. Polymorphonuclear leucocytes were present in oedematous fluid, most prominent at the tip of villi. (Fig. 45).

Liver: No lesion was observed. Histo-pathological study showed few areas of round cell infiltration near the blood vessels. Cloudy swelling was common. Focal areas of serum containing a very few protozoa were present.

Kidney: Normal.

Spleen: Normal.

Lung: Capillary congestion with haemorrhage in the air

sacs was present. A few heterophiles and macrophages were present.

DISCUSSION

Six different methods of producing Histomoniasis were attempted. The inoculum for each group consisted of diseased tissues and whole blood from known cases of Histomoniasis. (Table I).

Oral inoculation into the oesophagus of two grams of suspension of diseased sacs with their contents or two grams of suspension of diseased liver to three 16-day old poults in each group gave negative results. When two grams of suspension of combined liver and sacs with their contents were given by the same route to five 25-day old poults the disease developed in all poults after 7-14 days of exposure. Oral inoculation into the gizzard of three grams of suspension of diseased sacs with their contents produced Histomoniasis in 2 of 3 poults 40 days of age. When three grams of a suspension of diseased liver was given by the same route to three poults 40 days of age, only one developed Histomoniasis. The incubation period for this method was 7-12 days.

Rectal inoculation of two grams of suspension of diseased sacs with their contents produced Histomoniasis in all five 16 day old poults after 7-14 days exposure. When two grams of a suspension of infected liver was given to six 16 day old poults, five developed Histomoniasis.

CHAPTER V

DISCUSSION

Six different methods of producing Histomoniasis were attempted. The inoculum for each group consisted of diseased tissues and whole blood from known cases of Histomoniasis. (Table X).

Oral inoculation into the oesophagus of two grams of suspension of diseased ceca with their contents or two grams of suspension of diseased liver to three 16-day old poults in each group gave negative results. When two grams of suspension of combined liver and ceca with their contents were given by the same route to five 25-day old poults the disease developed in all poults after 7-14 days of exposure. Oral inoculation into the gizzard of three grams of suspension of diseased ceca with their contents produced Histomoniasis in 2 of 3 poults 40 days of age. When three grams of a suspension of diseased liver was given by the same route to three poults 40 days of age, only one developed Histomoniasis. The incubation period for this method was 7-12 days.

Rectal inoculation of two grams of suspension of diseased ceca with their contents produced Histomoniasis in all five 16 day old poults after 7-14 days exposure. When two grams of a suspension of infected liver was given to six 16 day old poults, five developed Histomoniasis,

7-13 days post inoculation. Two grams of a suspension of diseased liver and ceca with their contents produced Histomoniasis 7-9 days post inoculation in all five 25 day old poults.

Combined oral and rectal inoculation of two grams of suspension of diseased liver and ceca with their contents produced Histomoniasis in one of three poults 39 days of age. Typical Histomoniasis was not present.

In all the three methods mentioned above the diseased poult showed characteristic symptoms. Pathology of the disease followed a fairly well marked course. Infection spreads from the ceca to the liver and in later stages of the disease the spleen and kidney were involved in a few cases.

Subcutaneous inoculation of two grams suspension of diseased liver and ceca with their contents produced a local reaction in all the three 39 day old poults. Typical Histomoniasis was not present.

Intramuscular inoculation of one gram suspension of diseased ceca, liver, and combined liver and ceca produced local reactions in the pectoral muscle when given to thirteen 16-39 day old poults. Typical Histomoniasis was not present but in a few cases protozoa like bodies were observed in the liver tissue after 7-12 days of post exposure.

Whole blood inoculation of 0.2 ml. withdrawn from

posterior mesentric, hepatic and cecal veins of the diseased donor bird, when given intrahepatically through the body wall to five 35 day old poults, two poults developed the disease in 14-20 days of exposure. Typical lesions of Histomoniasis were present in the liver with atypical involvement of the lungs and spleen. In no instance was the ceca or any portion of the intestinal tract involved. A similar type of infected venous blood was injected into the wing vein to two 42 day old poults. Neither poult showed any lesion of the disease after 7-14 days of exposure. However a few protozoa like bodies were present in the liver tissue.

In view of the probable presence of parasites in tissue sections in the cecal blood vessels and later in hepatic vessels and even in the spleen and kidney, indicates clearly that protozoa pass in these organs by means of the blood streams. Whole blood inoculations indicate that the infective form of the protozoa is capable of entrance and survival in the hepatic, mesentric, and cecal blood vessels during the critical period of the disease. It also appears that Histomonas meleagridis may become established and cause pathology in nearly any type of tissue to which it can gain access.

In all of the methods of transmission used the protozoa observed in the diseased tissues presented a more or less uniform appearance, resembling the "vegetative phase"

of Histomonas meleagridis as described by Tyzzer. "Nests" and engulfed protozoa were also common in the later stages of the disease. Multiplication of the parasite within the tissues probably took place by binary fission as the divided phase of the protozoa were observed.

The foregoing experiments illustrate clearly that it is not a difficult matter to produce Histomoniasis artificially. These experiments have again shown that the "Rectal inoculation" of diseased liver and ceca with their contents give an incidence of 100 percent infection. This method is the most suitable for infecting turkeys to be used for experimental work in connection with the testing of drugs for prophylactic or therapeutic purposes.

SUMMARY

A series of experiments are described for producing artificial Histomoniasis in turkey poults 16-42 days of age. Material for the primary infection was obtained from two infected chicks. Experimental poults after developing Histomoniasis were at times used as a source of diseased liver and ceca with their contents and whole blood.

Tissue suspensions, one to three grams, were given once only by oral, rectal, combined oral and rectal, subcutaneous and intramuscular inoculations. Whole blood (0.2 ml.) was given intrahepatically through the body wall and intravenously into the wing vein. Histomoniasis was

TABLE X

The results of various methods of exposing poults to Histomonads.

Source of inoculum containing Histomonads	Route of Transmission	Age of recipient Days	Number of Poults	Amount of Inoculum	Successful Transmission	Incubation period Days
Group A						
(1) Ceca and contents	Oral inoculation into the oesophagus	16	3	2 gms.suspension	0	--
(2) Liver	Oral inoculation into the oesophagus	16	3	2 gms.suspension	0	--
(3) Liver, ceca and contents	Oral inoculation into the oesophagus	25	5	2 gms.suspension	5	7-14
(4) Ceca and contents	Oral inoculation into the gizzard	40	3	3 gms.suspension	2	7-12
(5) Liver	Oral inoculation into the gizzard	40	3	3 gms.suspension	1	7-12

TABLE X (continued)

Source of inoculum containing Histomonads	Route of Transmission	Age of recipient Days	Number of Poults	Amount of Inoculum	Successful Transmission	Incubation period Days
Group B						
(1) Ceca and contents	Rectal inoculation	16	5	2 gms.suspension	5 (local reaction)	7-14
(2) Liver	Rectal inoculation	16	6	2 gms.suspension	5	7-13
(3) Liver, ceca and contents	Rectal inoculation	25	5	2 gms.suspension	5	7-9
Group C						
Liver, ceca and contents	Combined oral and rectal inoculation	39	3	2 gms.suspension	1	--
Group D						
Liver, ceca and contents	Subcutaneous inoculation	39	3	2 gms.suspension	0 (local reaction only)	--

TABLE X (continued)

Source of inoculum containing Histomonads	Route of Transmission	Age of recipient Days	Number of Poults	Amount of Inoculum	Successful Transmission	Incubation period Days
Group E						
(1) Ceca	Intramuscular inoculation	16	5	1 gm. suspension	0 (local reaction)	--
(2) Liver	Intramuscular inoculation	16	5	1 gm. suspension	0 (local reaction)	--
(3) Ceca and liver	Intramuscular inoculation	39	3	1 gm. suspension	0 (local reaction)	--
Group F						
(1) Whole blood from mesentric, hepatic and cecal veins	Intrahepatic through body wall	35	5	0.2 ml.	2	14-20
(2) Whole blood from mesentric, hepatic and cecal veins	Wing vein	42	2	0.2 ml.	0	--

produced with variable success by different routes of transmissions.

The experimental poults died of Histomoniasis within 7 to 14 days after exposure to diseased tissue suspensions. When whole blood from infected poult was inoculated into healthy recipients death occurred within 14 to 20 days after exposure.

Diseased poults showed characteristic symptoms. Pathology of the disease followed a well marked course after oral, rectal and combined oral and rectal inoculations. Protozoa were observed in the ceca, liver, kidney, and spleen. Subcutaneous inoculation produced only a local reaction. Intramuscular inoculations produced lesions in the pectoral muscles and liver. In blood induced Histomoniasis the protozoa was observed in the liver, spleen, and lungs.

The parasite observed is spherical, without flagella, mostly with eccentric nucleus and having granular to non-granular cytoplasm. "Nests" of protozoa containing 2-8 parasites were observed in the later stages of the disease. Their reproduction in tissue appeared to be by binary fission.

Two new methods of producing Histomoniasis (described above) that is oral inoculation of diseased tissue into the gizzard and whole blood inoculation intrahepatically from the diseased donor bird gave encouraging

results. However further experiments are required to establish these routes of transmission on strong footings.

BIBLIOGRAPHY

Allen, R. A. 1936. A Pentatrichomonas associated with cases of Enterohepatitis or Blackhead of poultry. Trans. Am. Micr. Soc. 55:315-322.

_____. 1941. Microscopic differentiation of lesions of Histomoniasis and Trichomoniasis in turkeys. Am. J. Vet. Res. 2:214-217.

Allen, R. W., Olivier, L. J. and Peterson, H. C. 1942. The efficacy of Phenothiazine for the removal of the cecal worm of chicken. Vet. Med. 37:412-413.

Berger, E. H., Card, L. E., and Pomeroy, B. S. 1938. Diseases and Parasites of Poultry. 5th Ed. Lea & Febiger, Phil.

Boyce, H. F., and Bishop, A. 1937. Cultivation of Histomonas meleagridis from the liver lesions of a hen. Nature, Lond. 139:370.

BIBLIOGRAPHY

Bork, G. and Neal, R. 1952. The effect of some drugs upon Histomonas meleagridis in vitro. Ann. Trop. Med. Parasit. 46:68-71.

Biester, H. E., and Schwarte, L. H. 1959. Diseases of Poultry. 4th Ed. Iowa State Univ. Press, Ames: pp. 864-874, 1037, 1043.

Billings, W. A. 1928. Taking turkey. Minn. Ag. Exp. Div. Spec. Bull. 124.

Bishop, A. 1938. "Histomonas meleagridis" in Domestic Fowls (Gallus gallus). Cultivation and Experimental Infection. Parasit. 30(2):181-194.

Blount, W. P. 1938. New Arsenical preparation in the treatment of Blackhead in turkeys. Vet. J. 94:344-347.

Bolin, F. M., and Verdiman, F. N. 1941. Napharsen as a treatment for Enterohepatitis of turkeys. J. Am. Vet. Med. Assoc. 98:229-231.

Brackett, S., and Bliznick, A. 1949. The development of resistance to and the effect of some new chemotherapeutic agents on Enterohepatitis induced by the oral administration of cecal worm ova to chickens and turkeys. J. Parasit. 35 (Suppl):16.

BIBLIOGRAPHY

Allen, E. A. 1936. A Pentatrichomonas associated with cases of Enterohepatitis or Blackhead of poultry. Trans. Am. Micr. Soc. 55:315-322.

_____. 1941. Microscopic differentiation of lesions of Histomoniasis and Trichomoniasis in turkeys. Am. J. Vet. Res. 2:214-217.

Allen, R. W., Olivier, L. J. and Peterson, H. O. 1942. The efficacy of Phenothiazine for the removal of the cecal worm of chicken. Vet. Med. 37:412-415.

Barger, E. H., Cord, L. E., and Pomeroy, B. S. 1958. Diseases and Parasites of Poultry. 5th Ed. Lea & Febiger, Phil.

Bayon, H. P., and Bishop, A. 1937. Cultivation of Histomonas meleagridis from the liver lesions of a hen. Nature, Lond: 139:370.

Berk, G. and Neal, R. 1952. The effect of some drugs upon Histomonas meleagridis in Vitro. Ann. Trop. Med. Parasit. 46:68-71.

Biester, H. E., and Schwarte, L. H. 1959. Diseases of Poultry. 4th Ed. Iowa State Univ. Press. Ames: pp. 864-874, 1037, 1043.

Billings, W. A. 1928. Talking turkey. Minn. Ag. Exp. Div. Spec. Bull. 124.

Bishop, A. 1938. "Histomonas meleagridis" in Domestic Fowls (Gallus gallus). Cultivation and Experimental Infection. Parasit. 30(2):181-194.

Blount, W. P. 1938. New Arsenical preparation in the treatment of Blackhead in turkeys. Vet. J. 94:344-347.

Bolin, F. M., and Vardiman, P. H. 1941. Mapharsen as a treatment for Enterohepatitis of turkeys. J. Am. Vet. Med. Assoc. 98:229-231.

Brackett, S., and Bliznick, A. 1949. The development of resistance to and the effect of some new chemotherapeutic agents on Enterohepatitis induced by the oral administration of cecal worm ova to chickens and turkeys. J. Parasit. 35 (Suppl):16.

Brander, G. C., and Wood, J. C. 1955. Acetylamino 5 Nitrothiazole for the prevention and treatment of Blackhead in turkeys. Vet. Rec. 67:326.

Browne, T. G. 1922. Some observations on the digestive system of the fowl. J. Comp. Path. Therap. 35:13.

Calhoun, M. L. 1954. Microscopic anatomy of the digestive system of the chicken. Iowa State College Press: Ames, Iowa. Pp. 15, 23, 65-70, and 75-76.

Chester, F. D. 1900. Common diseases of fowls. Their control and treatment. Del. Col. Ag. Exp. Sta. Bull. 47.

_____, and Robin, A. 1900. Enterohepatitis or Blackhead of fowls. Twelfth Ann. Rep. Del. Ag. Exp. Sta. pp. 60-66.

Clarkson, M. J. 1961a. Some aspects of the chemical pathology of Histomoniasis in turkeys. Trans. Roy. Soc. Trop. Med. Hyg. 55 (1):2.

_____. 1961b. The blood supply of the liver of the turkey and the anatomy of the biliary tract with reference to infection with Histomonas meleagridis. Res. Vet. Sci. 2(2):259-264.

Cole, L. J., Hadley, P. B., and Kirkpatrick, W. F. 1910. Blackhead in Turkeys. A study in Avian Coccidiosis. Ag. Exp. Sta. R. I. State Col. Bull. 141:137.

Connell, R. 1950. Enterohepatitis (Blackhead) in turkeys VI. Abnormalities possibly caused by a stage of Histomonas meleagridis, occurring in second stage larvae of Blackhead transmitting Heterakis gallinae. Canad. J. Comp. Med. 14:331-337.

Costello, L. C. 1957. The efficacy of Furozolidone against Blackhead (Infectious Enterohepatitis) in turkeys. Proc. I. Nat. Symposium on Nitrofurans in Ag. Mich. Sta. Univ. 64-68.

_____, and Devolt, H. M. 1956. Studies on the Chemoprophylactic action of 'Furoxone' against Infectious Enterohepatitis (Blackhead) of turkeys. Poult. Sci. 35(4):952-955.

Cuckler, A. C., and Malanga, C. M. 1956. Nithiazide II. Effect on Enterohepatitis in turkeys. Proc. Soc. Exp. Biol. & Med. 92:485-488.

Curtice, C. 1907a. Notes on experiments with Blackhead of Turkeys. U. S. Dept. Ag. Bur. Anim. Ind. Circ. 119.

_____. 1907b. The rearing and management of turkeys with special reference to the Blackhead disease. R. I. Ag. Exp. Sta. Bull. 123:1-64.

_____. 1907c. Further experiments in connection with the Blackhead disease in turkeys. R. I. Ag. Exp. Sta. Bull. 124:67.

Cushman, S. 1893. Experiments with turkeys. R. I. Ag. Exp. Sta. Bull. 25:284.

Delaphane, J. P. 1931. Enterohepatitis (Blackhead) in turkeys. Thesis. Ohio State Univ. Lib.

_____. 1932. Etiological studies of Blackhead (Enterohepatitis) in turkeys. R. I. Ag. Exp. Sta. Bull. 233:2.

_____, and Stuart, H. O. 1933. Cecal obligation of turkeys by the use of clamps in preventing Enterohepatitis (Blackhead) infection. J. Am. Vet. Med. Assoc. 83:238-246.

Delappe, I. P. 1953a. Studies on Histomonas I. Use of antibiotics to facilitate in vitro isolation. Exp. Parasit. 2:79-86.

_____. 1953b. Studies on Histomonas II. Influence of age of original inoculum and pH on growth in various media. Exp. Parasit. 2:117-124.

_____. 1953c. Studies on Histomonas III. The influence of anaerobic versus aerobic environments on the growth of the organisms in vitro. Exp. Parasit. 2: 209.

_____. 1953d. Studies on Histomonas IV. A continuous automatic potentiometric method of measuring Eh of protozoan cultures. Exp. Parasit. 2:280.

Demke, D. D. 1954. A brief histology of the intestine of the turkey poult. Am. J. Vet. Res. 15:447-449.

Desowitz, R. S. 1950a. Protozoon Hyper parasitism of Heterakis gallinae. Nature. 165:1023-1024.

_____. 1950b. Enterohepatitis (Blackhead) of Turkeys. Trans. R. Soc. Trop. Med. Hyg. 44:2-3.

Desowitz, R. S. 1951. Age as a factor influencing fatal infection of Histomoniasis in chickens. J. Comp. Path. 61:231-236.

Devolt, H. M. 1950. The different effect of artificially and naturally induced Blackhead (Infectious Enterohepatitis) of turkeys on the prophylactic action of one quinolin derivative. Poult. Sci. 29:924-926.

_____, and Davis, C. R. 1936. Blackhead (Infectious Enterohepatitis) in turkeys with notes on other intestinal protozoa. Maryland Ag. Exp. Sta. Bull. 392:493-567.

_____, and Holst, A. P. 1948. Preliminary Report on the Preventive action of Vioform against Infectious Enterohepatitis (Blackhead) of turkeys. Poult. Sci. 27:356-358.

_____ _____. 1949. Comparative value of chlorohydroxy quinoline and vioform as Preventives of Blackhead (Infectious Enterohepatitis) of turkeys. Poult. Sci. 28:641-643.

Devolt, H. M., Trombe, F. C., and Holst, A. P. 1954. An investigation to determine whether immunity to Infectious Enterohepatitis (Blackhead) of turkeys develops during Enheptin treatment. Poult. Sci. 33:1256-1261.

Dickinson, E. M. 1930. Infectious Enterohepatitis in the pea fowl. J. Am. Vet. Med. Assoc. 76:567-568.

Dobell, C., and Laidlaw, P. P. 1926. On the cultivation of Entamoeba histolytica and some other entozoic amoebae. Parasit. 18:283.

Doyle, T. M. 1929. Infectious Enterohepatitis or Blackhead. of turkeys, J. Min. Ag. Lond. 36:349-352.

Drbohlav, J. J. 1924. The cultivation of the protozoon of Blackhead. J. Med. Res. 44:677-678.

Durant, H. J. 1930. Blackhead in turkeys--surgical control by cecal obligation. Mo. Ag. Exp. Sta. Bull. 123:1-32.

_____. 1937. Infectious Enterohepatitis. J. Am. Vet. Assoc. 90:596-602.

Enigk, K. 1935. The aetiology of Blackhead in Poultry. Arch. Wiss. Prakt. & Tierheilk. 69:410-438.

Eriksen, S. 1925. Blackhead in Chicks. Poult. Sci. 4: 250.

Eveleth, D. F. 1943. Histomoniasis in broilers. Vet. Med. 38:148-149.

Everett, R. W., Farr, M. M., McLoughlin, D. K. 1957. Chemotherapy of Blackhead in Poultry, 94th Annual Meet. Am. Vet. Med. Assoc. Cleveland, Ohio. 439-

Farmer, R. K. 1950. Infectious Enterohepatitis (Blackhead) in turkeys. A study of the prophylactic and therapeutic value of certain compounds. J. Comp. Path. Therap. 60:294-310.

_____, Huges, K. L., and Whiting, G. 1941. Infectious Enterohepatitis (Blackhead) in turkeys. A study of the pathology of the artificially induced disease. J. Comp. Path. Therap. 61:251-262.

_____, Stephenson, J. 1949. Infectious Enterohepatitis (Blackhead) in turkeys. A comparative study of methods of infection. J. Comp. Path. Therap. 59: 119-126.

Farr, M. M. 1956. Survival of the Protozoan parasite Histomonas meleagridis in feces of infected birds. Cornell Vet. 46:178-187.

_____. 1961. Further observations on survival of the protozoan parasite Histomonas meleagridis and eggs of poultry nematode in feces of infected birds. Cornell Vet. 51(1):3-13.

Frank, J. F. 1953. A note on the experimental transmission of Enterohepatitis of turkeys by arthropods. Canad. J. Comp. Med. 17:230-231.

Giudice, V. D. 1955. On certain histopathological aspects of Blackhead (Enterohepatitis in Poultry) (In Ital. with English summary) Soc. Ital. Delle. Sci. Vet. Atti. 78:488-490.

Graybill, H. W. 1921. The incidence of Blackhead and occurance of Heterakis papillosa in a flock of artificially reared turkeys. J. Exp. Med. 33:667.

_____. 1925. Blackhead and other causes of loss of turkeys in California. Univ. Calif. Ag. Exp. Sta. Circ. 291:1-14.

Graybill, H. W., and Smith, T. 1920. Production of fatal Blackhead in turkeys by feeding Embryonated eggs of Heterakis papillosa. J. Exp. Med. 31:647-655.

Grumble, L. C., Boney, W. A., and Turk, R. D. 1952a. Chemotherapy of Enterohepatitis of turkeys I. The value of 2-amino-5-nitro thiazole in prevention and treatment. Am. J. Vet. Res. 13:383-385.

_____, _____ _____ _____. 1952b. Chemotherapy of Enterohepatitis of turkeys II. The effect of 2-amino-5-nitro thiazole and 2-Acetylamino nitro-thiazole on egg production, fertility and hatchability in turkey hens. Ibid. 386-387.

_____, _____ _____ _____. 1952c. Chemotherapy of Enterohepatitis of turkeys III. Comparative value of 2-amino-5-nitro thiazole and 2-actylamino nitro-thiazole in prevention and treatment. Ibid., 572-574.

Hadley, P. B. 1916. The role of the flagellated Protozoa in Infective processes of the intestine and liver. Ag. Exp. Sta. R. I. State Col. Bull: 166.

_____, and Amison, K. 1911. Further studies on Black-head in turkeys. Zentralbl. f. Bakt. I. Orig. 58: 34.

Hall, M. C., and Shillinger, J. E. 1923. The removal of Heterakids from the ceca of chickens by rectal injection of anthelmintic. J. Am. Vet. Med. Assoc. 62:623.

Hall, W. J., and Wehr, E. E. 1953. Diseases and Parasites of Poultry. U. S. Dept. Ag. Farmer Bull. 1652:1-91.

Harrison, A. P., Jr. 1952. Bacteria as secondary invaders in Blackhead lesions of turkeys. Thesis. Univ. of Maryland Graduate School.

_____, Hansen, P. A., Devolt, H. M., Holst, A. P. and Trumbo, F. G. 1954. Studies on the pathogenesis of Infectious Enterohepatitis (Blackhead) of turkeys. Poult. Sci. 33:84-93.

_____, A., and Hansen, R. 1955. Characterization of the Lactobacteriaceae encountered as secondary invaders in Blackhead liver lesions of turkeys. Biol. Abs. 29(9):22070, p. 2202.

Harwood, P. D. 1954. Current research in animal Parasitology. Proc. Anim. Health Inst. 14th Ann. Meet. 61.

_____, and Stunz, D. I. 1954. Efficacy of Furazolidone, a new Nitrofuran against Blackhead and Coccidiosis. J. Parasit. (suppl.) 40:24-25.

Higgins, C. H. 1915. Enterohepatitis or Blackhead in turkeys. Canad. Dept. Ag. Health Anim. Branch Bull. 17.

Hinshaw, W. R. 1937. Diseases of turkeys, Univ. Calif. Ag. Exp. Sta. Bull. 613.

Horton-Smith, C. 1957. Histomoniasis (Blackhead) in Poultry. Ag. Res. vol. 2:30.

_____, and Long, P. L. 1955. The infection of chickens (gallus gallus) with suspension of Blackhead organism Histomones meleagridis. Vet. Rec. 67:478.

_____ _____. 1956a. Studies in Histomoniasis I. The infection of chickens (Gallus gallus) with histomonal suspensions. Parasit. 46:79-90.

_____ _____. 1956b. Furzalidone in the control of Histomoniasis (Blackhead) in turkeys. J. Comp. Path. Therap. 66:22-34.

_____ _____. 1956c. Further studies on the chemotherapy of Histomoniasis (Blackhead) in turkeys. Ibid. 378-388.

_____ _____. 1957. The effect of 2-amino-5-nitrothiazole, 2 acetylamino-5-nitrothiazole and furazolidone on the growth in vitro of Histomones meleagridis. Ann. of Trop. Med. Parasit. 51:117.

Hunt, S. 1955. Blackhead--Infectious Enterohepatitis. Red. Comb. Poult. J. 2(12):10.

Jerstad, A. C. 1957. Furazolidone for Infectious Enterohepatitis (Blackhead) of turkeys. Am. J. Vet. Res. 18:174-179.

Johnston, E., Andrews, F. N., and Schrewsbury, C. T. 1943. The preparation of musculer tissues for histological study. J. Anim. Sci. 2:244-250.

Johnson, E. P., and Lange, C. J. 1939. Blood alterations in typhlo hepatitis of turkeys with notes on the disease. J. Parasit. 25:157.

Jowett, W. 1911. Blackhead, Enterohepatitis or Typhlo hepatitis. A disease of young turkeys. J. Comp. Path. Therap. 24:289.

Joyner, L. P., and Kendall, S. B. 1955. The use of 2-amino-5-nitrothiazole in the control of Histomoniasis. Vet. Rec. 67:180-183.

Jungherr, E. L., and Winn, J. D. 1950. Field Experiments with Enheptin-T on the control of Histomoniasis (Blackhead) in turkeys. Poult. Sci. 29:462-465.

Kaupp, B. F. 1922. Poultry Diseases. 3rd Ed. Alexander Eger, Chicago.

Kendall, S. B. 1957. Some factors influencing resistence to Histomoniasis in turkeys. Brit. Vet. J. 113: 435-439.

_____. 1959. The occurance of Histomonas meleagridis in Heterakis gallinae. Parasit. 49:169-172.

Kuprowski, M. 1950. Entero-Hepatitis Infectiosa u Indykow na Terenii wedj Wroclawskugo. Med. Waterynary Jna. 6:461-463.

_____. 1955. Histopathologic studies on Infectious Enterohepatitis in turkeys (In Polish with English summary) Roez. Nank. Roln. Ser. E., Water. 67:69.

Lesser, E. 1960a. Cultivation of Histomonas meleagridis in a modified tissue culture medium. J. Parasit. 46(6):686.

_____. 1960b. Replacement of serum in the cultivation of Histomonas meleagridis. J. Parasit. 46(2):271.

Levine, P. P. 1947. Histomonas in a kidney of a turkey. Cornell Vet. 37:269-270.

Levine, N. D. 1961. Protozoan parasites of Domestic animals and of man. Burgess Pub. Co. Minn. pp. 74-81.

Lindquist, W. D. 1961. Some effect of Paramomycin Sulphate on Blackhead in turkeys. (Personal Communication).

Lucas, J. M. S. 1961. 1-2 dimethyl 5-nitroimidazole 8595 R. P. Part I. Prophylactic activity against experimental Histomoniasis in turkeys. Vet. Rec. 73(19): 465-467.

Lund, E. E. 1955. The progress of Histomoniasis (Blackhead) in turkeys as related to the size of the infective dose. Poult. Sci. 34:127.

_____. 1956a. Oral transmission of Histomonas in turkeys. Poult. Sci. 35:900-904.

_____. 1956b. Blackhead of turkeys and chickens. U. S. Dept. Ag. Yearbook of Agriculture. p. 441.

_____. 1956c. Blackhead of turkeys and chickens. How to control it. U. S. Dept. Ag. Leaflet No. 404.

_____. 1958a. Growth and development of Heterakis gallinae in turkeys and chickens infected with Histomonas meleagridis. J. Parasit. 44:297-301.

_____. 1958b. War on Blackhead still to be won. Turkey World 33 (May):12.

_____. 1959. Immunizing action of a non-pathogenic strain of Histomonas against Blackhead in turkey. J. Protozool. 6:182-185.

_____. 1960. Factors influencing the survival of Heterakis and Histomonas on soil. J. Parasit. 46 (5):38.

_____, and Burtner, R. H. 1957. Infectivity of Heterakis gallinae eggs with Histomonas meleagridis. Exp. Parasit. 6(2):189-193.

_____ _____. 1958. Effect of four embryonation media on the embryonation and infectivity to chickens of Histomonas bearing eggs of Heterakis. J. Parasit. 44:197-200.

Malewitz, T. D., and Calhoun, M. L. 1958. The Gross and Microscopic Anatomy of the Digestive tract, spleen, kidney, lungs and heart of the turkey. Poult. Sci. 37:388-398.

_____, Runnells, R. A., and Calhoun, M. L. 1958. The pathology of experimentally produced Histomoniasis in turkeys. Am. J. Vet. Res. 19:181-185.

McCulloch, E. C., and Nicholson, L. C. 1941. Mapharsen therapy in Enterohepatitis of turkeys. Vet. Med. 36:574-576.

McGregor, J. K. 1949. Observations on the prophylactic value of certain drugs for Enterohepatitis infection (Blackhead) in turkeys. Canad. J. Comp. Med. 13: 257-261.

_____. 1951. 2-amino-5-nitrothiazole in the Control of Enterohepatitis (Blackhead) in turkeys. J. Am. Vet. Med. Assoc. 118:394.

_____. 1953a. Further observations on the use of 2-amino-5-nitrothiazole as a prophylactic and therapeutic agent in the control of Blackhead (Enterohepatitis) in turkeys. Canad. J. Comp. Med. 17: 267-270.

_____. 1953b. Preliminary observations on the use of certain nitrofuran compounds in the control of Enterohepatitis (Blackhead) in turkeys. J. Am. Vet. Med. Assoc. 122:312-314.

_____. 1954a. Further observations on the control of Blackhead with Nitrofuran compounds. Canad. J. Comp. Med. 18:397-400.

_____. 1954b. Observations on the therapuetic value of Adreno corticotropic hormone in clinical Enterohepatitis (Blackhead) in turkeys. Canad. J. Comp. Med. 18:332.

McGuire, W. C. 1955. Blood induced Blackhead. J. Parasit. 41 (No. 6, Sec. 2):14.

_____., and Covett, J. W. 1952. Blood studies in Histomoniasis. Poult. Sci. 31:610-617.

_____, and Morehouse, N. F. 1952. Chemotherapy studies on Histomoniasis. Poult. Sci. 31:603-609.

_____ _____. 1958. Blood induced Blackhead. J. Parasit. 44:292-296.

McKay, F., and Morehouse, N. F. 1948. Studies on experimental Blackhead infection in turkeys. J. Parasit. 34:137-141.

Menzani, C. 1933. Osservazioni e ricerche su l'enteroepatite infettiva dei tacchini. La-clinica Vet. 56:508.

Moore, E. N. 1954. Diseases of turkeys. Vet. Med. 49: 314.

_____, Chamberlin, V. D., and Carter, R. D. 1954. The effect of Histomoniostatic agents upon the reproductive ability of turkeys. Poult. Sci. 33:1072.

Moore, V. A. 1896. The direct transmission of Infectious Enterohepatitis in turkeys. U. S. Dept. Ag. Bur. Anim. Ind. Circ. 5:1.

Morehouse, N. F. 1948. Experiments on the use of Quinoline Compounds for the prevention of Blackhead in turkeys. J. Parasit. 34(2):18.

_____. 1956. Blackhead still kills too many turkeys. Turkey World. 31(5):18.

_____, and McGuire, W. C. 1950. Experiments on the chemotherapy of Blackhead in turkeys. Proc. Iowa. Acad. Sci. 57:475-482.

Morgan, B. B., and Hawkins, P. A. 1952. Veterinary Protozoology. Rev. Ed. Burgess Publ. Co. Minn. pp. 93-97.

Noland, L. E. 1928. A combined fixative and stain for demonstrating flagella and cilia in temporary mounts. Science, 67:535.

Niimi, D. 1936. Studies on Blackhead I. Morphology development and pathogenicity of causal agent in body of host. J. Jap. Soc. Vet. Sci. 15:15.

_____. 1937. Studies on Blackhead II. Mode of infection. J. Jap. Soc. Vet. Sci. 16:183-239.

Pate soeast, D. D. 1955. Enterohepatitis (Blackhead). Vet. 7:24.

Pernot, E. F. 1907. Disease of turkeys. Oregon Ag. Exp. Sta. Bull. 95:1-8.

Picard, W. K. 1929. Bleackhead bij Kuikens (Ned. Indie Voorkomende Pluim VeeZiekten) Nederl-Indisch. Blad. Diergeneesk. 41:449-456.

Pomeroy, B. S. 1955. Management for Blackhead control. Poult. Dig. 14 (163):548.

Pullin, J. W. 1955. Observations on the use of cortisone in experimental Enterohepatitis in turkeys. Canad. J. Comp. Med. 19:67.

Rettger, L. F., and Kirkpatrick, W. F. 1927. An epidemiological study of Blackhead in turkeys. Storrs. Ag. Exp. Sta. Bull. 148:285-313.

Santos, J. A. 1944. Des-Entero-Hepatitis dos. Perus. Biol. Min. Ag. (Rio de Janeiro) 33:123-126.

Sautter, J. H., Pomeroy, B. S., and Roepke, M. H. 1950a. Histomoniasis (Enterohepatitis) in turkeys I. A procedure for the screening and testing of drugs. Am. J. Vet. Res. 11:115-129.

_____, _____ _____. 1950b. II. Chemotherapy of experimental Histomoniasis (Enterohepatitis) of turkeys. J. Am. Vet. Med. Assoc. 116:436-439.

Schlotthauer, C. E., and Essex, H. E. 1931. Control of Enterhepatitis in turkeys. Cornell Vet. 21:252-255.

_____, Mann, F. C., and Essex, H. E. 1944. Blackhead in turkeys: Notes on its occurance and its transmission. North Am. Vet. 25:603-608.

Shope, R. E. 1948. An unfamiliar mechanism for disease transmission. Proc. Am. Phil. Soc. 92:289-293.

Smith, T. 1895. An infectious disease among turkeys c caused by Protozoa (Infectious Enterohepatitis). U. S. Dept. Ag. Bur. Anim. Ind. Bull. 8:1-38.

_____. 1910. Amoeba meleagridis. Science. 32:509.

_____. 1915. Further investigations into the etiology of the Protozoan disease of turkeys known as Blackhead, Enterohepatitis, Typhlitis, etc. J. Med. Res. 33:243-270.

_____. 1917. Some field experiments bearing on the transmission of Blackhead in turkeys. J. Exp. Med. 25:405.

_____., and Graybill, H. W. 1920a. Epidemiology of Blackhead in turkeys under approximately natural conditions. J. Exp. Med. 31:633-645.

_____ _____. 1920b. Blackhead in chickens and its experimental production by feeding embryonated eggs of Heterakis papillosa. J. Exp. Med. 32:143-152.

Smith, T., and Smillie, R. W. 1917. Notes on Coccidia in sparrows and their relation to Blackhead in turkeys. J. Exp. Med. 25:415.

Starr, L. E. 1930. Blackhead in Turkeys. J. Am. Vet. Med. Assoc. 76:81-84.

Stephenson, J., Hughes, D. L. 1954. Observations on the Epizootiology of Enterohepatitis (Blackhead) in turkeys. World Poult. Cong. Rpt. Proc. 10 Sec. C:282.

Swales, W. E. 1948. Enterohepatitis (Blackhead) in turkeys II. Observation on transmission by the cecal worm (Heterakis gallinae). Canad. J. Comp. Med. 12:97-100.

_____. 1950a. Enterohepatitis (Blackhead) in turkeys. V. Further Experiment on chemotherapy. Canad. J. Comp. Med. 14:118-125.

_____. 1950b. Enterohepatitis (Blackhead) in turkeys VII. Experiments on transmission of the disease. Canad. J. Comp. Med. 14:298.

_____. 1952a. Enterohepatitis (Blackhead) in turkeys VIII. Further observations on the uses and mode of action of 2-amino-5-nitrothiazole. Canad. J. Comp. Med. 16:57-62.

_____. 1952b. Enterohepatitis (Blackhead) in turkeys IX. Miscellaneous tests of chemicals for possible therapeutic or prophylactic value. Canad. J. Comp. Med. 16:63-65.

_____, and Frank, J. F. 1948. Enterohepatitis (Blackhead) in turkeys III. Observation in the susceptibility of young poults. IV. Trails with chemotherapy. Canad. J. Comp. Med. 12:141-143.

Theobald, F. V. 1907. Parasitic liver disease in Poultry. Nat. Poult. Conf. Reading. Off. Rep. 2, p. 181.

Trombs, F. G. 1955. Introductory study of the effect of Enheptin (2 amino, 5 nitrothiazole) on the host parasite relationship in Blackhead. Md. U. Grad. Sch. Abs. Diss. 1952-54:91.

Tyzzer, E. E. 1919. Developmental phases of the protozoan of Blackhead in turkeys. J. Med. Res. 40:1-30.

Tyzzer, E. E. 1920a. Further studies on Blackhead in turkeys with special reference to transmission by inoculation. J. Inf. Dis. 27:207-239.

_____. 1920b. Observations on the transmission of Blackhead in turkeys. J. Med. Res. 41:219-237.

_____. 1920c. The flagellate character and reclassification of the parasite producing Blackhead in turkeys. Histomonas (gen. nov.) meleagridis (Smith) J. Parasit. 6:124-131.

_____. 1921. Further observations on Blackhead in turkeys. J. Inf. Dis. 29:258-286.

_____. 1922. A further inquiry into source of virus in Blackhead of turkeys together with observations on the administration of Epecac and sulphur. J. Exp. Med. 35(6):791-812.

_____. 1923. Arsenical Compounds in the treatment of Blackhead in turkeys. J. Exp. Med. 37:851-873.

_____. 1924. The chicken as a carrier of Histomonas meleagridis (Blackhead): The protozoan in its flagellated stage. J. Med. Res. 44:676.

_____. 1926. Heterakis vesicularis--Frolich 1791: A vector of an infectious disease. Proc. Soc. Exp. Biol. and Med. 23:708-709.

_____. 1927. Enterohepatitis in turkeys and its transmission through the agency of Heterakis vesicularis. Proc. Third. World Poult. Cong., p. 286.

_____. 1932. Problems and observations concerning the transmission of Blackhead infection in turkeys. Am. Phil. Soc. Proc. 71:407.

_____. 1934. Studies on Histomoniasis or Blackhead infection in the chicken and the turkey. Proc. Am. Acad. Arts & Sci. 69:189-264.

_____., and Collier, J. 1925. Induced and natural transmission of Blackhead in the absence of Heterakis. J. Inf. Dis. 37:265-276.

_____., and Fabyan, M. 1920. Further studies on Blackhead in turkeys with special reference to transmission by inoculation. J. Inf. Dis. 29:268.

Tyzzer, E. E., Fabyan, M., and Foot, N. C. 1921. Further observations on Blackhead in turkeys. J. Inf. Dis. 29:268.

U. S. Dept. of Agriculture. 1954. Losses in Agriculture. A Preliminary appraisal for review. U. S. D. A. Ag. Res. Serv. ARS-20-1, pp. 190.

Van Es, L., and Olney, J. F. 1941. Poultry diseases and Parasites. Nebr. Ag. Exp. Sta. Bull. 332:1-90.

Van Ness, C., and Hamilton, C. M. 1944. The use of Phenothiazine in the control of Enterohepatitis in turkeys. 54th Ann. Rep. Ag. Exp. Sta. State Col. of Wash. Bull. 455:152.

Wageforth, H. M., and Wageforth, P. 1921. Observation on the effect of Ipecac in the treatment of Infectious Enterohepatitis (Blackhead in turkeys) J. Pharm. Exp. Ther. 17:249.

Wales, N. S. 1956. Enterohepatitis or Blackhead in Turkeys. New South Wales, Dept. Ag. Div. Anim. Ind. Poult. Dis. Leaflet 18:p. 5.

Waletzky, E., Brandt, M. C., Blizniek, A., and Hughes, C. O. 1949. Some new chemotherapeutic agents in experimental Enterohepatitis (Blackhead) of turkeys. J. Parasit. 35 (Suppl.):16.

_____., Clark, J. H. and Marson, H. W. 1950. New chemotherapeutic agents in Enterohepatitis (Blackhead) of turkeys. Science. 111:720-721.

Walker, R. V. L. 1948. Enterohepatitis (Blackhead) in turkeys. I. Pentatrichomonas associated with Enterohepatitis and its propagation in developing chick embryo. Canad. J. Comp. Med. 12:43.

Weaver, C. H. 1930. Turkey rearing in confinement for the control of Blackhead (Enterohepatitis). Rpt. Dept. Ag. Canada. pp. 50-53.

Wehr, E. E., Farr, M. M., and Mcloughlin, D. K. 1958. Chemotherapy of Blackhead in Poultry. J. Am. Vet. Med. Assoc. 132:439-445.

_____, and Olivier, L. C. 1946. Limitations of phenothiazine in the control of cecal worms and Blackhead disease of turkeys. Poult. Sci. 25:199-203.

Welter, C. J. 1960. The effect of various stress upon Histomoniasis in chickens and turkeys. Poult. Sci. 39(2):361.

Welter, C. J., and Clark, D. T. 1961. The efficacy of pureidobenzenearsonic acid as a preventative of Histomoniasis in turkey poults. Poult. Sci. 40(1): 144-147.

Wenrich, D. H. 1943. Observations on the morphology of Histomonas (Protozoa-Mastigophora) from pheasants and chickens. J. Morph. 72:279-303.

Wetzel, R., and Knigk, K. 1939. Zur Aetiologie der Bluiddarm-Leberentzundung der Hühnervogel (Blackhead); 13 Internat. Vet. Cong. (Zurich-Interlaken, 1938): 2. p. 856.

APPENDIX

Fig. 1. Poult No. 627 showing enlarged ceca.

Fig. 2. Poult No. 634 showing 2-3 times enlarged ceca and abscesses in the liver.

Fig. 1a. Poult No. 627 showing some of the typical symptoms.

Fig. 3. Ceca. H. & E. x 500. Nests of protozoa.

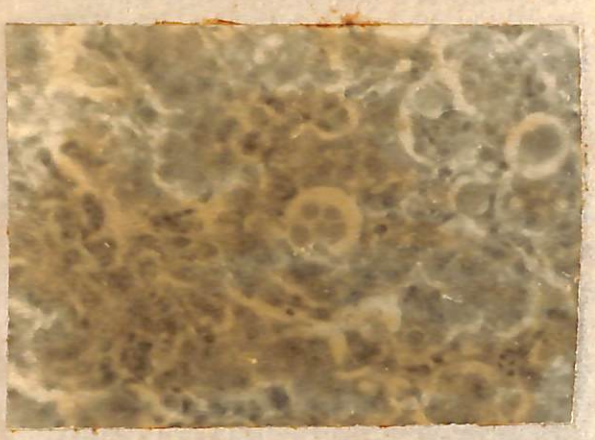

Fig. 4. Liver. H. & E. x 500. Nests of protozoa in the area of necrosis.

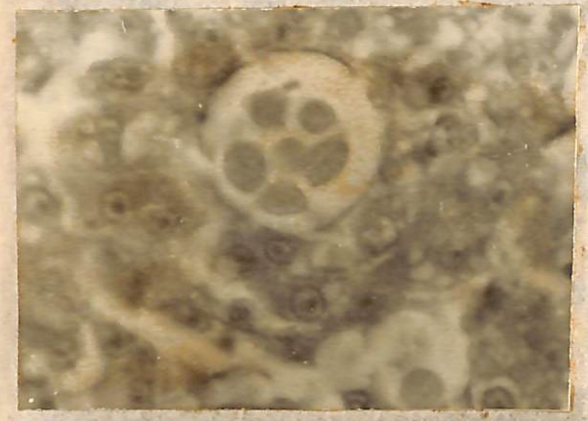

Fig. 5. Liver. H. & E. x 1200. Enlargement of a portion of Figure 4 showing nest containing six protozoa in a necrotic area.

Fig. 6. Liver. H. & E. x 1200. Notice Histomonas in the blood vessel.

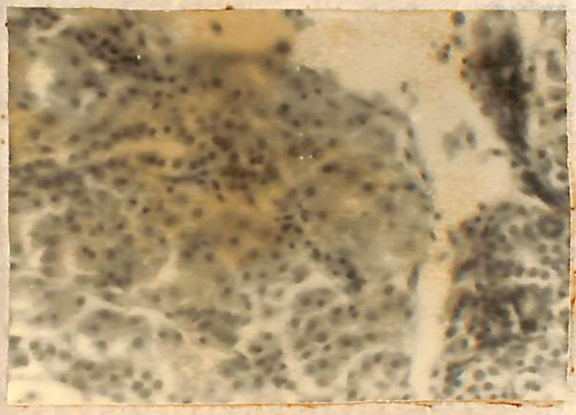

Fig. 7. Kidney. H. & E. x 500. Protozoa in blood vessel. (Top, right).

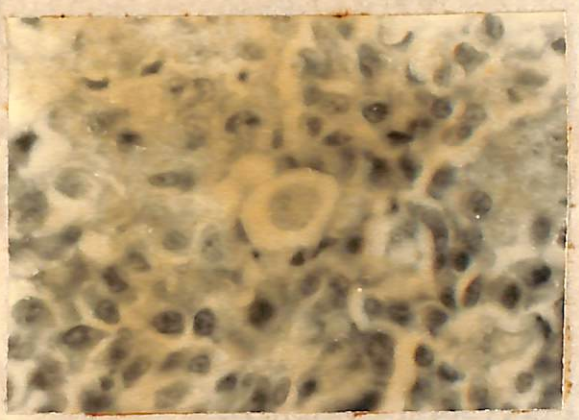

Fig. 8. Spleen. H. & E. x 1200. Protozoa in the tissue.

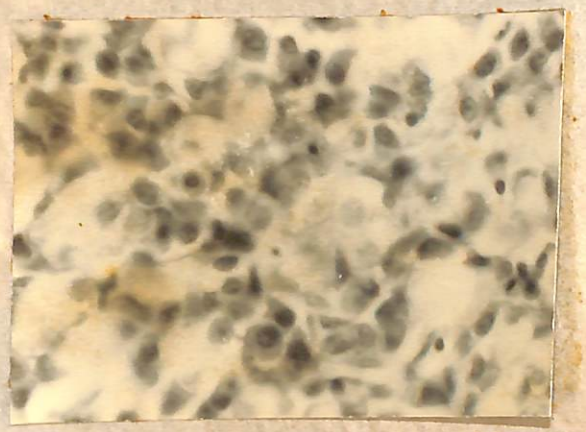

Fig. 9. Spleen. H. & E. x 1200. Protozoa with reactive cells.

Fig. 10. Spleen. H. & E. x 1200. Nests of protozoa in the tissue.

Fig. 11. Ceca. H. & E. x 500. Oedema of villus tip.

Fig. 12. Ceca. H. & E. x 125. Lamination of the contents in the lumen.

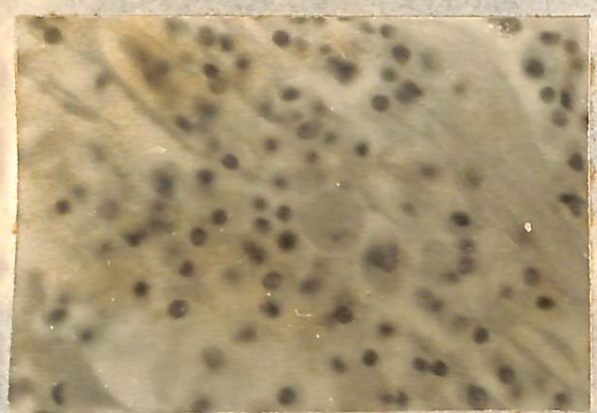

Fig. 13. Ceca. H. & E. x 1200. Protozoa in the muscle layer with reactive cells.

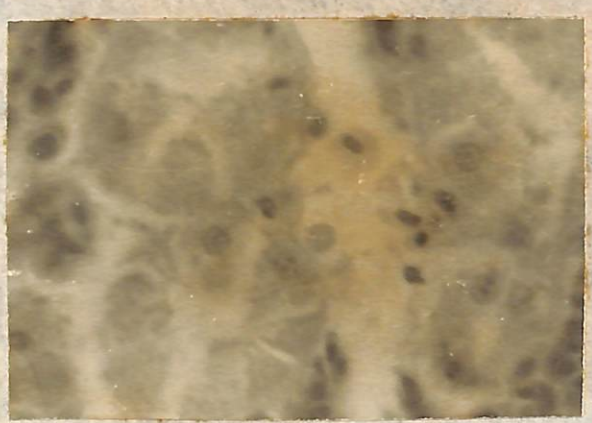

Fig. 14. Kidney. H. & E. x 1200. Notice protozoa (center) in the necrotic areas of the kidney tubules.

Fig. 15. Poult No. 611 showing drowsiness, weekness, and drooped wings.

Fig. 16. Ceca. H. & E. x 500. Notice swelling of the villi and fibrinous exudate.

Fig. 17. Poult No. 612 showing enlarged ceca and necrotic foci in the liver.

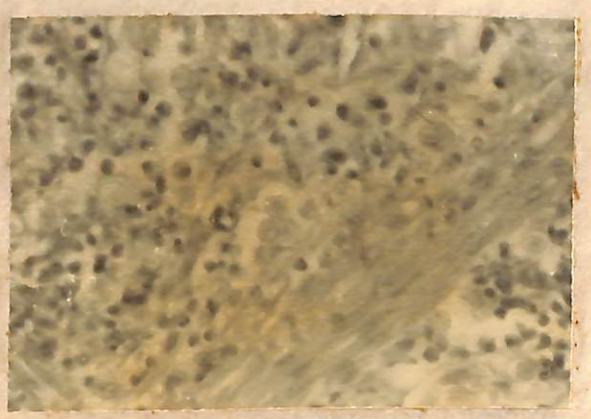

Fig. 18. Ceca. H. & E. x 500. Muscle layer showing necrosis, nests of protozoa, and reactive cells.

Fig. 19. Liver. H. & E. x 125. Necrosis of the hepatic triad.

Fig. 20. Poult No. 625 showing greatly enlarged ceca and necrotic foci in the liver.

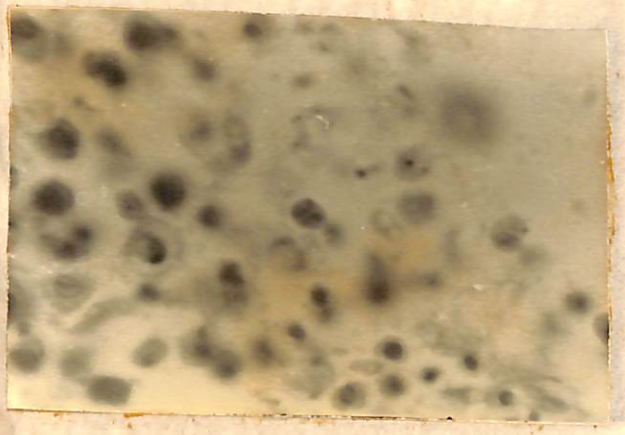

Fig. 21. Ceca. H. & E. x 1200. Protozoa in the lumen of the ceca with dead tissue and inflammatory cells.

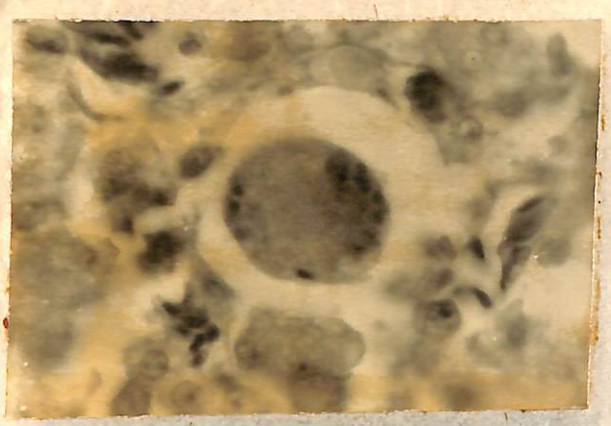

Fig. 22. Liver. H. & E. x 1200. Large protozoa containing many granules within a clear area of lysed tissue.

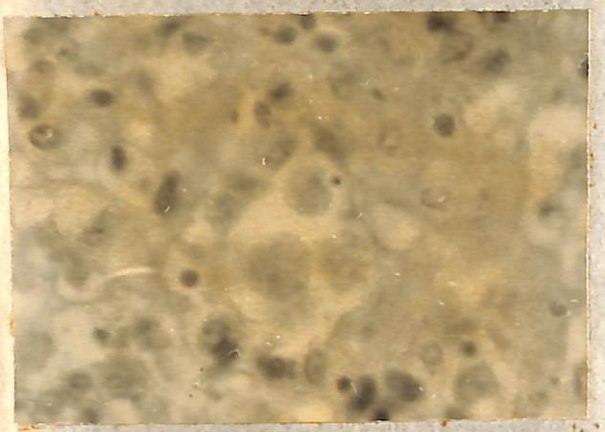

Fig. 23. Liver. H. & E. x 1200. Nests of protozoa and necrosed liver cells.

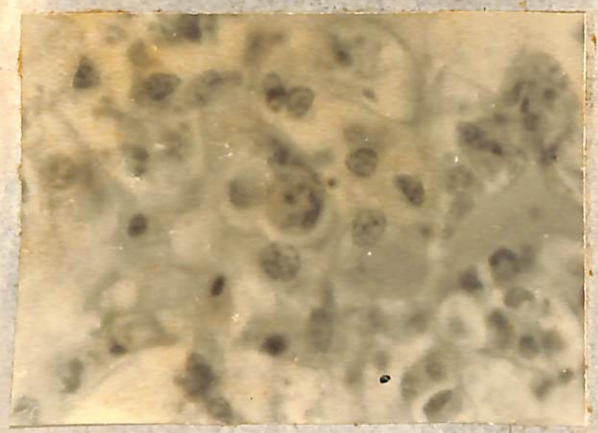

Fig. 24. Liver. H. & E. x 1200. Endothelial cells with protozoa.

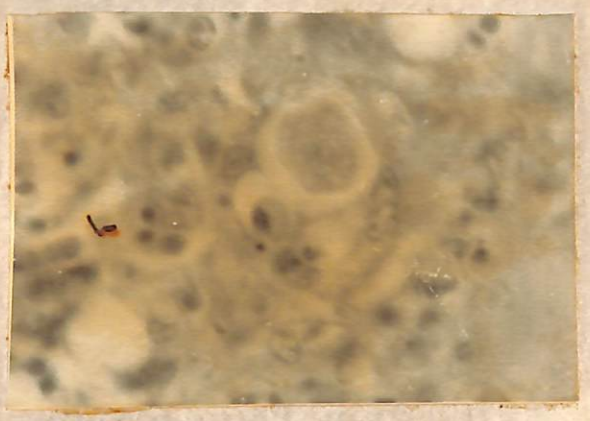

Fig. 25. Liver. H. & E. x 1200. "Vegetative phase" of protozoa.

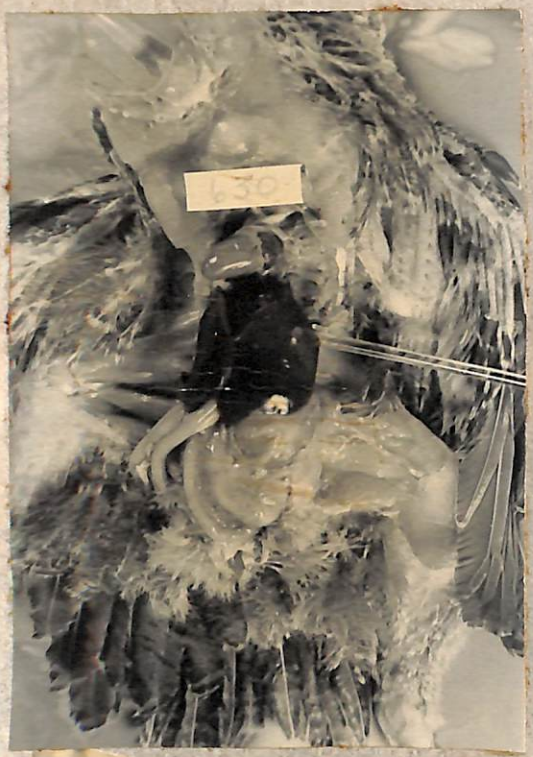

Fig. 26. Poult No. 630 showing enlarged ceca and a few necrotic foci in the liver.

Fig. 27. Ceca. H. & E. x 1200. Hyperplasia of lymphoid cells in the mucous layer.

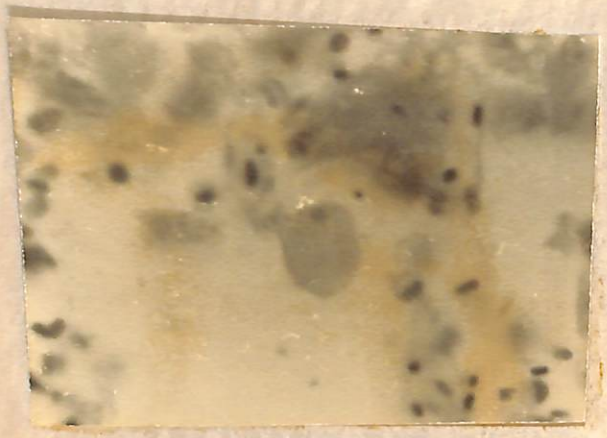

Fig. 28. Liver. H. & E. x 1200. Notice large protozoa.

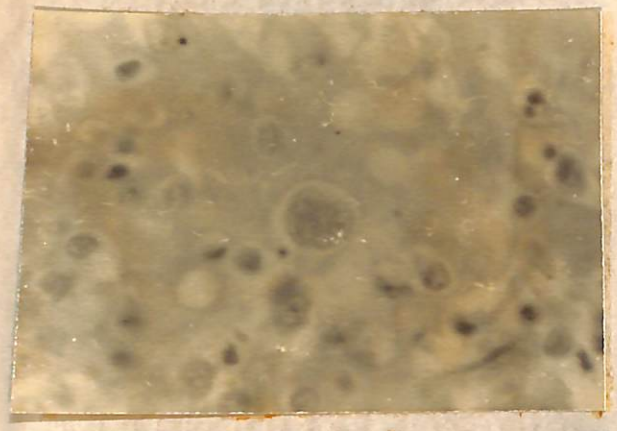

Fig. 29. Liver. H. & E. x 1200. Protozoa in a lysed area.

Fig. 30. Liver. H. & E. x 1200. Showing necrosis with protozoa and reactive cells.

Fig. 31. Pectoral muscle. H. & E. x 44. Showing a mass of necrosis with proteinacious fluid.

Fig. 32. Pectoral muscle. H. & E. x 125. A mass of necrosis with giant cells and oedematous exudate.

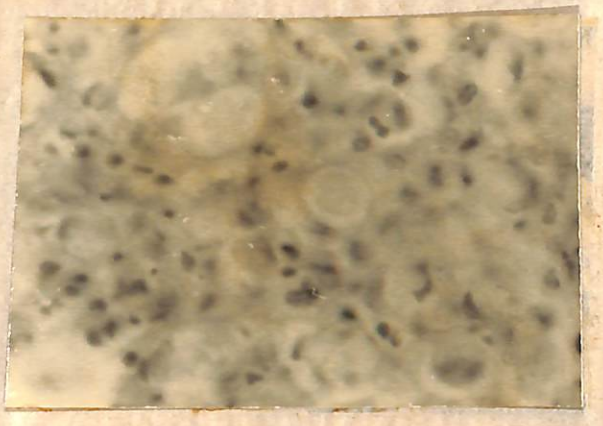

Fig. 33. Pectoral muscle. H. & E. x 1200. An enlargement of a part of Fig. 32, showing protozoa with reactive cells.

Fig. 34. Liver. H. & E. x 125. Necrosis.

Fig. 35. Liver. H. & E. x 1200. An enlargement of a part of Fig. 34, showing protozoa.

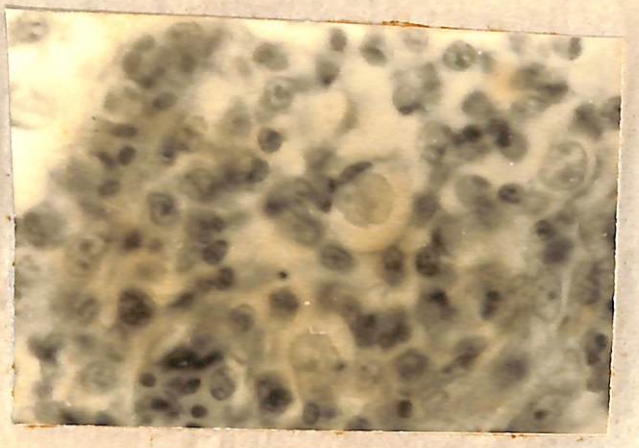

Fig. 36. Liver. H. & E. x 1200. Protozoa with reactive cells.

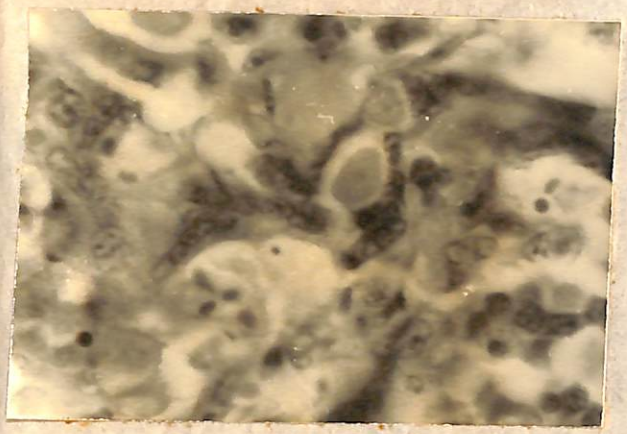

Fig. 37. Liver. H. & E. x 1200. Protozoa in nests.

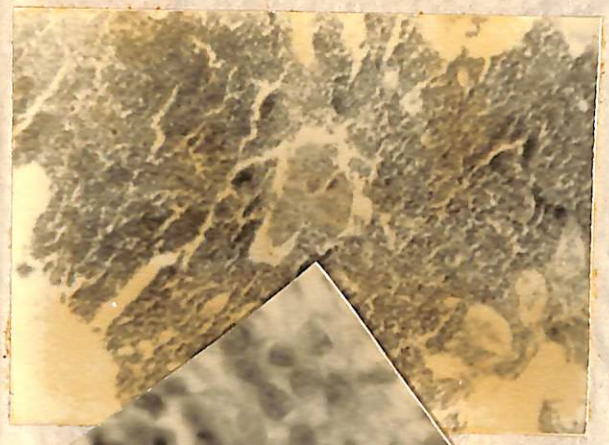

Fig. 38. Lung. H. & E. x 125. Necrosis and haemorrhage.

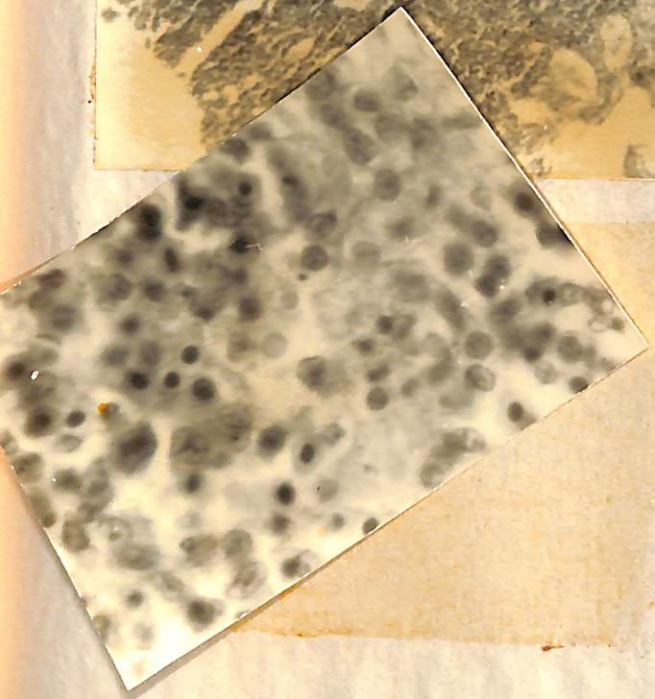

Fig. 39. Lung. H. & E. x 1200. An enlargement of a part of Fig. 38, showing protozoa (center) with reactive cells.

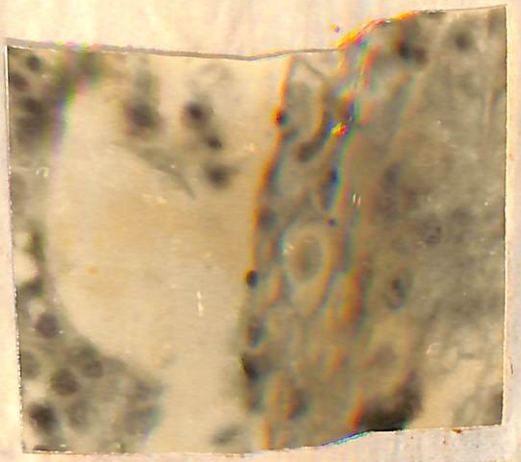

Fig. 40. Lung. H. & E. x 1200. Protozoa near the blood vessel.

Fig. 41. Liver. H. & E. x 125. Showing necrosis and haemorrhage.

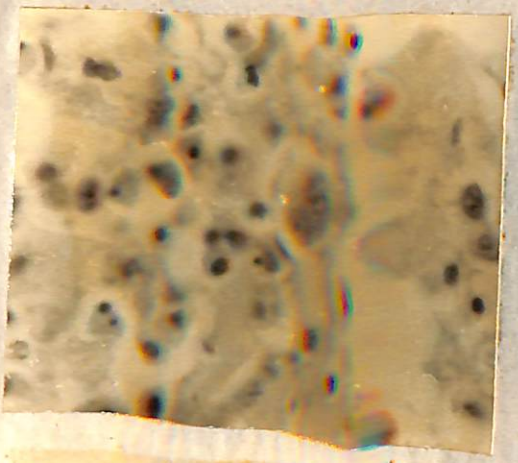

Fig. 42. Liver. H. & E. x 1200. An enlargement of a part of Fig. 41, showing protozoa with reactive cells.

Fig. 43. Spleen. H. & E. x 1200. Showing a prominent end artery (lower right).

Fig. 44. Spleen. H. & E. x 1200. Notice engulfed protozoae (center) with reactive cells.

Fig. 45. Cecum. H. & E. x 500. Oedema of villus with reactive cells.

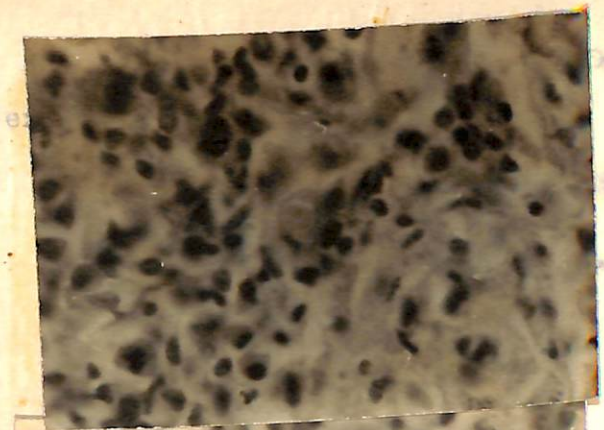

Fig. 44. Spleen. H. & E. x 1200. Notice engulfed protozoa (center) with reactive cells.

Fig. 45. Cecum. H. & E. x 500. Oedema of villus with reactive cells.

The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

A STUDY ON THE TRANSMISSION OF "HISTOMONAS MELEAGRIDIS" TO POULTS. (MELEAGRIS GALLOPAVO)

presented by J. S. AHLUWALIA

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is worthy of acceptance.

Harold C. McDougle, D. V. M.
Professor in Veterinary Bacteriology
and Parasitology

Donald C. Blenden, D. V. M.
Assistant Professor in Veterinary Bacteriology
and Parasitology