STUDIES ON EXPERIMENTAL HISTOMONIASIS IN TURKEYS

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CHAPTER I

INTRODUCTION

Since the discovery that histomoniasis or blackhead in turkeys could be produced by the ingestion of embryonated Heterakis gallinae eggs, investigators have successfully initiated experimental infections by the administration of these eggs. Demonstration of the presence of the causative organism, Histomonas meleagridis, in the larvae and the eggs of the nematode has not been as successful. Even though some positive results have been reported, because of the highly pleomorphic nature of the protozoa in the nematode tissues, acceptance of these findings has not been unanimous. If the causative agent of blackhead is in the nematode eggs, as is generally accepted, it should be possible to infect turkeys by administering protozoa that have been freed from the larvae unless there are non-infective developmental stages of the protozoan within the larvae. In order to determine if such infections could be induced, Heterakis gallinae eggs were broken up in a tissue grinder to release the protozoa from the larvae or the eggs. Therefore, one of the purposes of this research was to

see what the infectivity of ground embryonated and ground unembryonated Heterakis gallinae eggs would be for turkeys. This was followed by observing macroscopic and microscopic pathological changes, as well as studying the effect of the inocula on the serum protein picture of the birds by using cellulose acetate electrophoresis.

Because very little research has been done to determine the nature of the resistance to histomoniasis it was also desired to observe the effects of different intensities of infection on the serum protein pattern of birds by inoculating them with different numbers of Heterakis gallinae eggs. By using an immunizing infection, an attempt was made to establish resistance to further infections. Challenge inoculations of infective material were administered to the turkeys and the effects on the liver and caeca as well as on the serum proteins were studied.

CHAPTER II

REVIEW OF THE LITERATURE

Histomoniasis, or blackhead, in turkeys in an infectious disease resulting in a high number of mortalities. Its economic importance is and has been significant. As early as 1894 blackhead threatened to destroy the turkey industry in the United States (Moore, 1896). Doyle, Cable and Moses (1947) reported a similar situation existing in the mid 1940's.

In addition to its occurrence in the United States, the existence of blackhead has also been reported in Poland (Kuprowski, 1950); Japan (Nimi, 1930); South Africa (Martingala, 1930); Australia (Harwood, 1953); the Netherlands (Van Heelsberger, 1923); France (Viege, 1923); India (Craig, 1932); Germany (Enigk, 1935); and Italy (Del Guidice, 1954).

Although it is not known how long histomoniasis existed in the United States prior to 1893, it was not until this time that the disease was described (Cushman, 1893). Smith (1895) was the first investigator to study the nature of blackhead. He named the disease "infectious enterohepatitis." Its cause was attributed to Amoeba meleagridis. The amoeba, as studied from sections of liver, varied in

size from six to ten microns in diameter and possessed a nucleus two microns in diameter. Later studies by Smith (1910) of parasites from the liver showed the presence of pseudopodia and organisms both singly and in groups. The latter observation suggested that reproduction of the organisms took place either by simple division or by multiple agamic division.

Controversy as to the true nature of the organism causing blackhead started when Cole, Hadley, and Kirkpatrick (1910) stated that the organisms described by Smith were, in actuality, schizogonous stages or coccidia. Hadley and Amison (1911) and Hadley (1916 a, b) stated that a trichomonad was the causative organism. They advocated that a trichomonad which usually lives a harmless existence in the gut may, under certain circumstances, become pathogenic, ". . .having experienced its first taste of blood its whole nature is changed; it becomes an animal raging through the tissues. . . ." Hadley (1916, b) also contended that infection of the liver could not be considered to be an infectious disease, since the trichomonad usually existed as a faculative parasite and that its ability to produce disease was dependent upon its ability to become pathogenic. Jowett (1911) in South Africa also believed that a trichomonad, Trichomonas eberthi Kent, a normal inhabitant of the caeca, could become pathogenic and initiate blackhead. However,

investigations by Tyzzer (1919, 1920), discredited the ideas that coccidia or trichomonads were the causative organisms.

In addition to Smith (1910), who described the presence of pseudopodia in the protozoa, the nature of the organism was further studied by Tyzzer, (1919). He noted an "extra-nuclear body" lying close to the nucleus of the parasite. Filament-like rays or axonemes were seen to radiate from this body and pass over the surface of the nuclear membrane. Division of this body into two portions connected by a dark staining line was thought to initiate nuclear division. Tyzzer (loc. cit.) made no mention of the flagellate nature of the organism. However, he later deomonstrated the presence of flagella and renamed the causative agent of blackhead Histomonas meleagridis Smith (Tyzzer, 1920). In the same paper he contended that the organism migrated through the tissues by amoeboid movement.

Natural transmission of the histomonads to turkeys can occur directly by the ingestion of feces containing the organisms and by the ingestion of embryonated Heterakis gallinae eggs. Although the infection of turkeys by ingestion of histomonads in feces occurs, it is not an important avenue of infection. Tyzzer and Collier (1925) stated that the organism cannot survive for long periods of time after being expelled, but if immediately ingested, infection can be produced. Graybill and Smith (1920), and

Nimi (1937) failed to produce infection when droppings were fed to turkeys. Graybill and Smith (loc. cit.) first demonstrated Heterakis gallinae, a nematode that commonly inhabits the caeca of chickens, turkeys, and other fowl, as the primary agent responsible for the transmission of histomoniasis. Swales (1948) conducted a series of experiments in order to clarify the role of the caecal worm. He used ground embryonated and ground unembryonated Heterakis gallinae eggs and artifically hatched larvae. Upon inoculation of two turkeys with an unspecified number of ground unembryonated Heterakis gallinae eggs, he found that the birds remained healthy. Similar results were obtained when he used ground embryonated Heterakis gallinae eggs. Blackhead was produced when the aritficially hatched larvae were inoculated into the birds. From these sparse data, he concluded that initiation of histomoniasis is dependent upon the presence of living larvae in the turkeys.

Inoculation of turkeys with caecal worm eggs has shown the causative agent of blackhead to be present only within the eggs. Tyzzer and Fabyan (1922) treated embryonated Heterakis gallinae eggs with 1.5 per cent nitric acid for three days. After this period of time, even though the medium was bacteriologically sterile, infection was initiated when the eggs were fed to incubator-hatched turkeys. Droppings from turkeys containing protozoa did not initiate disease after they had been treated

with nitric acid. Lund and Burtner (1957) found that only one out of two hundred Heterakis gallinae eggs contained histomonads. Kendall (1959) stated that only one of 1,000 caecal worm eggs was infected.

Despite the fact that Histomonas meleagridis is generally recognized to be the etiological agent in blackhead, there have been and are those that do not think so. Enigk (1935) reported that in 56 cases of blackhead in turkeys and five in chickens, the condtion was due to a budding fungus, "Blastocystis." He successfully cultivated the fungus from the livers of 33 poults. Inoculation of these organisms grown on maltose agar produced blackhead in normal birds. He speculated that injury to the caecal mucosa caused by Heterakis gallinae permitted the penetration of the fungus into the tissues, although causes other than the caecal worm may be instrumental in determining if the fungus will invade the tissues. Menzani (1933) had also considered a fungus to be the primary invader. Wetzel and Enigk (1938) identified the fungus to be Monilia (Candida) albicans. Kuprowski (1955, 1960) shared the same views on the etiology. He contended that the moniliasis was due to parasitic infection. He corroborated his earlier findings that "the apparent encysted forms of Histomonas meleagridis (resistant phases, tissue phases) are the cells of the initiated reticulo-endothelial system, undergoing necrosis, and that the characterisitc liver lesions in typhlo-hepatitis (blackhead) are white infarcts."

Although it is almost universally accepted that Histomonas meleagridis is present in the caecal worm egg, its demonstration in these eggs has been questioned (Desowitz, 1950, Kendall, 1959). Tyzzer (1926) examined a large number of Heterakis gallinae eggs but was unable to demonstrate any protozoa within them. However, examination of the half grown worms taken from animals suffering from blackhead showed the presence of protozoa in the worms. The presence of protozoa in the gut of three-day and twelve-day old worms that were hatched from Heterakis gallinae eggs was shown by Tyzzer (1934). These findings were not readily accepted because of the failure of other workers to confirm them. Connell (1950) observed refractile swellings in the cuticle of hatched second-stage larvae which he attributed to the presence of Histomonas meleagridis. He contended that these forms may possess the ability to change into infective organisms. Kendall (1959) studied sections from larval infected tissues and showed the presence of organisms resembling Histomonas meleagridis in the larvae. Although the exact position was difficult to ascertain, they were generally found to be in the cells lining the gut or within the gut lumen. The latest investigator to look for Histomonas meleagridis in Heterakis gallinae eggs was Gibbs (1962). He demonstrated protozoa in both male and female caecal worms. In male worms, the histomonads were seen to be in the lumen of the gut and the reproductive tract. Infected

female worms also harbored the protozoa in the reproductive tract. These forms were morphologically similar to the tissue-invading stages of the protozoa. The histomonads found in the uterine eggs differed in that they had larger nuclei and reduced cytoplasm.

The early clinical symptoms of blackhead include a loss of appetite, drowsiness, and general unthriftiness (Steiner, 1924). Appearance of slight diarrhea with evidence of some hemorrhage occurred approximately nine days postinfection. At this time there was a general loss of body weight resulting from reduced food intake. About 11 days after the infection sulfur-colored droppings appeared and the clinical symptoms became more severe. Deaths can occur from 14 days on, the incidence being greater 19-21 days after infection (Clarkson, 1962, b).

Macroscopic pathological changes occurring as a result of the disease have been reported by a number of investigators Steiner (1924), Malewitz, Runnels, and Calhoun (1958), Welter (1960), and Kendall (1959). The liver and caeca were the primary organs involved, but kidneys and the spleen have been shown to harbor the organisms. Liver lesions vary in size from 1 mm to about the size of a half-dollar. The lesions are usually circular and depressed below the surface of the liver, and show a yellow-white or gray center.

Caecal involvement is generally first observed five days after infection, at which time the caecal contents are white but still liquid. As the infection proceeds, the caecal contents become gelatinous and finally the caeca are distended and the bloody necrotic material within it forms a hard core. The cores may be in one or both of the caeca.

Study of the progressive histopathological changes by Kendall (1959) revealed the liver to be normal up to six days after infection. At this time lymphocytic infiltration and a very slight heterophilia were noted. "From the eighth to eleventh days of infection, vessels were thrombosed and contained liver cells." Histomonads were not seen until 12 days after infection even though macroscopic liver lesions were seen as early as 10 days. As the infection progressed there was increased lymphocytic infiltration and plasma cells appeared. The number of protozoa increased and there was focal necrosis of the liver. The presence of histomonads in the caeca was not shown until five days after infection. At this time the protozoa were seen beneath the epithelium at the tips of the folds of the caecal mucosa. In addition to the progressive lymphocytic infiltration, the histomonads were seen to extend to columns down to the muscularis mucosa. Near this area were many heterophils. Finally the organisms invaded the serosa of the caecum.

A number of techniques, other than the use of embryonated Heterakis gallinae eggs, have been used to produce experimental infection of blackhead in turkeys. Infections have been produced by the inoculation of infected liver and caecal material by Tyzzer and Collier (1925), Wegeforth and Wegeforth (1921), Delaplane (1932) and Farmer and Stephenson (1948). Although the probability of infection is not high when turkeys are placed on histomonad contaminated ground, Rettger and Kirkpatrick (1927), DeVolt and Davis (1936) and Farr (1955) were able to achieve positive results by raising birds on soil containing contaminated feces. The other most common method of experimental infection has been by the use of cultured histomonads. DeVolt and Davis (1936) obtained high percentages of infections when cultured material was administered orally to turkeys. Tyzzer (1934) obtained similar results when hen chicks were inoculated rectally with culture material. More unusual but successful methods were tried by Tyzzer, Fabyan, and Foot (1921). They implanted ground diseased tissues subcutaneously into turkeys. Even though infections were produced, atypical lesions occurred. The same was true of intrasinal inoculation of infected liver and caecal material. Tyzzer and Fabyan (1920), and McGuire and Morehouse (1958) attempted to induce blackhead by using blood taken from infected animals. They were able to produce blackhead in 25 per cent of the inoculated turkeys by injecting them

with citrated whole blood obtained from the veins draining the caeca of infected animals. Typical blackhead lesions were produced with involvement of lungs, heart, pancreas, and proventriculus. In no instance was there involvement of the caeca or the lower intestinal tract. These results seem to indicate that the protozoa can survive and reproduce in any type of tissue to which they can gain access.

Despite the fact that numerous and extensive studies have been carried out to elucidate the nature of the macroscopic and microscopic pathological changes induced by experimental infections, very little has been done to determine the blood changes that occur during the course of the disease. McGuire and Cavett (1952) found that blood non-protein nitrogen and uric acid decreased in the early stages of the disease, but returned to near-normal values before death of the infected birds. Blood glucose concentration increased during the incubation period. As the infection progressed, hypoglycemia developed which could have been the immediate cause of death. Packed cell volumes, hemoglobin tests, and erythrocyte counts indicated that an anemic condition is established near the end of the disease. Clarkson (1962, a) found that the bromsulphthalein (BSP) liver function test showed a sharp decrease in the per cent plasma BSP load transferred to the liver in unit time. Analyses of blood levels of carontenids, bile pigments and serum proteins of normal birds and those

dying of histomoniasis showed that the carotenids fell from a normal vlaue of 300 micrograms per 100 ml to 80 micrograms per 100 ml. A reversal of the albumin to globulin ratio was seen. Bile pigments were not present in normal birds but were present in a concentration of 1.2 mgm per 100 ml in infected turkeys.

Johnson and Lange (1938), McGuire and Cavett (1952) and Venkataratnam and Clarkson (1962) observed that as the disease progressed, there was a steady and marked fall in the number of erythrocytes. A steady increase in the number of leukocytes, to a level three to four times that of normal birds, was observed. The increase in leukocytes was made up mainly of heterophils and monocytes. Lymphopenia occurred prior to death.

In recent years, electrophoretic techniques have been used quite extensively for clinical purposes (Smith, 1960, Gronwall, 1952; and Ribiero, 1955). These techniques have proven reliable in aiding in the diagnosis of many diseases (Brente, 1952; Hardwicke, 1952; and Smith, 1960). However, serum electrophoresis has not been used extensively in the study of histomoniasis. The effect of blackhead on the serum proteins as determined by the use of cellulose acetate electrophoresis has been reported only by Clarkson (1959). He found a greatly reduced albumin fraction and an increased amount of gamma globulin which he attributed to liver damage and an immunological response respectively.

Experimental studies dealing with resistance and immunity in turkeys resulting from histomoniasis have not been extensive. Although blackhead can occur in birds of varying ages, it it more prevalent in turkeys ranging in age from eight to 14 weeks. Swales and Frank (1948), and Tyzzer (1934) noted that adult birds were living in an environment that would have been fatal to younger birds. On the basis of the preceding, Desowitz (1951) postulated that an age resistance to blackhead was present in turkeys. However, contradictory results were obtained by Kendall (1957). He found no difference in susceptibility to histomoniasis, regardless of the age of the birds.

Conflicting opinions have been put forth as to the development of acquired resistance to balckhead. Tyzzer (1936) reported that he was able to induce resistance in turkeys by repeated inoculations with attenuated strains of Histomonas meleagridis. Immunization of turkeys by the use of nonpathogenic strains of histomonads proved to be unsatisfactory when whole embryonated Heterakis gallinae eggs were administered to the birds as a challenge. Increased resistance was evident when birds were challenged with protozoa taken from the caeca of infected birds. Waletzky (1950) contended that there was no certainty the turkeys recovering from blackhead became immune. Kendall (1957) demonstrated that resistance to reinfection can occur. He concluded that turkeys could acquire a resistance by having a relatively non-pathological infection. In-

fections with avirulent strains of Histomonas meleagridis or infection by a small number of virulent organisms may stimulate some degree of resistance.

The survey of literature illustrates that the concentrated extensive research in the past has mainly dealt with the elucidation of the macroscopic and microscopic pathology, as well as the determination of the etiological agent of blackhead. Further possibilities in experimental studies exist in deciding what if any role is played in the infection by Monilia albicans, the manner in which the protozoa are released from the nematodes and the physiological processes related to mechanisms of resistance and immunity to blackhead in turkeys.

CHAPTER III

MATERIALS AND METHODS

Chicken entrails obtained from a local poultry abattoir were the source of adult Heterakis gallinae worms. The caeca, after being separated from the rest of the intestines, were opened and the contents were washed into a screen using a stream of cold water. The screen containing the worms was backwashed into a casserole. Gravid female Heterakis gallinae worms were separated and ground in a Ten-broeck tissue grinder. The ground material was then placed in Petri dishes with a small amount of one per cent formalin and incubated at room temperature until the eggs were embryonated. The eggs were usually embryonated at the end of 21 days. The embryonated Heterakis gallinae eggs were refrigerated at approximately 7° C. until they were used. Caecal worm eggs which were to remain umembryonated were washed in water, placed in one per cent formalin, and immediately refrigerated at approximately 7° C. until used.

The turkeys used in these experiments were either Broad Breasted Bronze or Beltsville White. With the

exceptions of Experiments I and III, the birds were purchased from a commercial hatchery. Turkeys used in other experiments were obtained from the Poultry Department of Michigan State University. Poults were received on the day of hatching and were raised in wire cages to reduce the possibility of accidental infection. Uninoculated control birds were kept as a check to see it accidental infection occurred.

Prior to being administered to the birds, whole Heterakis gallinae eggs were placed in 1.5% nitric or 2.0% peracetic acid for 15 minutes. The eggs were then washed in sterile water. Bacteriological sterility against contamination with aerobic bacteria was checked by inoculating nutrient broth and observing for bacterial growth after incubation at approximately 37° C for two to four days. The eggs were suspended in sterile o.85% saline before being inoculated into the turkeys.

Both the embryonated and the unembryonated caecal worm eggs that were to be ground were first placed into 1.5% nitric acid and then washed in sterile tap water and placed in sterile 0.8% saline. The eggs were ground up in a Ten-broeck tissue grinder until examination of 0.02 ml of the suspension under the low power of the microscope showed that none of the eggs or larvae were intact.

Twenty-four hours prior to inoculation, the birds were taken off feed and placed randomly into various

groups, after which they were wing-banded. Following inoculation, food and water were available ad libitum. The desired number of Heterakis gallinae eggs to be introduced per bird was contained in 1 ml of 0.85% saline. Per os inoculations were made by inserting a 1 ml volumetric pipette containing the infective material into the crop of each turkey. Intra-rectal inoculations were made by inserting one end of a #8 French catheter into the rectum. The other end of the catheter was attached to a 1 cc syringe containing the infective material. After inoculating the ground Heterakis gallinae eggs, the syringe was filled with 1 cc of sterile 0.85% saline and this too was injected into the rectum. Following the inoculation, the birds were hung upside down for approximately 20 minutes so that the inoculum would be able to reach the caeca and not be expelled. The turkeys were then placed in cages.

Blood samples were obtained by using a 5 cc syringe fitted with a 22 gauge needle. Two cc of blood were drawn from a jugular vein. The blood was put into test tubes and allowed to clot in the incubator at approximately 37° C. for approximately 30 minutes. After loosening the clots with a wooden applicator stick, the tubes were refrigerated at approximately 5° C. until the clots had retracted. The serum was then harvested and refrigerated at approximately 5° C. As soon as adequate quantities of serum were obtained electrophoresis was performed.

A Universal electrophoresis apparatus, manufactured by Shandon Scientific Co., was used to carry out the cellulose acetate electrophoresis. A discontinous buffer system as described by Goldberg (1959) was used. Tris (hydroxymethyl) amino-methane-disodium ethylene-diamine tetraacetate, dihydrate-boric acid buffer (tris-EDTA-boric acid buffer) pH 9.1 was the strip buffer. A barbital buffer pH 8.6 was used in the electrophoresis chamber. The electrophoresis procedure followed was similar to that described by Smith (1960). The cellulose acetate strips were placed in the strip buffer. After impregnation, the strips were removed from the buffer, blotted lightly between paper towels and then were placed on the birdge holders of the electrophoresis pan. Three micromilliliters of sera were applied to the strips by means of a micropipette. A Vokam power unit, manufactured by Consolidated Laboratories Inc., producing constant voltage and constant amperage, set at 250 v was the power source. However, the constant voltage across the strips was 100 v. Electrophoresis, which took place in the refrigerator at approximately 7° C., lasted approximately 45 minutes. After electrophoresis was complete, the strips were marked and then placed in 3% trichloroacetic acid for 15 minutes so that the proteins would be fixed to the strips. Staining with Ponceau S for 10 minutes followed next. The strips were washed in 5% acetic acid until the background was clear of excess stain. Following this, the strips

were placed between several sheets of paper towelling and were put under a piece of plate glass until dry.

Analyses of the electrophoresis strips were done by placing the cellulose acetate strips with translumination on a Thermolyne Lab Lite, manufactured by Thermolyne Corporation, and visually comparing and counting intensities of the different bands. Representative electrophoresis strips from each group were placed in the Chromoscan, manufactured by Joyce, Loeble and Co. Ltd., and the relative concentrations of the different fractions were recorded and calculated. These methods were adequate to demonstrate quantitative changes that might have occurred.

Experiment I: Inoculation of Turkeys with Ground Embryonated and Ground Unembryonated Heterakis gallinae Eggs

Review of the literature revealed that the presence of Histomonas meleagridis in Heterakis gallinae eggs has been shown by the use of histologic techniques. The purpose of this experiment was to see if the presence of protozoa in caecal worm eggs could be demonstrated experimentally by inoculating turkeys rectally and orally with ground embryonated and unembryonated Heterakis gallinae eggs.

Five, 39 day-old turkeys, (Broad Breasted Bronze), were placed in each of the following groups:

Group A = Uninoculated control turkeys

Group B = Whole embryonated eggs--orally

Group C = Ground embryonated eggs--orally

Group D = Ground unembryonated eggs--orally

Group E = Ground embryonated eggs--rectally

Group F = Ground unembryonated eggs--rectally

Four hundred caecal worm eggs were contained per ml of the ground and whole embryonated material, and 1,500 eggs per ml were contained in the ground unembryonated material administered per bird in each group. One ml of the inoculum was given to each turkey.

Thirty-one days after initiation of the experiment, the birds were killed and the livers and caeca were examined for lesions. Portions of the livers and caeca from some of the birds showing lesions were placed in Bouin's fixative and histologic sections were stained with haemotoxylin and eosin. Microscopic examination of the tissues for the presence of Histomonas meleagridis was undertaken.

Experiment II: Contined Studies on the Effects of Inoculating Turkeys with Ground Embryonated and Ground Unembryonated Heterakis gallinae eggs

Forty, 29-day old Broad Breasted Bronze turkeys, were put into the following groups:

Group A = Uninoculated control turkeys

Group B = Whole embryonated eggs--orally

Group C = Whole unembryonated eggs--orally

Group D = Ground unembryonated eggs--orally

Group E = Ground unembryonated eggs--rectally

Group F = Ground embryonated eggs--rectally

There were five birds in Groups A, B, C, and F. Groups D and E were comprised of 10 birds each.

A new batch of Heterakis gallinae eggs was used in this experiment and therefore no direct comparison of the results can be made. One ml of inoculum contained either 500 whole or ground embryonated Heterakis gallinae eggs of 2,800 whole or ground unembryonated caecal worm eggs.

The turkeys were killed 32 days after inoculation. The liver and caeca were examined for lesions. Pieces of liver and caeca from all birds were placed in Bouin's fixative for histologic study.

Experiment III: Continued Studies on the Effects of Inoculating Turkeys with Ground Embryonated and Ground Unembryonated Heterakis gallinae eggs

Fifty, 35 day-old Beltsville White turkeys were placed into the following groups:

Group A = Uninoculated control turkeys

Group B = Whole embryonated eggs--orally

Group C = Whole unembryonated eggs--rectally

Group D = Ground unembryonated eggs--rectally

Group E = Ground unembryonated eggs--orally

Group F = Ground embryonated eggs--rectally

There were 12 birds each in Groups D, E, and F. Groups B and C contained five birds each. Four turkeys were present in Group A.

A fresh supply of caecal worm eggs was used in this experiment. One ml of the inoculum containing 4,000 eggs was administered to all of the birds.

The turkeys were killed 31 days after initiation of the experiment. Gross examination was performed to see if liver or caecal lesions were present. Pieces of tissue from the liver and caeca were placed in Bouin's fixative to prepare them for histologic study.

Experiment IV: A Study of the Effects of the Ground Embryonated and Ground Unembryonated Heterakis gallinae Eggs on the Serum Proteins of Turkeys

Forty, 25 day-old Beltsville White turkeys were separated into the following groups:

Group A = Uninoculated control turkeys

Group B = Whole embryonated eggs--orally

Group C = Ground embryonated eggs--rectally

Group D = Ground unembryonated eggs--rectally

Group E = Ground unembryonated eggs--orally

Groups C, D, and E had 10 birds. Groups A and B were comprised of five birds each.

There were 500 Heterakis gallinae eggs in the inoculum administered to each of the turkeys receiving the embryonated eggs. Twenty-five hundred eggs were contained in the inoculum given to the turkeys inoculated with the unembryonated Heterakis gallinae eggs.

Blood samples were taken from the jugular veins of the birds 15 and 36 days after inoculation and electrophoresis of the sera was performed.

Thirty-seven days after initiation of the experiment, the turkeys were killed and the liver and caeca were examined macroscopically for alterations.

Experiment V: Electrophoretic Studies of Sera Taken from Turkeys Inoculated Orally with Varying Numbers of Whole Embryonated Heterakis gallinae Eggs

Thirty-seven, day-old Beltsville White turkeys, were put into the following groups:

Group A = Uninoculated control turkeys

Group B = Five embryonated eggs--orally

Group C = Fifty embryonated eggs--orally

Group D = One-hundred embryonated eggs--orally

Group E = Five hundred embryonated eggs--orally

There were nine birds in Group A and seven birds in each of the other groups.

The eggs were contained in one ml of saline and were administered per os into the crop by means of a one ml volumetric pipette.

Blood samples were obtained 14 and 38 days after inoculation. Electrophoresis of the sera was performed.

Experiment VI: Electrophoretic Studies on the Effects of Challenge Inoculations of Turkeys Previously Inoculated Orally with Varying Numbers of Whole Embryonated Heterakis gallinae Eggs

Forty-one days after turkeys were inoculated in Experiment V, the surviving 20 turkeys were reinoculated with 500 whole embryonated Heterakis gallinae eggs. The following groups of birds remained:

Group A = Uninoculated control turkeys

Group B = Five embryonated eggs--orally

Group C = Fifty embryonated eggs--orally

Group D = One hundred embryonated eggs--orally

Group E = Five hundred embryonated eggs--orally

(Inoculated controls)

There were six birds in Group B and two birds each in Groups C and D. The control groups each contained 5 turkeys.

Twenty-five days after the reinoculation or 66 days after the initial inoculation (Experiment V), the turkeys were bled and electrophoresis was run on the sera. A second challenge inoculation of the turkeys using 500 embryonated Heterakis gallinae eggs was made 25 days after the first reinoculation. The turkeys were bled 22 days after the second challenge inoculation and electrophoresis of the sera was performed.

The experiment was concluded 49 days after its initiation, at which time the surviving birds were killed and macroscopic examination of the liver and caeca was made.

CHAPTER IV

RESULTS

Experiment I: Inoculation of turkeys with Ground Embryonated and Ground Unembryonated Heterakis gallinae Eggs

The turkeys used in this experiment were inoculated with ground embryonated and ground unembryonated Heterakis gallinae eggs rectally and orally into the crop to learn if infection could be initiated in the absence of the living larvae.

Ten days after initiation of the experiment, control turkeys (Group B), inoculated orally with whole embryonated eggs showed typical symptoms of histomoniasis. At this time, the other experimental birds did not show any visible symptoms. Thirty-one days after inoculation, sulfur colored droppings were noticed in three groups of birds inoculated with ground embryonated and ground unembryonated eggs (Groups D, E, and F). The observed diarrhea was not nearly as serious as was seen in the infected controls. At this time, the remaining birds were killed and examination was made of the liver and caeca (See Table I). This examination revealed the presence of atypical liver lesions in the groups inoculated rectally (Group F) and orally

TABLE I.--The effects of inoculating turkeys with ground embryonated and ground unembryonated Heterakis gallinae eggs.

Group	Nature of Inoculum	Route of Inoculation	Per Cent Mortality	Caecal Lesions	Number of Liver Lesions	Number of Birds With Liver Lesions	Histomonads Present in Liver Sections
A	---	---	0	---	---	0/5	---
B	400 Whole Embryonated Heterakis gallinae eggs	Oral	100	+	too many to count	5/5	+
C	1500 Ground unembryonated Heterakis gallinae eggs	Oral	0	---	---	0/5	0
D	1500 Ground unembyonated Heterakis gallinae eggs	Oral	0	---	4-20	3/5	+
E	400 Ground Embryonated Heterakis gallinae eggs	Rectal	0	---	2-12	3/5	+
F	1500 Ground unembryonated Heterakis gallinae eggs	Rectal	0	---	3-10	3/5	+

(Group D) with ground unembryonated caecal worm eggs and rectally with ground embryonated eggs (Group E). The lesions were white, had irregular borders, measured from 2 to 6 mm in diameter, and were not depressed below the surface of the liver. The caeca of all of the birds at gross examination were normal. Microscopic examination of liver sections made from the tissues of the birds exhibiting the atypical liver lesions showed organisms resembling Histomonas meleagridis (See Plate I, Figure 1, Plate II, Figure 2, Plate III, Figure 3, Plate IV, Figure 4, Plate V, Figure 5). The protozoa were not nearly as numerous as in the livers of infected control turkeys.

The results of this experiment indicated that blackhead can be initiated experimentally by inoculating turkeys rectally with ground embryonated caecal worm eggs and rectally and orally with ground unembryonated caecal worm eggs.

Experiment II: Continued Studies on the Effects of Inoculating Turkeys with Ground Embryonated and Ground Unembryonated Heterakis gallinae Eggs

The turkeys used in this experiment were inoculated orally and rectally with ground embryonated and ground unembryonated Heterakis gallinae eggs. The primary differences between this experiment and Experiment I were the source and size of the inoculum. Because of the availability of a greater number of eggs and the unknown in-

PLATE I

Photomicrographs of liver sections from turkeys inoculated 32 days earlier with ground embryonated or ground unembryonated Heterakis gallinae eggs.

Figure 1.--Liver. H. and E. x 950. Portion of liver from an uninoculated control turkey.

PLATE I

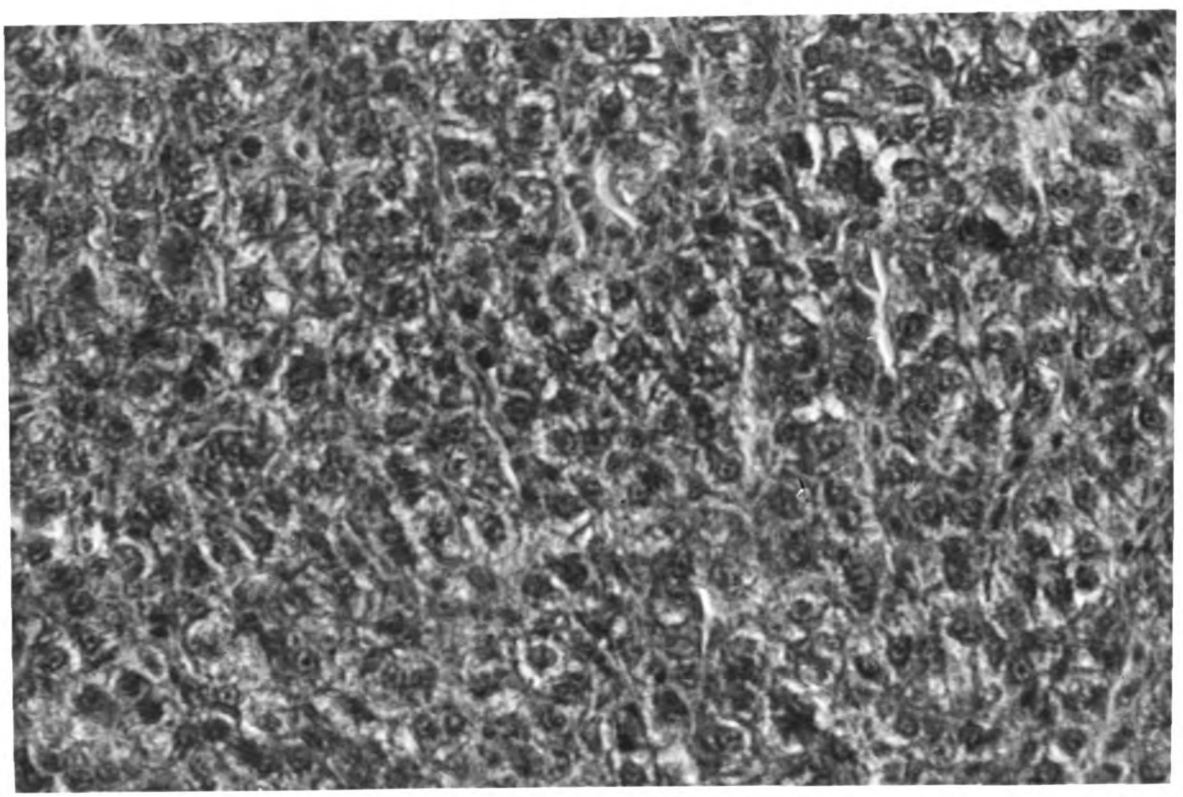


Figure 1

PLATE II

Photomicrographs of liver sections from turkeys inoculated 32 days earlier with ground embryonated or ground unembryonated *Heterakis gallinae* eggs.

Figure 2.--Liver. H. and E. x 950. Portions of liver from a turkey inoculated rectally with ground embryonated *Heterakis gallinae* eggs showing organisms resembling *Histomonas meleagridis* in the tissues. The arrow points to a cluster of organisms.

PLATE II

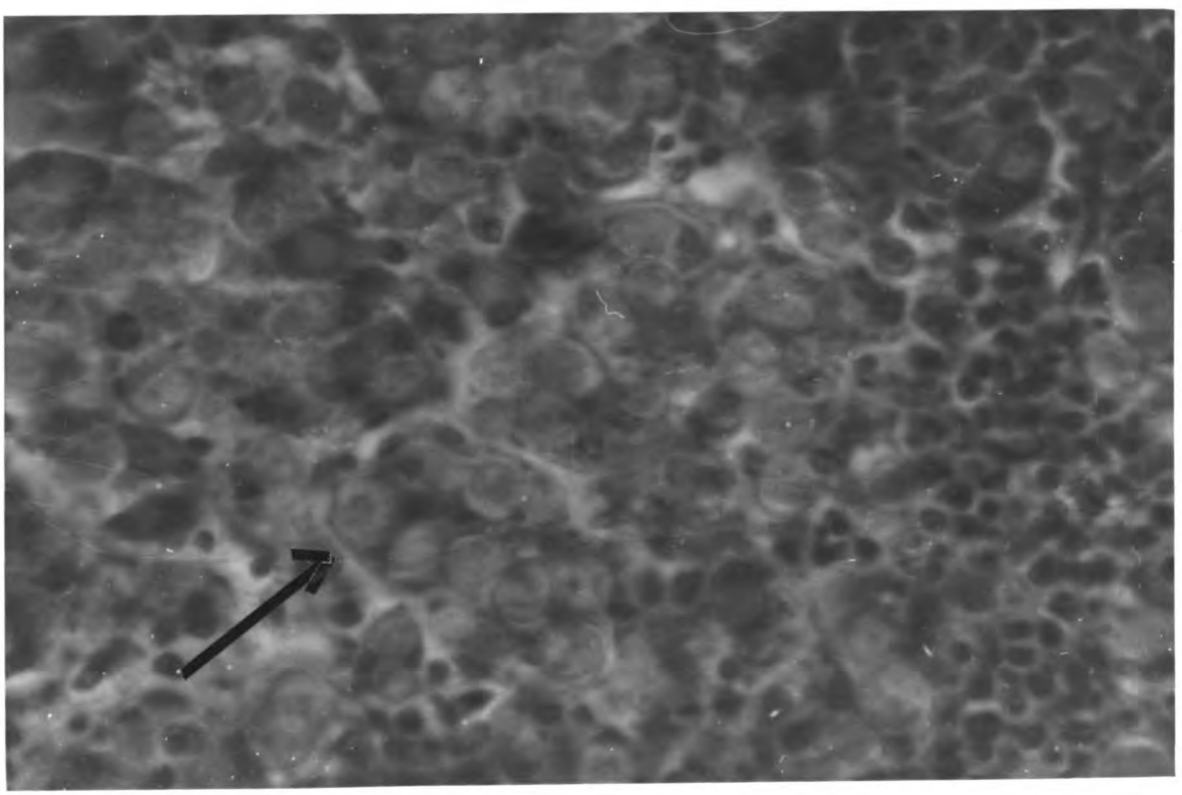


Figure 2

PLATE III

Photomicrographs of liver sections from turkeys inoculated 32 days earlier with ground embryonated or ground unembryonated *Heterakis gallinae* eggs.

Figure 3.--Liver. H. and E. x 950. Portions of liver from a turkey inoculated orally with ground unembryonated *Heterakis gallinae* eggs showing organisms resembling *Histomonas meleagridis* in the tissues. The arrow points to a cluster of organisms.

PLATE III

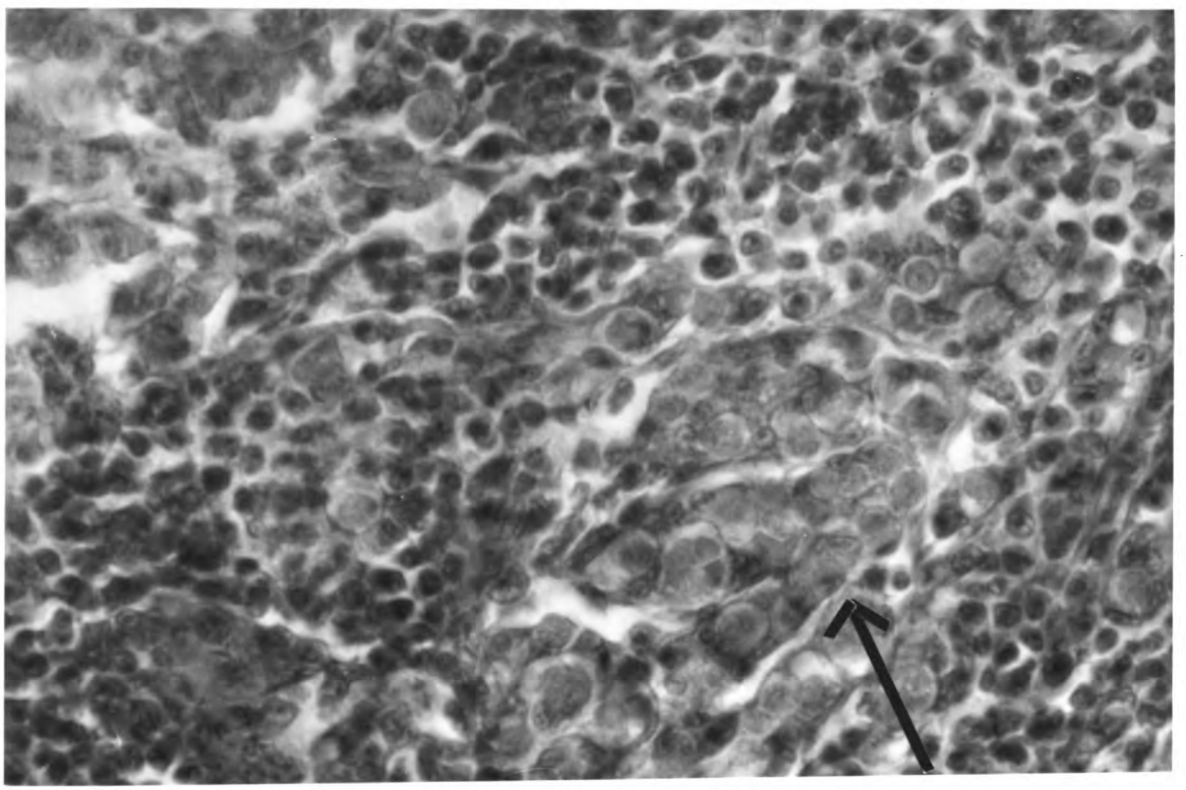


Figure 3

PLATE IV

Photomicrographs of liver sections from turkeys inoculated 32 days earlier with ground embryonated or ground unembryonated Heterakis gallinae eggs.

Figure 4.--Liver. H. and E. x 950. Portions of liver from a turkey inoculated rectally with ground embryonated Heterakis gallinae eggs showing organisms resembling Histomonas meleagridis in the tissues. The arrow points to a cluster of organisms.

PLATE IV

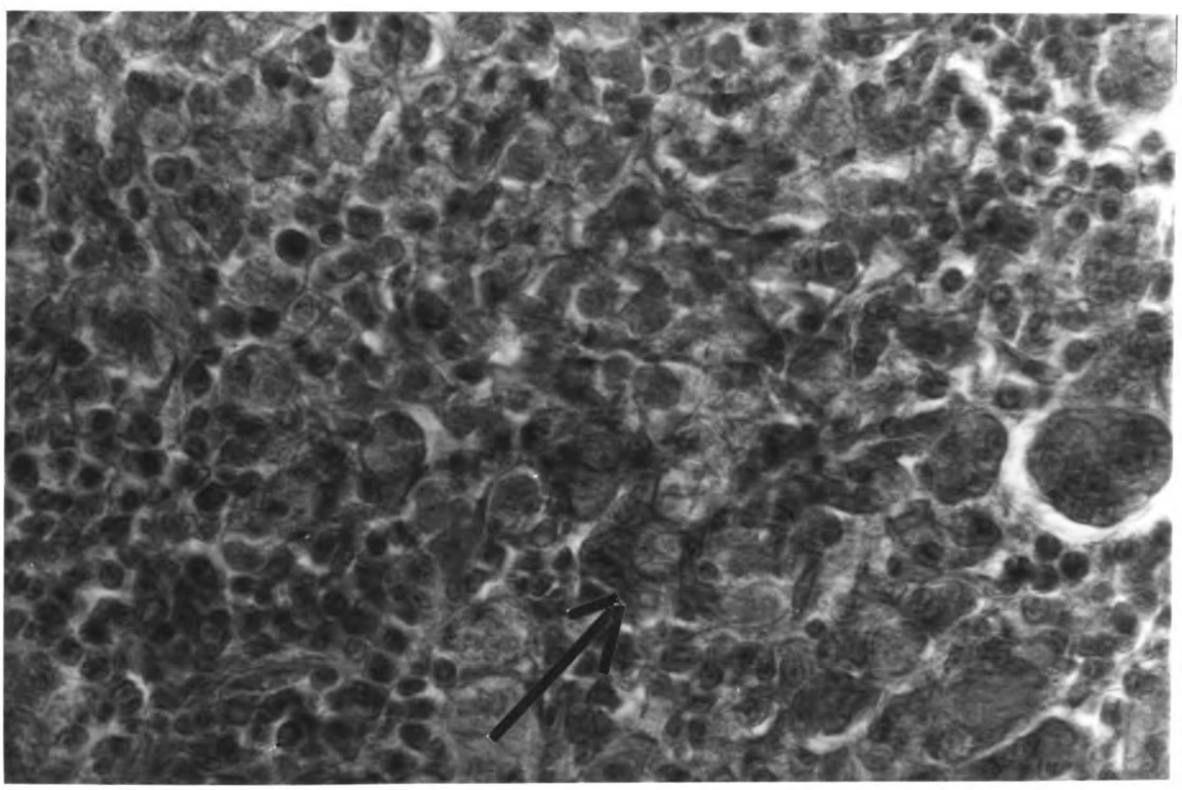


Figure 4

PLATE V

Photomicrographs of liver sections from turkeys inoculated 32 days earlier with ground embryonated or ground unembryonated Heterakis gallinae eggs.

Figure 5.--Liver. H. and E. x 950. Portions of liver from a turkey inoculated orally with whole embryonated Heterakis gallinae eggs showing "nests" of protozoa and giant cells with ingested protozoa. The arrow points to a cluster of organisms.

PLATE V

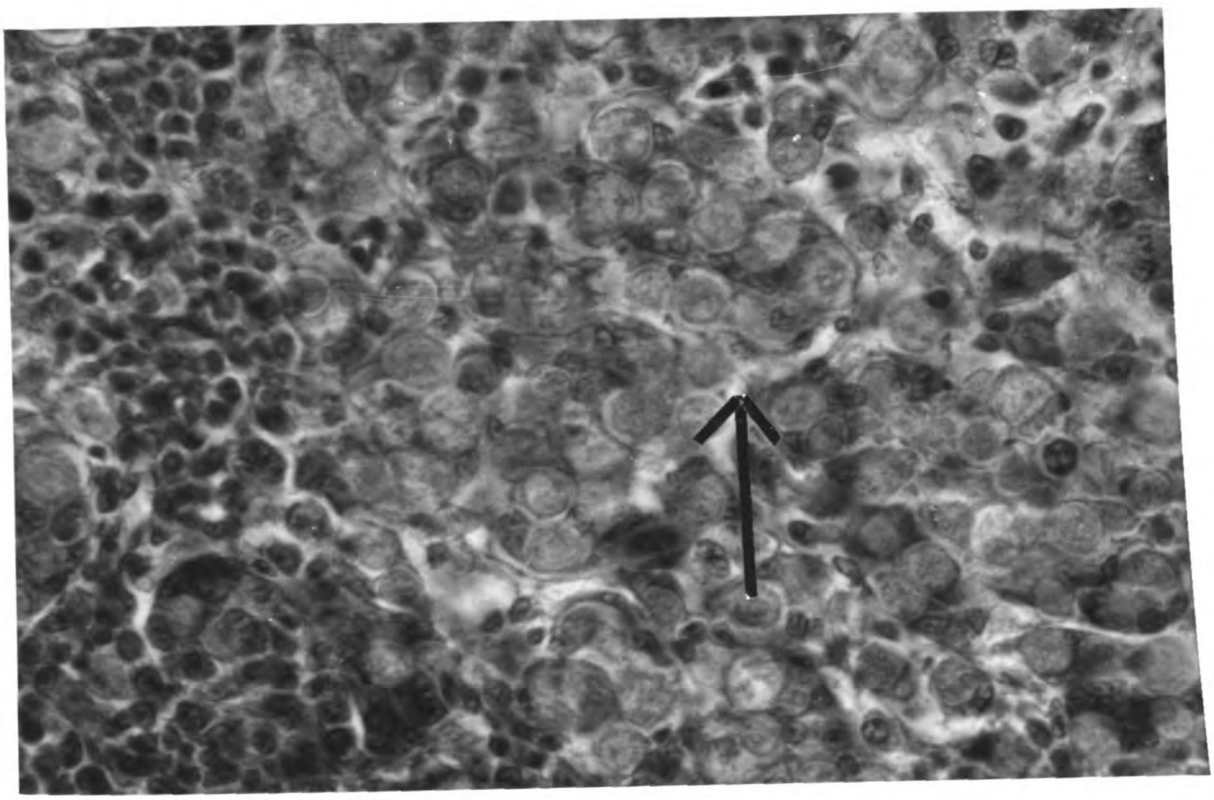


Figure 5

fectivity of the histomonads a larger inoculum was administered to the birds.

Sulfur colored droppings and the general clinical symptoms were seen only in the group of birds inoculated orally with whole embryonated caecal worm eggs. All of these birds died, and examination of the livers and caeca revealed typical liver lesions and caecal cores. There were no outward appearances that the birds inoculated with the ground embryonated or ground unembryonated eggs were infected. Examination of the livers and caeca did not reveal the presence of typical blackhead lesions. There were however, petechial hemorrhagic foci on the livers of turkeys that had been inoculated orally and rectally with the ground embryonated and ground unembryonated caecal worm eggs. These hemorrhagic foci were not seen in the livers of uninoculated controls nor in the birds infected with the whole embryonated Heterakis gallinae eggs. Histologic examination of liver sections did not show the presence of recognizable histomonads.

The results obtained in this experiment were in direct contrast to those obtained in Experiment I in that there were no typical or atypical lesions produced and no histomonads were demonstrable in the liver sections examined.

Experiment III: Continued Studies on the Effects of Inoculating Turkeys with Ground Embryonated and Ground Unembryonated Heterakis gallinae eggs

The turkeys in this experiment were inoculated orally and rectally with ground embryonated and ground unembryonated caecal worm eggs. A different source of heterakis material was used to obtain histomonads and a larger inoculum was employed. Diarrhea, sulfur colored droppings, general emaciation and mortalities were observed only in the group of turkeys inoculated orally with whole embryonated eggs. There were no visible indications that any of the birds that had been inoculated with ground embryonated or ground unembryonated caecal worm eggs became infected. Post-mortem examination of livers and caeca of birds inoculated with the ground material showed them to be normal when compared with the uninoculated control birds. Because of the normal appearance of the livers, histologic examination was not undertaken. One hundred per cent mortalities and liver lesions and caecal involvement were seen in the birds inoculated with the whole embryonated eggs.

Results of this experiment agreed with those of Experiment II in that no liver lesions were produced in turkeys inoculated with the ground embryonated or ground unembryonated caecal worm eggs.

Experiment IV: The Study of the Effects of the Inoculation of Ground Embryonated and Ground Unembryonated Heterakis gallinae Eggs of the Serum Proteins of Turkeys

Turkeys were inoculated with ground embryonated and ground unembryonated Heterakis gallinae eggs both

orally and rectally. Sulfur colored droppings were first observed in the infected control birds ten days after initiation of the experiment. There was however, no visible evidence that the birds inoculated with ground embryonated or ground unembryonated eggs had become infected. Blood samples were taken from the turkeys 15 and 36 days after inoculation and serum electrophoresis was performed.

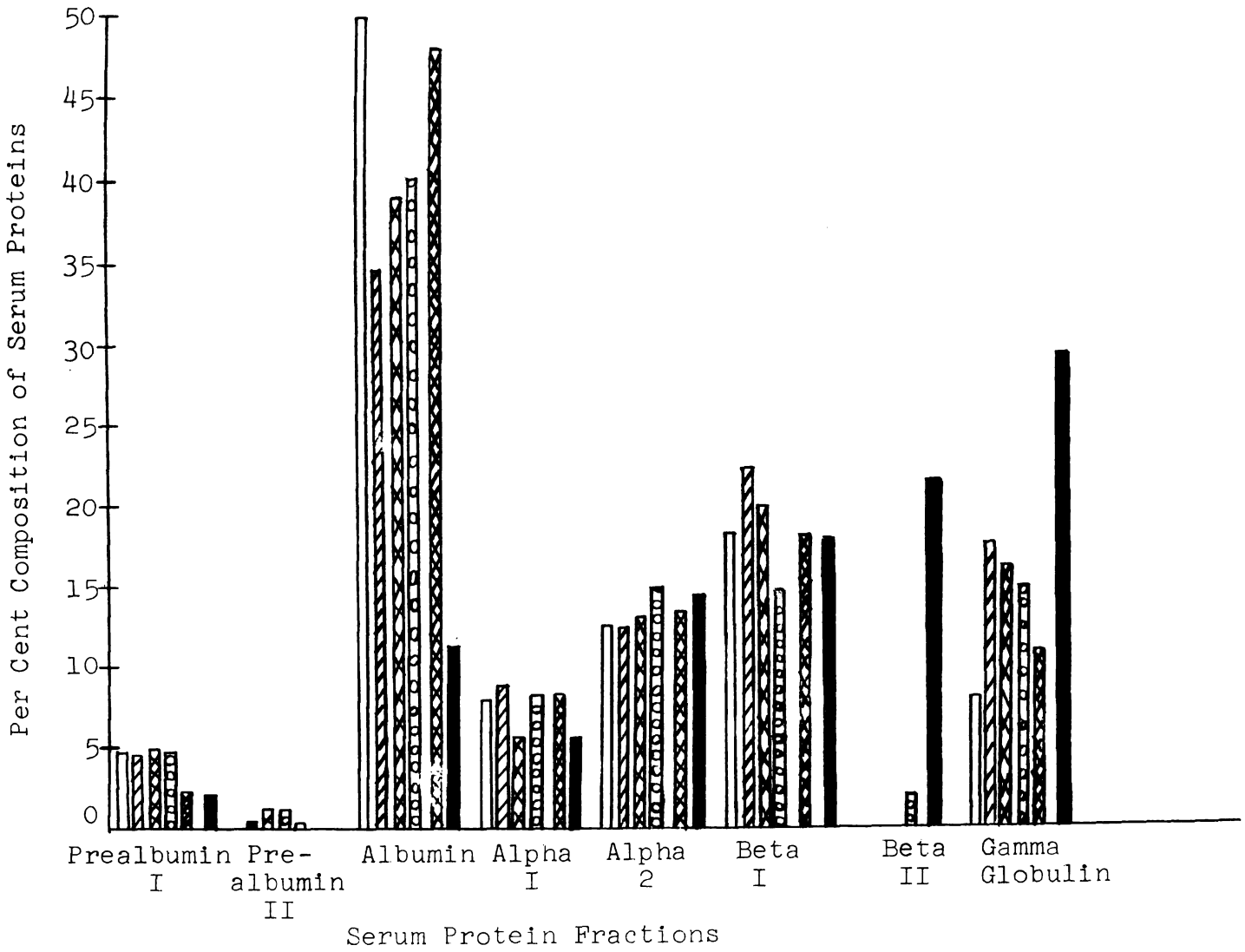
Optical scanning, integration and calculation of line intensities of the electrophoresis after 15 days is shown in Figure 6. An increase in the beta fraction was noted in that a beta-2 appeared in the sera of the inoculated controls and the turkeys inoculated rectally with ground unembryonated caecal worm eggs. The gamma globulin concentration also increased. There were no major changes in the serum proteins of birds inoculated orally with ground embryonated caecal worm eggs except for the absence of the alpha-1 globulin and an increase in the alpha-2 globulin. Increases in the beta-1 and gamma globulin concentrations were noted in the sera of birds inoculated rectally with ground embryonated eggs and inoculated orally with ground unembryonated eggs. (See Table II).

Blood samples were taken from turkeys 36 days after inoculation. Optical scanning, integration, and calculation of line intensities of the electrophoresis strips is shown in Figure 7. There was an increase in the beta and gamma globulins when the sera of birds inoculated rectally with ground embryonated eggs and orally with ground unem-

Figure 6.--Summary of optical scan and integration and calculation of line intensities of electrophoresis strips of turkeys inoculated with ground embryonated and unembryonated Heterakis gallinae eggs 15 days after inoculation in Experiment IV.

Legend:

- Uninoculated control turkeys.
- Turkeys inoculated rectally with ground embryonated Heterakis gallinae eggs.
- Turkeys inoculated orally with ground unembryonated Heterakis gallinae eggs.
- Turkeys inoculated rectally with ground unembryonated Heterakis gallinae eggs.
- Turkeys inoculated orally with ground embryonated Heterakis gallinae eggs.
- Inoculated control turkeys.


Per Cent Composition of Serum Proteins
50
45
40
35
30
25
20
15
10
5
0
Prealbumin I
Pre-albumin II
Albumin
Alpha I
Alpha 2
Beta I
Beta II
Gamma Globulin
Serum Protein Fractions

bryonated eggs were compared to the sera of the uninoculated control birds. A slight gamma globulin increase was noted in the sera of birds inoculated orally with ground embryonated Heterakis gallinae eggs. A beta-2 band and an increase in the gamma globulin were seen on the electrophoresis strips of sera of birds inoculated rectally with ground unembryonated caecal worm eggs. In no case were the increases in the gamma globulin as great as in the inoculated controls and the albumin concentration was lowest in the inoculated control birds. (See Table III).

Post-mortem examination of turkeys inoculated with the ground embryonated or ground unembryonated caecal worm eggs 37 days post inoculation revealed no gross alterations. Small hemorrhagic areas were noted on the livers of turkeys inoculated rectally with ground embryonated and ground unembryonated Heterakis gallinae eggs, and orally with ground unembryonated eggs. Turkeys inoculated with whole unembryonated caecal worm eggs, those inoculated orally with ground embryonated eggs, and the uninoculated control birds had livers that were normal in appearance.

Experiment V: Electrophoretic Studies of Sera Taken from Turkeys Inoculated Orally with Varying Numbers of Whole Embryonated Heterakis gallinae Eggs

TABLE II.--Average per cent changes in serum protein fraction concentrations within each group of turkeys 15 days after inoculation.

	Prealbumins	Albumin	Alpha-1	Alpha-2	Beta-1	Beta-2	Gamma Globulin
Uninoculated controls	+1.0	+ .1	+ .6	+3.2	-4.8	--	+1.0
Turkeys inoculated rectally with 500 ground embryonated *Heterakis gallinae* eggs	+2.0	+1.9	-9.6	0	+2.8	--	+4.5
Turkeys inoculated orally 2,500 ground unembryonated *Heterakis gallinae* eggs	+1.3	-5.5	-4.9	+4.0	+1.8	--	+3.4
Turkeys inoculated rectally with 2,500 ground unembryonated *Heterakis gallinae* eggs	+ .3	-2.5	-5.3	+ .4	-6.9	--	+2.2
Turkeys inoculated orally with 500 ground embryonated *Heterakis gallinae* eggs	+3.2	+ .3	-9.6	+7.9	-5.3	--	+1.3
Turkeys inoculated orally with 500 whole embryonated *Heterakis gallinae* eggs	+ .4	-22.6	-7.9	+1.8	-6.7	+16.0	+31.8

Turkeys in this experiment were inoculated with different number of embryonated Heterakis gallinae eggs to see if changes in the serum proteins would occur.

There was 100 per cent mortality in the birds inoculated with 500 caecal worm eggs each. No deaths occurred in the group of birds inoculated with five embryonated eggs each. One bird in this group was killed during the course of the experiment because of a broken leg sufferred during handling. Post-mortem examination of the liver and caeca revealed no pathological changes. In the groups inoculated with 50 and 100 embryonated eggs per birds, five of the seven died. Examination revealed typical liver lesions and the presence of caecal cores. It was interesting to note that the rapidity of death was not correlated with size of the inoculum. Death first occurred in the turkeys which were inoculated with 50 embryonated Heterakis gallinae eggs; other fatalities occurred randomly in the three groups (See Table V).

Fourteen days after inoculation, turkeys from each group were bled and electrophoresis was performed on the sera. The serum protein picture of the uninoculated controls showed the presence of the following bands; two prealbumins, albumin, alpha-1, alpha-2, beta-1, and gamma globulin (Plate VI, Figure 8) Results of optical scanning, integration and calculation of electrophoresis strips of sera from turkeys inoculated with varying numbers of

TABLE IV.--Effects of orally inoculating turkeys and varying numbers of embryonated *Heterakis gallinae* eggs.

Groups	Number of Embryonated Eggs in Inoculum	Number of Deaths Per Group	Number of Days After Infections When Deaths Occurred	Caecal Cores	Liver Lesions
A	0	0/9	---	---	---
B	5	1/7*	---	---	---
C	50	5/7	14,21,24,24,32	+	+
D	100	5/7	18,19,24,27,28	+	+
E	500	7/7	19,21,21,23,32,39	+	+

*The turkey was killed during the experiment because of injury suffered during handling.

TABLE V.--Average per cent changes in serum protein fraction concentrations within each group of turkeys 38 days after inoculation.

	Prealbumin I	Prealbumin II	Prealbumin III	Albumin	Alpha-1	Alpha-2	Beta-1	Beta-2	Gamma Globulin
Uninoculated Controls	+3.5	+1.7	---	+3.6	-5.1	+2.2	-7.8	---	+2.0
Turkeys Inoculated with 5 embryonated Heterakis gallinae eggs.	0	+1.5	---	+7.3	+1.5	-4.4	-6.3	---	+1.0
Turkeys Inoculated with 50 embryonated Heterakis gallinae eggs	+1.5	-1.2	---	+15.9	+1.6	+ .4	+ .5	-16.3	-1.5
Turkeys Inoculated with 100 embryonated Heterakis gallinae eggs	+5.5	---	---	+21.9	+2.1	-3.8	+4.9	-18.1	-12.5
Turkeys Inoculated with 500 embryonated Heterakis gallinae eggs	-2.4	---	---	-6.0	---	+5.2	+2.4	-20.6	+20.2

PLATE VI

Line graphs of optical scan of serum electrophoretic patterns of turkeys bled 14 days after inoculation with varying numbers of embryonated Heterakis gallinae eggs.

Figure 8.--Serum electrophoretic pattern of an uninoculated control turkey.

Figure 9.--Serum electrophoretic pattern of a turkey inoculated orally with five embryonated Heterakis gallinae eggs.

Figure 10.--Serum electrophoretic pattern of a turkey inoculated orally with 100 embryonated Heterakis gallinae eggs.

Figure 12.--Serum electrophoretic pattern of a turkey inoculated orally with 500 embryonated Heterakis gallinae eggs.

PLATE VI

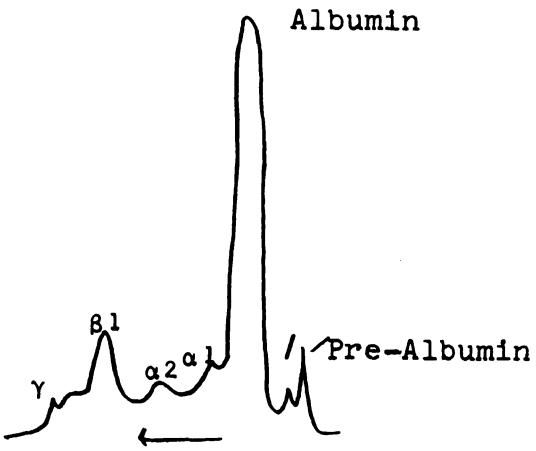


Figure 8

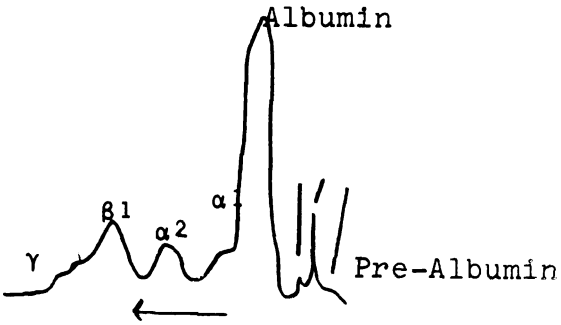


Figure 9

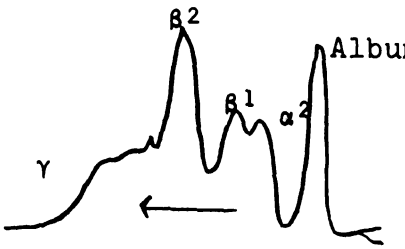


Figure 10

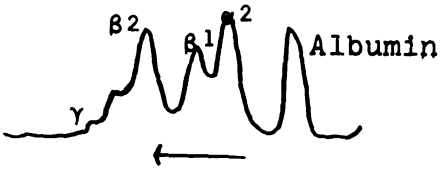


Figure 11

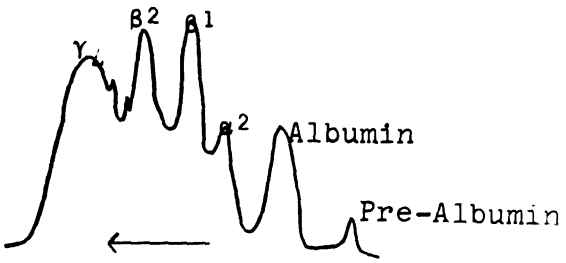


Figure 12

Heterakis gallinae eggs is shown in Figure 13. The data shows a decrease in the albumin concentration in all inoculated groups and an increase in the gamma globulin. A new band, beta-2 appeared in the sera of birds inoculated with 50, 100, and 500 caecal worm eggs each (Plat VI, Figures 10, 11, 12). This band was absent in the electrophoretic strips of the uninoculated controls and those birds inoculated with five embryonated Heterakis gallinae eggs (Plate VI, Figures 8, 9).

Thirty-eight days after inoculation, the surviving turkeys were bled and electrophoresis was performed on the sera (Plate VII, Figures 14, 15, 16, 17, 18). Results of optical scanning, integration and calculation of line intensities from the cellulose acetate strips is shown in Figure 19.

The average per cent changes of the serum proteins that occurred after 38 days are shown in Table V. Except in the group of birds inoculated with 500 embryonated eggs, an increase in the albumin was noted after 38 days. At this time, the beta-2 was no longer present. With the exception of the groups of birds inoculated with five and 500 caecal worm eggs each, and the uninoculated controls, the gamma globulin concentrations decreased after the 14 days bleeding. The group of birds inoculated with five embryonated Heterakis gallinae eggs had a gamma globulin change that was similar to that seen in the uninoculated controls. The birds inoculated with 500 embryonated eggs had a much larger increase of gamma globulin.

Figure 13.--Summary of optical scan integration and calculation of line intensities of electrophoresis strips of sera taken from turkeys 14 days after inoculation with varying numbers of embryonated Heterakis gallinae eggs.

Legend:

- Uninoculated controls.
- Turkeys inoculated with 5 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 50 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 100 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 500 embryonated Heterakis gallinae eggs.

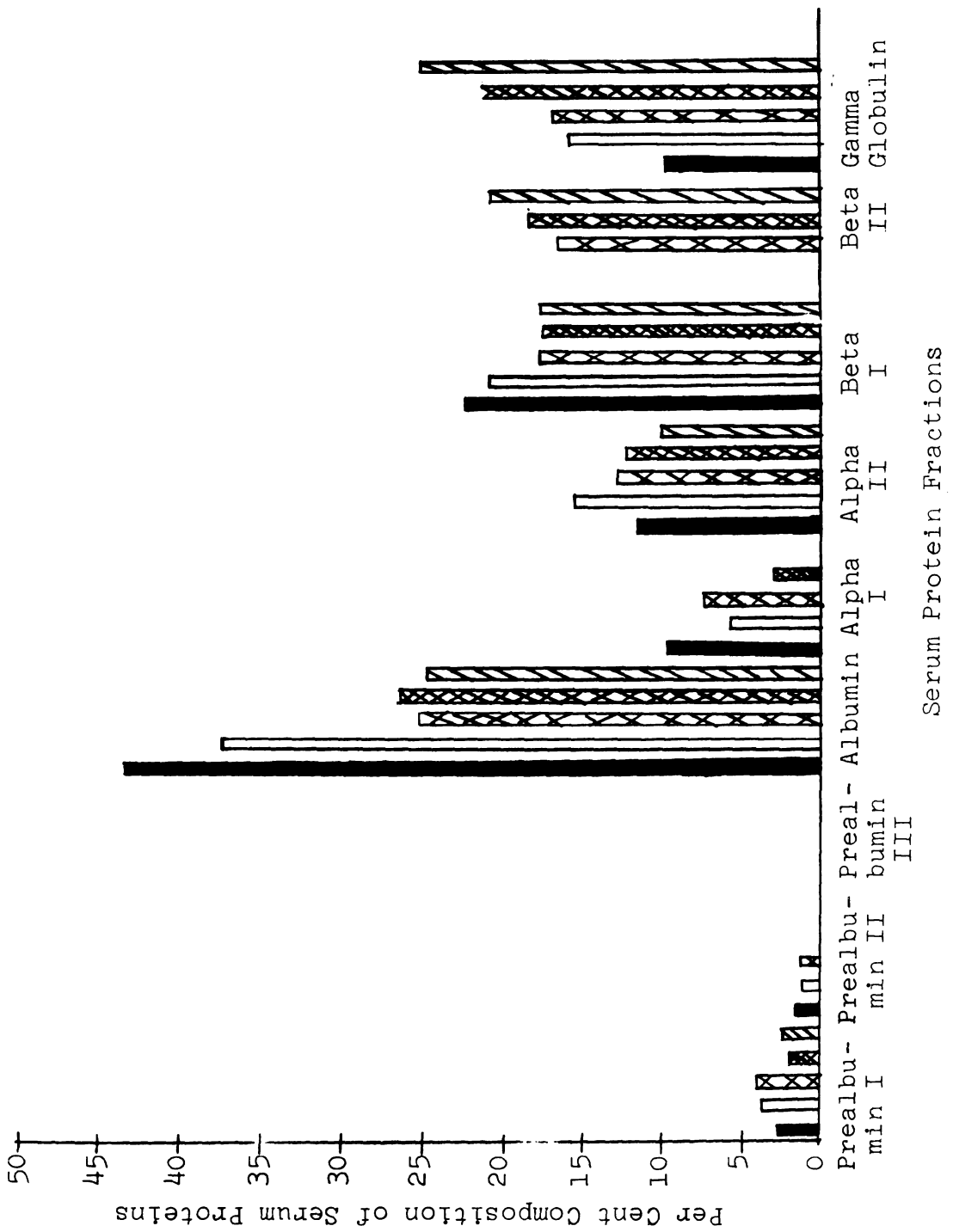

Per Cent Composition of Serum Proteins
50
45
40
35
30
25
20
15
10
5
0
Prealbu- Prealbu- Preal- Albumin Alpha Alpha Beta Beta Gamma
min I min II bumin I II I II Globulin
III
Serum Protein Fractions

PLATE VII

Line graphs of optical scan of electrophoretic patterns of turkeys bled 38 days after inoculation with varying numbers of embryonated Heterakis gallinae eggs.

Figure 14.--Serum electrophoretic pattern of an uninoculated control turkey.

Figure 15.--Serum electrophoretic pattern of a turkey inoculated orally with five embryonated Heterakis gallinae eggs.

Figure 16.--Serum electrophoretic pattern of a turkey inoculated orally with 50 embryonated Heterakis gallinae eggs.

Figure 17.--Serum electrophoretic pattern of a turkey inoculated orally with 100 embryonated Heterakis gallinae eggs.

Figure 18.--Serum electrophoretic pattern of a turkey inoculated orally with 500 embryonated Heterakis gallinae eggs.

PLATE VII

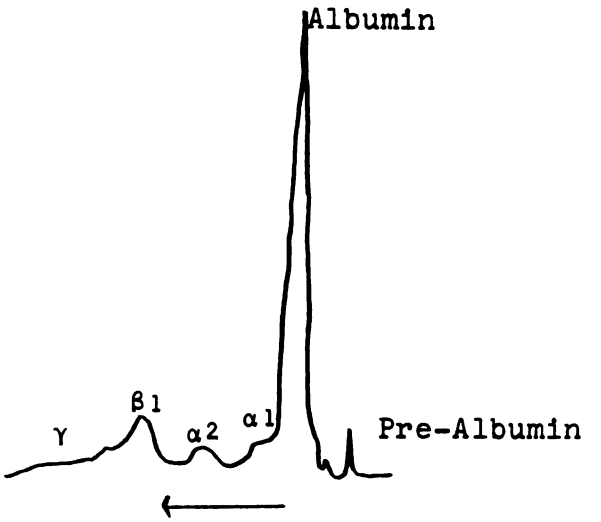


Figure 14

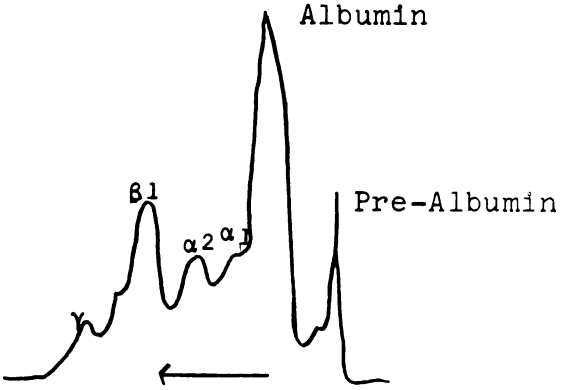


Figure 15

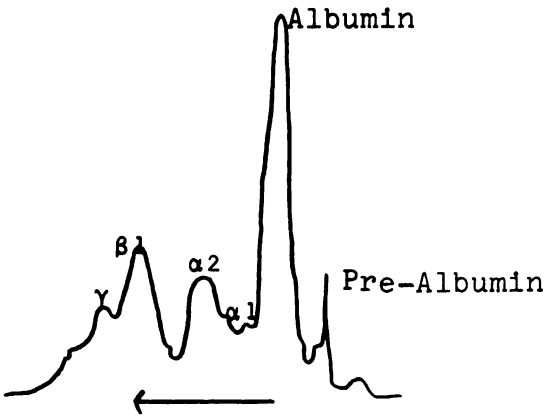


Figure 16

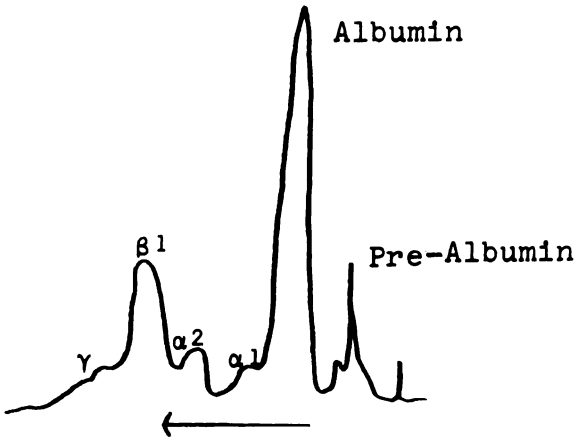


Figure 17

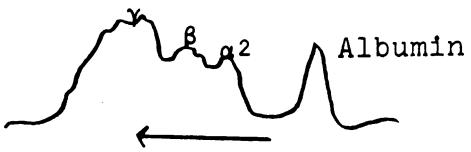
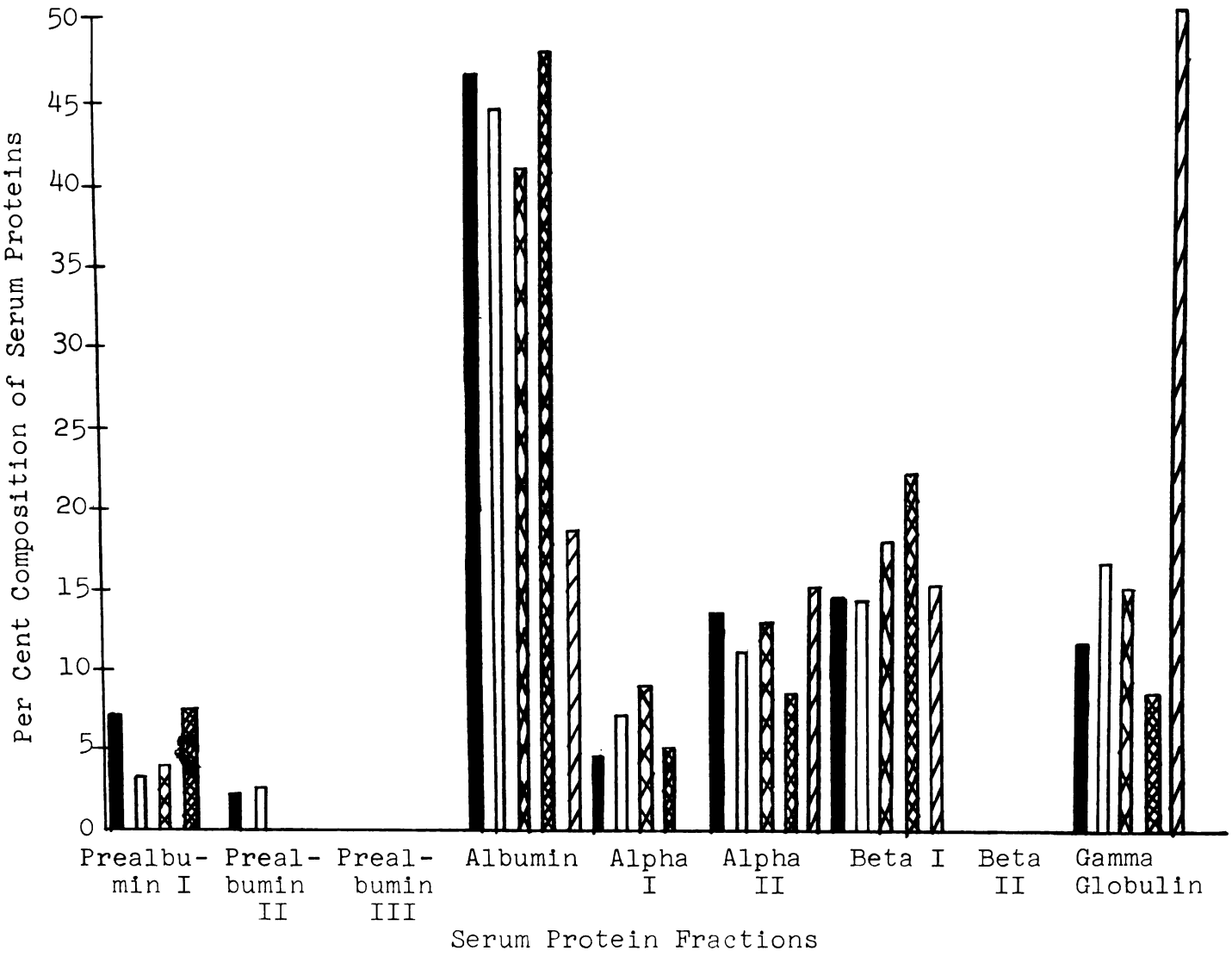


Figure 18

Figure 19.--Summary of optical scan integration and calculation of line intensities of electrophoresis strips of sera taken from turkeys 38 days after inoculation with varying numbers of Heterakis gallinae eggs.

Legend:

- Uninoculated controls.
- Turkeys inoculated with 5 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 50 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 100 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 500 embryonated Heterakis gallinae eggs.


Per Cent Composition of Serum Proteins
50
45
40
35
30
25
20
15
10
5
0
Prealbu- min I
Preal- bumin II
Preal- bumin III
Albumin
Alpha I
Alpha II
Beta I
Beta II
Gamma Globulin
Serum Protein Fractions

Experiment VI: Electrohpretic Studies on the Effects of Challenge Inoculations in Turkeys Previously Inoculated Orally with Varying Numbers of Whole Embryonated Heterakis gallinae Eggs

A total of 20 turkeys survived Experiment V. (See Table II for distribution). With the exception of a new uninoculated control group, all of the survivors were inoculated with 500 embryonated Heterakis gallinae eggs. A new inoculated control group was made up from the uninoculated survivors. In contrast to the results obtained in Experiment V, where 500 embryonated caecal worm eggs produced severe clinical symptoms and 100 per cent mortality, no mortalities nor clinical symptoms had appeared 25 days after the reinoculation. This was also true for the inoculated control birds. Turkeys from all groups were bled at this time and cellulose acetate electrophoresis of the sera was performed. The results are shown in Figure 20. None of the changes that occurred were great when compared to the control sera (See Table VI).

The animals were again reinoculated with 500 embryonated Heterakis gallinae eggs. A new inoculated control group was made up at this time. The first mortality in the experimental group occurred 16 days after the second reinoculation. This was one of the birds originally inoculated with 50 embryonated eggs. The

Figure 20.--Summary of optical scan integration and calculation of line intensities of electrophoresis strips of sera taken from turkeys 25 days after the first reinoculation with 500 embryonated Heterakis gallinae eggs.

Legend:

- Uninoculated controls.
- Turkeys inoculated with 5 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 50 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 100 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 500 embryonated Heterakis gallinae eggs.

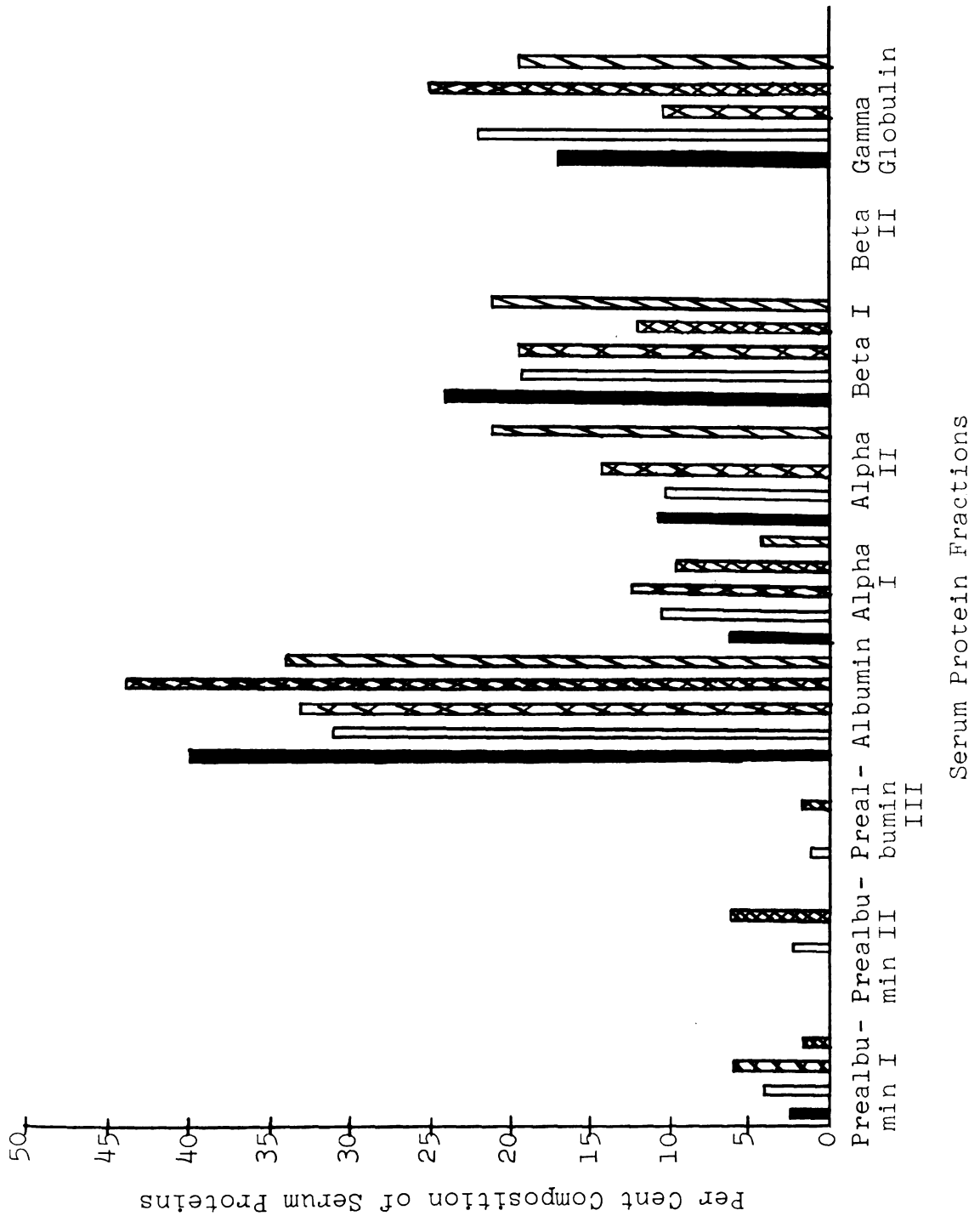

Per Cent Composition of Serum Proteins
50
45
40
35
30
25
20
15
10
5
0
Prealbu- Prealbu- Preal- Albumin Alpha Alpha Beta I Beta Gamma
min I min II bumin I II II Globulin
III
Serum Protein Fractions

TABLE VI.--Average per cent changes in serum protein fraction concentrations within each group of turkeys 25 days after the first re-inoculation with 500 embryonated *Heterakis gallinae* eggs.

	Prealbumins	Albumin	Alpha-1	Alpha-2	Beta-1	Beta-2	Gamma Globulin
Uninoculated controls	+ .4	+ 7.1	-3.5	-5.3	+5.7	--	+ 2.4
Turkeys inoculated with 5 embryonated *Heterakis gallinae* eggs	0	-12.1	+ .9	-5.7	+ .8	--	+12.4
Turkeys inoculated with 50 embryonated *Heterakis gallinae* eggs	+4.1	-10.0	+2.8	-1.7	+ .9	--	+ 5.5
Turkeys inoculated with 100 embryonated *Heterakis gallinae* eggs	+1.3	+ 1.1	- .1	-15.8	-6.4	--	+15.6
Turkeys inoculated with 500 embryonated *Heterakis gallinae* eggs	-2.0	- 9.1	-5.4	+6.4	+2.9	--	+10.4

inoculated control birds were dead 26 days after inoculation. Two turkeys initially inoculated with 5 embryonated caecal worm eggs died 25 and 26 days post-inoculation (See Table VII).

Twenty-two days after the second challenge inoculation birds from each group were bled and electrophoresis was performed on the sera. The results are shown in Figure 21. A decrease in the albumin concentration of all the inoculated groups of birds was seen. Increases in the gamma globulin fraction also occurred in the groups of birds originally inoculated with 100 and 500 embryonated eggs showing the greatest increases. The beta-2 fraction was present in the sera of all the groups except the uninoculated controls and the birds inoculated with 500 embryonated eggs. (See Plate VIII, Figures 22, 23, 24, 25, 26).

Forty-nine days after initiation of the experiment, the only surviving birds were those originally inoculated with five embryonated Heterakis gallinae eggs. These birds were killed and examinations were made to see to what extent caecal and liver involvement had progressed. Of the six birds in the group when the experiment began, four turkeys survived the two challenge inoculations. Examination showed that in one bird only there were neither liver lesions nor caecal cores, even though thickening of the caecal walls was noted. The second birds examined showed a greatly enlarged liver with typical blackhead

TABLE VII.--Effects of challenge inoculations of 500 whole embryonated Heterakis gallinae eggs on turkeys.

Groups	Number of Embryonated Eggs in Initial Inoculation	Number of Deaths per Group	Number of Days After Infectum when Deaths Occurred	Caecal Cores	Liver Lesions
A	0	0/5	--	-	--
B	5	2/6	25,26	-,+,+,-	-,-,+,-
C	50	2/2	16,18	+,+	+,+
D	100	2/2	17,18	+,+	+,+
E	500	5/5	17,18,18,25,26	+,+,+,+,+	+,+,+,+,+

Figure 21.--Summary of optical scan integration and calculation of line intensities of electrophoresis strips of sera taken from turkeys 22 days after the second reinoculation with 500 embryonated Heterakis gallinae eggs.

Legend:

- Uninoculated controls.
- Turkeys inoculated with 5 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 50 embryonated Heterakis gallinae eggs.
- Turkeys inoculated with 100 embyonated Heterakis gallinae eggs.
- Turkeys inoculated with 500 embryonated Heterakis gallinae eggs.

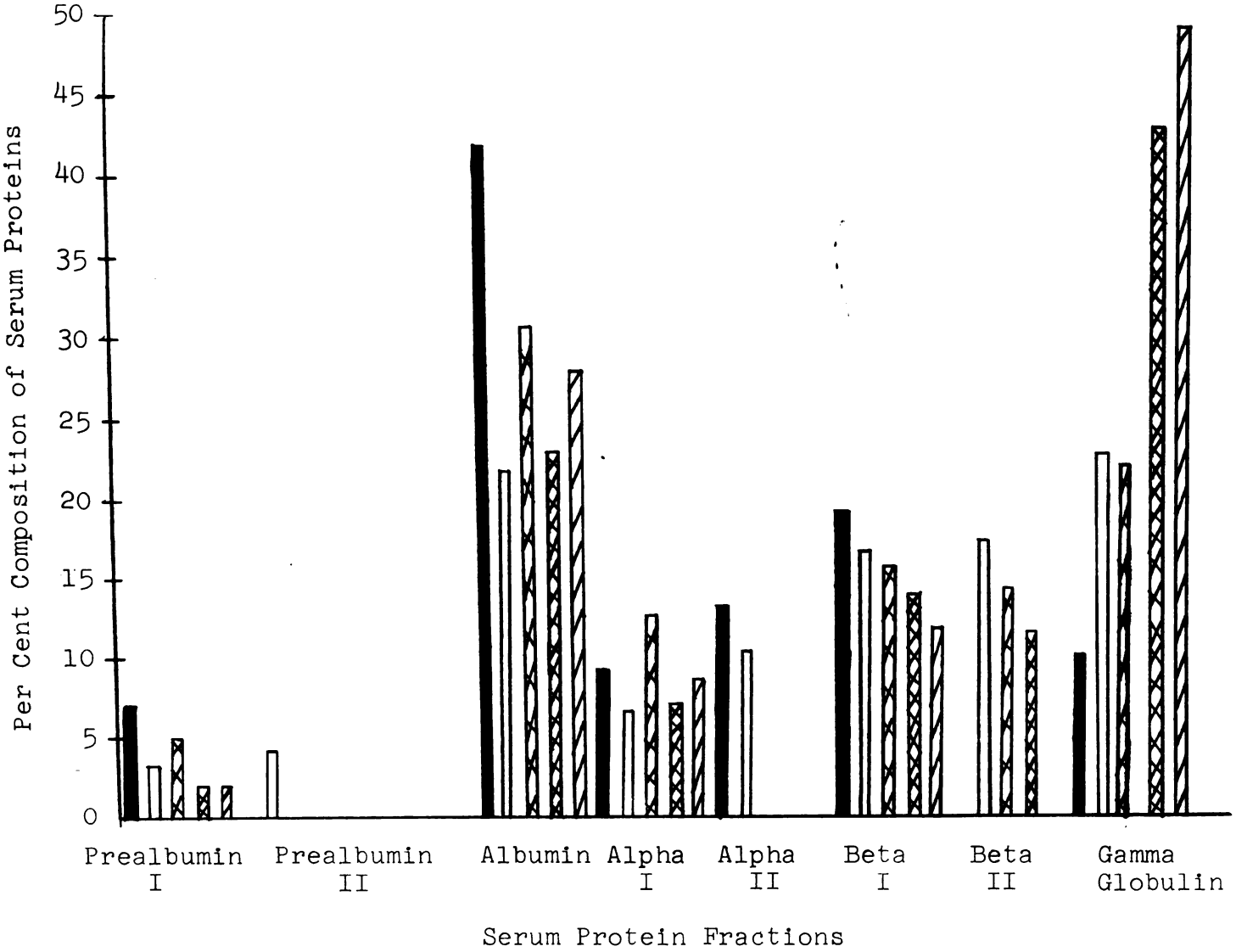

Per Cent Composition of Serum Proteins
50
45
40
35
30
25
20
15
10
5
0
Prealbumin I
Prealbumin II
Albumin
Alpha I
Alpha II
Beta I
Beta II
Gamma Globulin
Serum Protein Fractions

PLATE VIII

Line graphs of optical scan of serum electrophoretic patterns of turkeys bled 22 days after the second reinoculation with 500 embryonated Heterakis gallinae eggs.

Figure 22.--Serum electrophoretic pattern of an uninoculated control turkey.,

Figure 23.--Serum electrophoretic pattern of a turkey initially inoculated with five embryonated Heterakis gallinae eggs followed by two challenge inoculations.

Figure 24.--Serum electrophoretic pattern of a turkey initially inoculated with 50 embryonated Heterakis gallinae eggs followed by two challenge inoculations.

Figure 25.--Serum electrophoretic pattern of a turkey initially inoculated with 100 embryonated Heterakis gallinae eggs followed by two challenge inoculations.

Figure 26.--Serum electrophoretic pattern of a turkey inoculated with 500 embryonated Heterakis gallinae eggs.

PLATE VIII

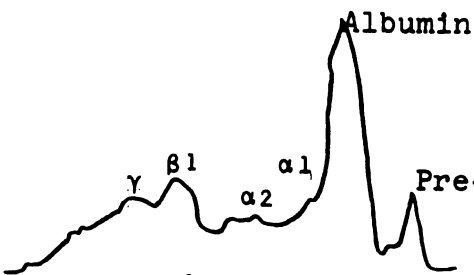


Figure 22

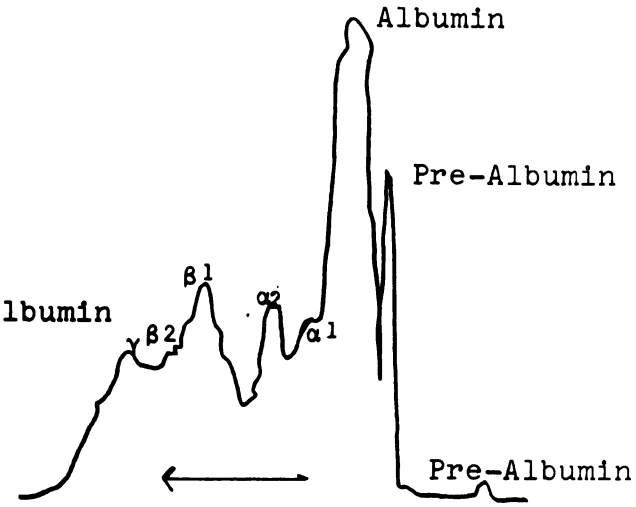


Figure 23

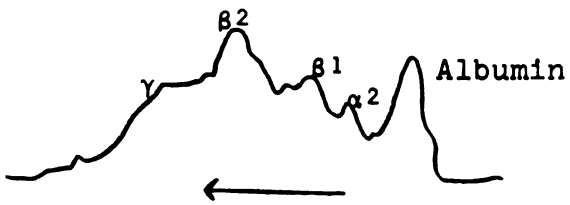


Figure 24

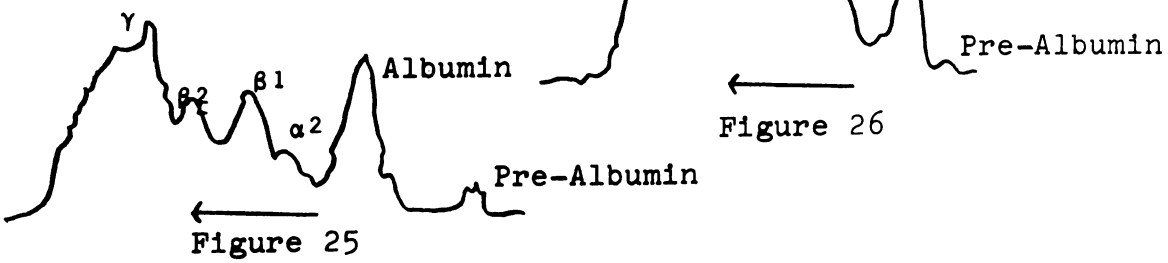


Figure 26

Figure 25

lesions. The caeca appeared to be normal. The third bird, while not having the usual diffuse liver lesions, had a single large nodular lesion, the size of an acorn on the cardiac lobe of the liver. In this bird, cores were present in both caeca. The final bird examined showed scars on the liver, but no lesions. The caeca even though swollen, did not contain cores.

The some degree of immunity was confirmed upon the turkeys inoculated with five embryonated Heterakis gallinae eggs is shown by the consistent and constant rise of the gamma globulin and the slight reduction of the albumin during Experiment V and VI (See Figure 27). Production of anti-bodies to the histomonads or the nematodes may have been responsible for four birds surviving in this group.

Figure 27.--Albumin and gamma globulin relationships in turkeys inoculated with 5 embryonated Heterakis gallinae eggs followed by two challenge inoculations with 500 Heterakis gallinae eggs.

Legend:


Albumin concentrations in uninoculated control turkeys.

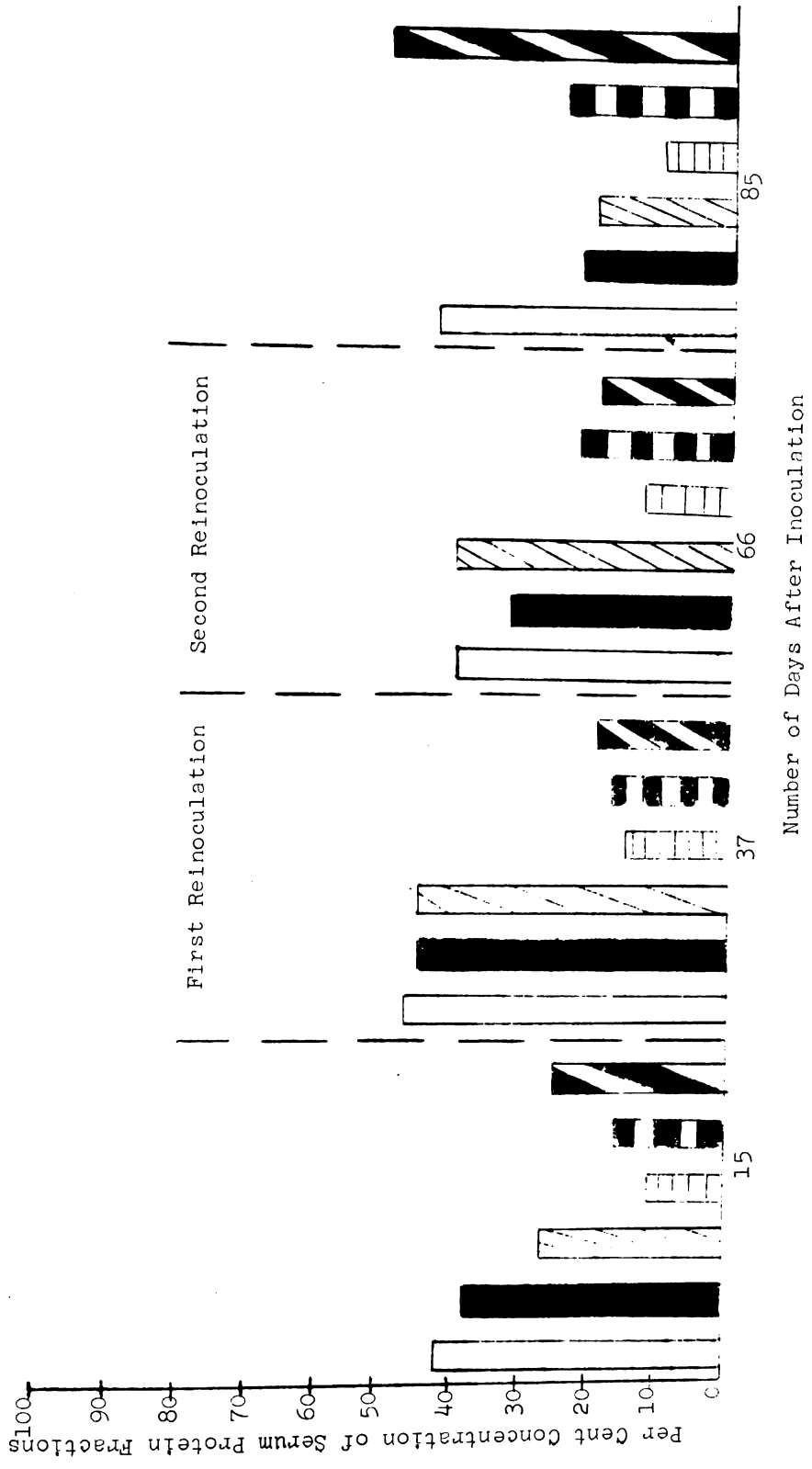

Gamma globulin concentrations in uninoculated control turkeys.


Albumin concentrations of turkeys inoculated with 5 embryonated Heterakis gallinae eggs followed by two challenge inoculations.


Gamma globulin concentrations of turkeys inoculated with 5 embryonated Heterakis gallinae eggs followed by two challenge inoculations.


Albumin concentrations in inoculated control turkeys.


Gamma globulin concentrations in inoculated control turkeys.


Per Cent Concentration of Serum Protein Fractions
100
90
80
70
60
50
40
30
20
10
0
First Reinoculation
Second Reinoculation
15
37
66
85
Number of Days After Inoculation

CHAPTER V

DISCUSSION

Since the presence of Histomonas meleagridis-resembling organisms has been shown in Heterakis gallinae eggs by a number of investigators and since the disease can be induced by the oral administration of whole embryonated Heterakis gallinae eggs, it was desired to learn if histomoniasis could be induced in turkeys by the oral and rectal administration of ground embryonated and ground unembryonated caecal worm eggs.

The finding of typical blackhead liver lesions in turkeys in Experiment I differed from the results obtained by Swales (1948). Initiation of infection with ground embryonated and ground unembryonated Heterakis gallinae eggs in Experiment I may have been due to a number of factors. The resulting infection may be attributed to the fact that the turkeys were taken off feed 24 hours prior to inoculation. Horten-Smith and Long (1956) showed that successful infection by histomonads and the incidence of lesions were directly affected by the alkalinity of the gizzard. The absence of food in the birds may have resulted in a lowered pH of the gizzard and upper intestine and therefore facilitated tissue in-

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vasion. The 20 birds used in Experiment I as compared to the two used by Swales (lec. cit.) could have afforded a greater probability that infection could be initiated. Swales did not state in his paper the length of time turkeys were kept before being killed and examined for liver lesions. He stated that some experiments were terminated at the end of two weeks. Since it took over four weeks before any visible signs of infection appeared in Experiment I, the possibility exists that in birds, in which histomonads are administered in the absence of living larvae, a longer incubation period is needed before lesions appear and before valid conclusions can be made with regard to the initiation of infection.

The production of liver lesions resulting after inoculation of ground unembryonated Heterakis gallinae eggs can also be explained on the basis of research done by Gibbs (1962). He demonstrated the presence of Histomonas meleagridis in uterine eggs of Heterakis gallinae worms. The protozoa were also present in cleaving cells of developing eggs. In the absence of living larvae, grinding of the caecal worm eggs would be a means of releasing the protozoa and making them available to invade the tissues of the birds.

Although post-mortem examination of inoculated birds in Experiments II, III, and IV showed no liver lesions typical of blackhead, the fact that petechial hemorrhages were seen only in the birds inoculated with

ground embryonated and ground unembryonated Heterakis gallinae eggs in Experiment II can not be discounted. It is possible that an infection or invasion of the liver by the protozoa had been effected in all or some of the birds. Clarkson (1962, a) stated "that the absence of demonstrable protozoa suggests that the early liver lesions may be caused by something other than the direct action of the histomonads." The possibility exists therefore, that lesions of "precursors" of typical blackhead lesions may be present without protozoa being demonstrated or present in the tissues.

In Experiment IV, electrophoretic analyses of sera taken from turkeys inoculated rectally with ground embryonated Heterakis gallinae eggs showed consistent increases in the beta and gamma globulin fractions. These changes, even though not as great as in the birds inoculated with 500 whole caecal worm eggs (the inoculated control birds), were evident when compared to sera taken from uninoculated control animals. An increase in the concentration of the beta globulins is an indication of disturbance in the serum lipoproteins. This change is thought to be directly related to disturbances of enteric fat resorption and disturbed liver function. Beta globulins also increase when an inflammatory process or nephrotic syndrome are present Wuhrmann and Wunderly, (1960). According to Leland (1961), increases in beta globulin are attributed to "antibody" formation. But that this evidence is in-

direct. In infections with protozoa and nematodes, one generally sees initial increases in the beta globulin, after a period of time beta globulin concentration decreases and gamma globulin concentrations are seen to rise gradually. However, it is not known if the increase in the beta and gamma globulins are due to antibody formation or are the result of a nonspecific type of response resulting from trauma caused by the invading organisms. Osserman and Lawler (1961) point out that in addition to the major gamma fraction, the immune globulins also include the beta 2A (7S) fraction which is distinguished from the major gamma fraction by a higher content of conjugated carbohydrate, and the higher molecular weight antibody fraction, beta 2M (synγ ., 19S, B_2 macroglobulin). It must be noted that all three of these immunoglobulin fractions have a broad range of electrophoretic mobilities extending from the slow gamma through the beta and into the alpha-2 region.

Since the increases in gamma globulins containing antibodies are known to exist in animals with chronic inflammations due to the presence of protozoa, the possibility exists that histomonads may have managed to successfully invade and damage tissues in all or some of the birds in Experiment IV. However, the possibility also exists that the noted response could have been due to extracellular antigens without the accompanying tissue destruction.

The great differences in results between Experiment I and Experiments II, III and IV are difficult to explain. The occurrence of a typical blackhead lesions in the livers induced by the inoculation of ground embryonated and ground unembryonated Heterakis gallinae eggs in Experiment I indicates that protozoa in both unembryonated and embryonated caecal worm eggs are capable of producing infection if they can reach the tissues. Doll and Franker (1963) have suggested that a synergistic relationship may exist between the protozoa and the bacterial flora of the host. The possibility exists that the turkeys in which blackhead lesions did not appear lacked the necessary microbial factors that would allow tissue penetration or, perhaps they had some factor which prevented invasion and infection. Also, the possibility exists that the protozoa within the nematode eggs must be at a certain stage of physiological development before they become infective to the host. Even though a typical blackhead lesions were produced with ground caecal worm material, other experimental methods may be more successful in producing consistent results. Staining techniques employed to determine if the protozoa within the nematode and nematode eggs undergo stages of development before reaching an infective stage may be fruitful. The possibility exists that factors concerned with the developing larvae determine the infectivity of the protozoa. The inoculation of turkeys using Heterakis

gallinae eggs in various developmental stages may be another method of determining at what stage the protozoa become infective. In order to eliminate variables involved when using animals, tissue culture methods, or inoculation into chick embryos could be employed to see what effects are casued by histomonads released from developing nematode eggs.

The studies of Tyzzer (1934), Swales (1950), and Kendall (1957) have indicated that acquired resistance to histomoniasis may occur as a result of infection. By using varying numbers of whole embryonated caecal worm eggs, it was desired to learn if a persistent but nonlethal infection could be induced in inoculated turkeys that would also result in the production of protective antibodies.

The over-all results in Experiment V showed that as the size of the inoculum increased, an electrophoretic pattern indicative of progressively severe disease appeared. This was expressed by the consistent decrease in the albumin and a corresponding increase in the gamma globulin. Rama and Colby (1953) studied the effects of Plasmodium berghei on the serum proteins and also reported a decrease in albumin and an increase in gamma globulin. They attributed the albumin decrease to liver and kidney damage and felt that the gamma globulin increase was probably immunological in nature.

Inspite of the fact that Heterakis gallinae eggs used in Experiment VI were from the same batch that produced 100 per cent mortality in the inoculated controls (Group E, 500 embryonated eggs administered orally) in Experiment V, there were no clinical manifestations in the inoculated controls of Experiment VI. the lack of development of a severe infection in the animals previously inoculated may have been due to the presence of caecal cores which caused the larvae to be passed through the gastro-intestinal tract without their being able to establish themselves in the host. However, this is inadequate to explain the lack of mortalities in the inoculated controls (Group E). The possibility exists that since these eggs were embryonated at a different time, there were fewer viable larvae or only a small percentage of nematode eggs contained infective histomads. The strongest evidence to give indication of any degree of infection was the demonstration that the alpha-2 fraction had doubled in concentration in the sera of the inoculated controls (Group E). It was also seen that there were no consistent changes in the electrophoretic patterns of sera from the inoculated animals. The responses which were noted were probably due to the combined effected of the reinoculation as well as the initial infection.

The observed alpha-2 increases are also related to infection. This serum fraction is thought to be non-

immunological in nature and is considered to be completely non-specific because it always increases when there is a disturbed cellular protein metabolism. Although the alpha-2 globulin appears to be electrophoretically homogenous, it is chemically heterogenous because lipoproteins and glycoproteins are present (Wuhrmann and Wunderly, 1960). Conditions that can increase or decrease the serum glycoporteins will obviously produce a change in the alpha globulin concentration. Increase in the glycoproteins results when they are released from injured or inflammed tissues into the blood stream, or they may arise as a response to tissue injury, or proliferation of damaged liver cells. A mechanism may be present which initiated the production of glycoproteins which may have a physiological role in the defense mechanism of the host. The rapid increase of the glycoproteins after liver damage may be attributed either to their release from damaged cells or by increased biosynthesis in the liver.

The appearance of the beta-2 fraction in turkey sera in Experiment IV, V, and VI indicated that there were derangements of the serum lipoproteins. Under normal conditions, only a beta-1 fraction is observed. The absence of the beta-2 fraction after 38 days in Experiment VI indicated that reparative processes were operative and the factors causing the alterations in the serum lipoproteins were being corrected. Another

indication that reparative processes were operative is seen when comparison was made of the per cent changes in the serum protein fractions after 38 days. In all turkeys surviving Experiments V and VI an increase in the albumin concentration was noted.

In addition to the assault on the host by the combined efforts of the nematodes and the protozoa, various physiological disturbances in the effected animal influence the serum protein pattern. Among some influenceing factors are malnutrition, negative water balance and loss of protein through the gut (Leland, 1961). In addition to the preceding, McGuire and Cavett (1952) have shown that blood non-protein nitrogen and uric acid decreased in the early stages of the disease. Blood glucose concentration increased during the incubation period. As the infection progressed, a hypoglycemia developed. It is therefore possible, that the electrophoresis results were either directly or indirectly influenced by the nematodes and the protozoa.

The failure of any of the birds inoculated with five embryonated Heterakis gallinae eggs to die was probably due to the small size of the inoculum. However, a persistent and constant increase in the gamma globulin concentration after 38 days indicated that some physiological response to the nematodes or protozoa was occuring.

Four of six birds inoculated with five embryonated Heterakis gallinae eggs withstood the two challenge inoculations of 500 embryonated caecal worm eggs. This may indicate the resistance to infection was induced by an immunological mechanism that could be associated to the fairly constant and consistent increase of gamma globulin that was noted throughout the course of Experiments V and VI. The immunizing infection may have elicited antibodies specific for both the caecal worm larvae and for the histomonads.

The fact that severe pathological changes and 100 per cent mortality occured in all inoculated birds in Experiment VI except for those birds previously inoculated with five caecal worm eggs showed that severe invasion of the livers and caeca by the histomonads had been prevented. The possibility exists that a result of the immunizing infection, immunological processes were set into operation and that the gamma globulin increase may have been due to the increased production of antibodies against the caecal worm larvae and the histomonads.

SUMMARY

Turkeys were inoculated orally and rectally with ground embryonated and ground unembryonated Heterakis gallinae eggs to see if experimental histomoniasis could be produced. In one experiment, typical blackhead liver lesions were seen. Histologic studies of tissues from the experimentally inoculated turkeys showed organisms resembling Histomonas meleagridis. Electrophoretic studies were also undertaken to see what effects the ground heterakis material would have on the serum protein picture of the inoculated turkeys. The results showed an increase in the beta and gamma globulin concentrations in the sera of birds inoculated rectally with ground embryonated and orally with ground unembryonated eggs. In no case were the increases as great as in the inoculated control birds.

Turkeys were also inoculated with varying numbers of whole embryonated Heterakis gallinae eggs to see if a level of infection could be induced that did not result in mortalities. Five caecal worm eggs inoculated orally accomplished this. Electrophoretic studies indicated that there was a correlation between the size of the inoculum and the resulting serum protein changes.

Challenge inoculations of 500 embryonated Heterakis gallinae eggs to birds previously inoculated with varying numbers of embryonated caecal worm eggs indicated that resistance mechanisms may have been established in the birds initially inoculated with five embryonated Heterakis gallinae eggs. The resulting immunity against fatal infection may have occurred as a result of antibodies specific for the caecal worm larvae or for the histomonads.

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